Pascual Ángel Gargiulo
Humberto Luis Mesones-Arroyo
Editors

Psychiatry and Neuroscience Update

A Translational Approach, *Volume II*

Springer

Psychiatry and Neuroscience Update - Vol. II

Pascual Ángel Gargiulo
Humberto Luis Mesones-Arroyo
Editors

Psychiatry and Neuroscience Update - Vol. II

A Translational Approach

Springer

Editors
Pascual Ángel Gargiulo
National University of Cuyo
Catholic University of Argentina
Guaymallen Mendoza
Argentina

Humberto Luis Mesones-Arroyo
Institute of Neurobiology Foundation
National Academy of Medicine
Buenos Aires
Argentina

ISBN 978-3-319-53125-0 ISBN 978-3-319-53126-7 (eBook)
DOI 10.1007/978-3-319-53126-7

Library of Congress Control Number: 2015945938

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

Since the explosive growth in discoveries by neuroscientists, the fields of neurology and psychiatry have changed dramatically. Theoretical hypotheses that had more to do with poetry and mythology have left the stage, and new actors have begun to explain how human behavior is conditioned by DNA (Desoxiribonucleic acid) and neurotransmitters. Fascination with the details of metabolic molecular interactions has changed the whole picture of human behavior.

Specialization in medicine has made it impossible to be the kind of general practitioner who formerly had the time and the will to relate to each patient as a person, as a member of a family, and as a member of a social group, with their own life history, moral standards, and manners.

Psychiatry, and its younger sibling clinical psychology, have tried to bridge the gap between personal relationships and medical specialization, but again scientific experience and objective evidence have rejected the validity of philosophical deduction. The very complexity of the anatomy, histology, and physiology of the nervous system should alert us to avoid making simple materialistic explanations.

We must continue with this effort, initiated by a group of colleagues who are trying to preserve the personal relationship between the person who suffers and their medical help.

Acquiring scientific and technical competence is becoming more and more demanding and time-consuming, and the range of abilities that are needed by those with a medical vocation has expanded. Teamwork can help medical practitioners to relate to their patients and their peers, and to accept different views. Guidelines established by the therapeutic community that stress sensitivity training and living learning situations should be part of medical education, but the chance to start near a teacher from whom one can absorb experience and humanity is a prized gift from the gods.

Anthropology is the cornerstone of understanding human beings. Love is the force that moves the Hippocratic spirit. Empathy is the feeling that has to be mastered by health professionals. Humanization has to surpass ideologies that deny human rights based on the purported freedom to apply technological advances without bioethical boundaries. The present time is one of risks and challenges. We assume the commitment to enforce human dignity in its psychological, social, and spiritual uniqueness.

Buenos Aires, Argentina
November 14, 2016

Humberto Luis Mesones-Arroyo

Preface

As in the previous volume, the present state of the art in the knowledge of motivation or causality and the treatment of mental disorders is reviewed by researchers working in different fields who are trying to communicate what, classically, has not been well communicated, that is, the integration of basic neuroscience findings and clinical approaches to mental disorders. Furthermore, the correlation between clinical approaches and their anthropological basis is outlined here. Once again we try to avoid the lack of integration between anthropology, clinical practice, and basic neuroscience.

Here we offer an opportunity for the meeting of different disciplines that study human beings and their normal behavior. In these pages, laboratory findings, clinical practice, and comprehensive anthropology are integrated. Emerging ideas, different from the mere chemical-physical-mathematical concept, are proposed here. Also, the difference between understanding and explaining human mental illness is emphasized. Our main intention is to provide a bridge between different disciplines, integrating different points of view.

As in the previous volume, new trends in different fields converge in the pages of this volume. Basic studies, clinical evidence, and heuristic discussions are here drawn into a multidisciplinary confluence that opens a rich dialogue. An open forum is proposed here, with the aim of consolidating integration. Illustrative examples of lines of research originating from different areas are shown here. Additionally, invited authors come from different geographical areas and very different disciplines, providing the opportunity for an open ambit to study human behavior as a whole.

The book has been designed, as was the previous one, with the aim of reaching a large number of readers. Primarily oriented to researchers and mental health professionals, the material here may prompt the actualization or initiation of studies of human behavior. In this sense, the present pages are directed to academics and students. Some chapters also deal with education strategies that are related to the application of neurosciences in teaching practice. Physicians may find here representative information in the field of neurosciences and its application to everyday practice. The effort to integrate knowledge is a difficult preoccupation for us.

As in the previous volume, the book is divided into four main sections. The first section is dedicated to the philosophical, epistemological, and anthropological basis of the study of human behavior. The intention is, again, to avoid reductionism, offering a place for comprehensive psychology. This

first section starts with a significant chapter by Miriam Dolly Arancibia de Calmels, who writes regarding the phenomenology of the encounter. Commenting on the ideas of Jozéf Tischner, she draws an interesting reflection on some recent manifestations of human individualism. A Manichean position related to fundamentalism, xenophobia, and other violent phenomena is here described and lucidly analyzed. The notion of encounter in Tischner is analyzed in the context of his proposal; i.e., giving to the other human being in the encounter his or her full value and meaning. This encounter with another and the involved variables of the other person are the subject of an exhaustive analysis. Some expressive windows are opened here by Dolly Arancibia de Calmels.

The second chapter, by Ivana Anton Mlinar, is dedicated to the role of corporality in psychopathology. It is postulated here that the notions of self and intersubjectivity have a clear link with corporality, and that these notions began to develop in pre-linguistic periods. It is postulated that these notions are present in psychiatric illnesses, even in its more dramatic forms. The relevance of these notions is highlighted, resolving the dichotomies between bodily functions and mental states, enhancing the value of concrete, bodily existence and suggesting its possible role in therapy. Ivana Anton Mlinar provides interesting reflections here.

In the third chapter, Professor Pablo Emanuel García proposes a bridge between neurosciences and philosophy, similar to that initiated between neurosciences and psychiatry. The proposal is highly interesting, avoiding the reduction of the first to the second, and vice versa. He proposes Dan Zahavi's phenomenology of mind as a valid way to superate the analytic tradition in the field of the philosophy of mind. The Zahavi proposal is presented in four steps. Firstly, García clarifies the term "phenomenology". Secondly, he makes a distinction between first- and third-person perspectives, the first related to the phenomenology proposal, the second related to a neuroscience framework. As the third step, Pablo García explains the methodological stages assumed by Zahavi from the Husserlian phenomenological tradition. As the fourth step, a *naturalization of phenomenology* is proposed, aiming to facilitate a dialogue between science and philosophy. The chapter is dense, rigorous, and relevant. Pablo García reviews a renewal of the phenomenological approach and establishes a bridge between science and phenomenology, incorporating the criteria of Zahavi.

The fourth chapter, written by Professor Ricardo F. Crespo, is dedicated to the exploration of a possible philosophical proposal, tending to a non--physicalist conception of mind, here considered reductionist. Crespo proposes a classical philosophical frame. He refers to Aristotelian hylomorphism, considering it as providing an adequate non-reductionist perspective. Finally, Crespo maintains that, in the opinion of contemporary Anglo-Saxon professional philosophers, a physicalist reductionist position in the philosophy of neurosciences is not unanimous. The position defended by Ricardo Crespo, written in an elegant and precise style, prevents excesses of reductionism.

In the fifth chapter, Jorge Martínez Barrera analyses the question of the soul in Aristotle, drawing a comparison with proposed contemporary body-mind relationships. Physicalist reductionism is analyzed, and proposed

limitations are discussed. Martínez Barrera maintains that one limitation of this concept is that it reduces the explanation of mental activity and free choice to neurological conditions. Additionally, he postulates that physicalist reductionism does not allow other possible explanations that would lead beyond the determinism of the neurosciences. The chapter is well designed and written with rigor and precision, in a pure style. It constitutes a relevant examination of the true extent of the attempt to explain mental events and human actions neurologically.

The sixth chapter, by Ricardo Aranovich, analyzes the ideas of Ortega y Gasset about what that author defines as the *project of life*. This proposition starts with Ortega's ideas concerning what is man. Ortega maintains that man is his life. The life project must be analyzed in this context. Here, the life project is what a man is *already* doing in his life. The notion of vocation is analyzed in this framework. Vocation is a *doing* responding to a defined wish, in form and objectives. When a vocation is followed, it leads to satisfaction, independently of results. Aranovich analyzes the loss of contact with us, with ourselves, as underlying the current cultural crisis. He concludes by analyzing the notions of vocation and the life project. He maintains that the life project must be the expression of vocation, and that it should be considered as a fundamental objective in psychotherapy. It should be, in a wider sense, an objective of *living* in general. Aranovich's analysis constitutes an interesting effort to use the philosophy of Ortega y Gasset in psychotherapy.

In the seventh chapter, Gilberto Gamboa-Bernal proposes a brief bioethical perspective of work in healthcare. He considers that the delivery of medical services has become a real business and this is considered an actual distortion in the field of health. The emergence of this kind of new healthcare system is analyzed from a bioethical point of view. Scientific and medical requirements, such as technical competence and human skills, that are necessary in the persons dedicated to health services are analyzed here, together with a remembrance of the Hippocratic tradition and its implications in contemporary medicine. This chapter is, interestingly, dedicated to the humanization of health services, and Gamboa-Bernal outlines here a very interesting proposal.

The eighth chapter is dedicated to a discussion of the instrumentalization of human faculties. Luis Echarte maintains that, in the present post-emotional society, human faculties (rationality, affectivity, and will) are instrumentalized, with a new modern moral paradigm leading to this instrumentalization. Echarte analyzes three types of psychological problems dealing with inauthenticity: those related to: (a) the artificial origin of emotions, (b) the physical nature of emotions, and (c) the episodic coherence of emotions. Finally, he proposes solutions to these problems. In this context, the presence of cosmetic psychopharmacology is deplored. The chapter, well documented, is an example of rigor.

The first section of the book ends with the ninth chapter. This chapter is an invitation to rethink two terms in classical psychology: "identity" and "personality". The chapter, written by Francisco Guell, Javier Bernacer, Pilar de Castro-Manglano, Gonzalo Arrondo, and José Ignacio Murillo, constitutes an interesting discussion of these topics. Authors from a Mind-Brain research

group, a department of psychiatry, and a department of philosophy converge in the consideration of these relevant concepts. Psychiatric diagnoses and classifications are analyzed. The case of dissociative identity disorder (DID) is a particular focus, emphasizing the relevance of a dialogue between philosophy and psychiatry. The authors postulate that the concept of *person* in John Locke's philosophy is a relevant influence in this regard. This philosopher conceived the person as an apparent expression of consciousness and memories. The authors of the chapter maintain that this concept has led to some problems and misunderstandings, dissociating mental activities from the body. A dialogue between psychiatry and philosophy is postulated and favored here, in a very profound and interesting chapter.

The second section of the book is dedicated, as in the first volume, to basic neuroscience. Some paradigmatic examples of lines of research that are conducive to interesting extrapolations are included here. They may serve as examples of the continuity that we are trying to show in building the bridges between basic and clinical findings. The preclinical value of these lines of research is evident. They are closely related to the recently postulated mechanisms and preclinical evidences of mental illnesses, and may contribute to the clarification of several variables related to them. Extrapolation of the research findings to clinical conditions is evident here. These lines of research may contribute to the explanation of somatically based illnesses, and they may also contribute to the understanding of some aspects of psychoreactive disorders. Considering the near future, these studies may also contribute to current research projects in these areas.

The second section of the book starts with the tenth chapter which is dedicated to exploring the effects of emotional stress on astrocytes. The implications of these effects in stress-related disorders are also considered. The authors of the chapter, Christian Bender, Gastón Calfa, and Víctor Molina, note the role of stress in the etiology of several psychiatric disorders, where it is considered a major risk factor. The authors review evidence on the role of morphological and functional changes of astrocytes in basic stress-related models and the extrapolation of this evidence to mental disorders. The concept of behavioral sequelae and their relationships with astrocyte changes, and the corresponding relationship with psychiatric illnesses, are here developed in an interesting manner. Important new lines of evidence are considered, giving remarkable value to this chapter.

In the eleventh chapter, Jorge Aquino adds significantly to the information provided in the previous chapter, writing on the role of the glia in health and disease. New unexpected roles of the main glial subtypes are discussed. The role of glia in learning, memory, fear conditioning, long-term potentiation, and some complex neurocognitive functions is developed here. The mechanisms involved at the cellular and systemic levels led the author to review glial-neuron and glial-glial interactions. Additionally, Aquino postulates that human brain evolution required the concomitant evolution and specialization of glia, in order for these cells to interact with neurons. The role of glia in psychiatric disorders is reviewed and considered in the light of recent evidence, including that in the field of embryogenesis. The chapter is precise, elegant, and informative.

In the twelfth chapter, Walter Manucha describes the protective role of nitric oxide pathways on the neurotoxicity mediated by glutamate-induced apoptosis. Important lines of evidence suggest the involvement of oxidative stress in neurodegenerative diseases. The author postulates, giving solid evidence, a clear deregulation of the mitochondrial respiratory mechanism in patients with neurodegeneration. Protection against neurotoxic effects is related to mitochondrial respiratory mechanisms. In this context, inflammation and apoptosis are clearly related. The nitric oxide pathways appear to be mediating modulation, preventing oxidative damage to neurons, inhibiting apoptosis. This chapter is solid and well documented, constituting a relevant source of new findings.

The thirteenth chapter, written by Gustavo Tafet, is dedicated to exploring the interaction of psychoneuroendocrinological and cognitive variables in the interface of chronic stress and depression. The chapter is directed to the explanation of converging stress factors. The role of chronic stressful experiences, in adulthood and early-life events, may produce activation of the hypothalamic-pituitary-adrenal (HPA) axis. This activation leads to the increased synthesis and release of corticotrophin-releasing hormone (CRH) and cortisol. Furthermore, chronic stressful experiences appear to be associated with functional changes in certain limbic structures, such as the amygdala and hippocampus, and changes in different monoamine and indoleamine systems. Evidence regarding these mechanisms is provided here in an elegant and interesting form by Professor Tafet.

In the fourteenth chapter, written by a group led by Claudia Bregonzio and Gustavo Baiardi, the cognitive alteration induced by psychostimulants is studied, focusing mainly on the role of angiotensin AT1 receptors. The other members of the group are: Natalia Marchese, Osvaldo Martin Basmadjian, and Victoria Belén Ochieppo. These authors outline the role of dopamine and other monoamines and their relationship with psychostimulants. The actions of these drugs on learning and memory are also presented, in a novel form. The effects of amphetamines on several brain areas, and the corresponding effects on learning and memory, are detailed here. The authors comment here on their own lines of research, with recent evidence, and a solid bibliography.

In the fifteenth chapter, written by some members of the same group that wrote the fourteenth chapter (Claudia Bregonzio, Natalia Andrea Marchese, María Costanza Paz, Emilce Artur de la Villarmois, Gustavo Baiardi, and Mariela Fernanda Pérez), the previous notions are expanded. The extent of the neuroadaptive responses to psychostimulant drugs is presented. In this chapter, dopamine innervated areas (the caudate putamen, nucleus accumbens, substantia nigra, hypothalamus and ventral pallidum) that express high AT1 receptor density are studied. The recent findings of the authors show the relevant role of angiotensin II AT1 receptors induced by amphetamines in neuroadaptative behavioral and neurochemical changes. The authors report alterations in the components of the renin angiotensin system (RAS) and in the functionality of AT1 receptors observed after amphetamine exposure. This chapter constitutes an interesting and bright example of preclinical lines of research explaining drug effects.

In the sixteenth chapter, the team directed by Carlos Tomaz develops present evidence linking habit learning and addiction. This study was written by Antonella Gasbarri, Enrico Patrono, Asunta Pompili, Hisao Nishijo and Carlos Tomaz. The relevance of the mesocorticolimbic reward system is emphasized and analyzed in a detailed form. The involvement of different brain areas and circuits in regard to cognitive functions is studied, and the authors outline the relation of motivation and memory to the above reward system. For years, this research group has made continuous and transcendent contributions to the theme of drug effects on the brain. The effects of addiction on different behavioral patterns and their corresponding brain structures are here lucidly considered, in a very original manner.

The seventeenth chapter is dedicated to the role of stress in the dynamic of fear memory. This chapter, written by Gastón Calfa, Marcelo Giachero, and Víctor Molina, constitutes an interesting approach from the synaptic-cellular phenomena observed in preclinical approaches to psychiatric illnesses. The role of mnemonic processing is studied in close relationship to aversive neuronal circuitries. The role of these structures in the modification of behavioral responses is developed, and the implications of long-lasting emotional memories are discussed and analyzed. The fact that in some cases perturbations of the modulator mechanisms involved in adaptive responses become excessive or inappropriate is here brightly analyzed, and the relationship of these perturbations to clinical reality are interestingly commented on.

In the eighteenth chapter, written by Renata Duarte, Aline Caron Borges, and Marilia Barros, an interesting effort is made aiming to establish a relationship between palatable foods and drug addiction. The intention of these authors is to show evidence of the mechanisms underlying addiction-like behaviors. The roles of hormones and the hedonic drive are analyzed as being related to addictive processes. The authors focus on “food addiction” and “food-related binge-like” and “craving” behaviors, and a very interesting parallelism is established. The role of the hedonic drive in “overriding” a homeostatic system is postulated. An interesting group of different animal models is then discussed. The relationship between food addiction, binge eating, and reward, and the correlation to findings in animal models, constitute the main line of discussion in this interesting chapter.

The nineteenth chapter, written by Laura Perez-Caballero, Sonia Torres Sanchez, Juan Antonio Mico, and Esther Berrocoso, constitutes an interesting approach to deep brain stimulation (DBS) as a tool for use in patients with severe depression. Since statistics show that a high percentage of patients with depression are refractory to treatment, new strategies should be tried in this population. The postulated mechanisms of DBS are interestingly analyzed in view of recent evidence. The effect of DBS applied to different brain areas (ventral capsule/ventral striatum, nucleus accumbens, subgenual cingulate cortex, lateral habenula, medial forebrain bundle, and inferior thalamic peduncle) is here detailed in an interesting manner by a group with wide experience in this research area.

In the twentieth chapter, written by Jens Helmert and Sebastian Pannasch, parameters and mechanisms of eye movements are interestingly described and analyzed. The dynamics of the complex interaction of several types of

eye movements are studied and discussed. The involvement of several components of these movements with different tasks (orienting in space, identifying objects, interacting with persons) is also discussed. The main characteristics of fixations and saccades in the context of active vision are developed. An analysis of the duration of fixations and amplitude is developed as a possibility to gain insights into the processing of visual information. Attention is a parameter closely related to these phenomena. Perception of context is involved in this discussion by a solid research team.

The twenty-first chapter constitutes, in some senses, an extension of the previous chapter. In this chapter, Gerardo Fernandez, Paola Buedo, David Orozco, and Osvaldo Agamennoni analyze eye movement as a strategy to study cognitive performance. The relationship of this line of research to pathologies is also considered. Eye movements are analyzed. The interest, as signaled in the immediate posterior sentences, is to have applications in clinical research in mild cognitive impairment, Alzheimer disease and schizophrenia. The value of these techniques in the early diagnosis of mental illnesses is discussed. This research group opens interesting windows to clinical research.

In the twenty-second chapter, the last chapter in section two of the book, psychotropic medication in the elderly is interestingly analyzed. This chapter, written by Alicia del Carmen Panini, Mauricio Roberto Teves, Emiliano Giraudo, Marisa Hilda Garraza, and Claudia Patricia Calderón, constitutes a very interesting approach to clinical pharmacology in a very vulnerable patient group. Problems in the use of different medications are analyzed in a very strict manner. This is an elegant study of treatment approaches in the elderly, in terms of polypharmacy, adverse drug reactions, drug interactions, medicalization, and the rational use of medicines. The authors discuss different areas of pharmacology in regard to drug use in elderly people and the consequences of this drug use. This study shows the wide knowledge and experience of this group.

The third section of the book, consisting of the twenty-third and twenty-fourth chapters, is mainly dedicated to the relationship between neurosciences, education, and the dialogue with the social environment. Topics such as learning and teaching are discussed in relation to studies of the relevance of social variables. The study in the first chapter of this section develops ideas about cognitive stimulation to be used mainly in those who are socially vulnerable. The second chapter reports research on psychological suffering in the world of work. Some ways to improve learning are delineated in the first study, and possibilities of improving work conditions are delineated in the second. Both give interesting ideas to prevent the relevant problems.

The twenty-third chapter, written by Celina Korzeniowski and Mirta Ison, provides an interesting description of cognitive stimulation programs in children. Some interventions are proposed for children growing up in disadvantaged socioeconomic conditions. The objective is to stimulate cognitive control capacities, with the idea of counteracting the adverse effects of poverty on children in need. Here two cognitive stimulation programs promoting executive functions (EFs) in Argentine children are reported. The efficacy of this treatment, which aims to narrow the gaps in cognition associated with

conditions of poverty, is discussed. The implications of the treatment reported here are very relevant for future generations.

The twenty-fourth chapter is oriented to the study of the world of work. The authors, Melisa Mandolesi, Carlos Bonantini, Víctor Quiroga Calegari, Maria Romina Cattaneo, and Miguel Gallegos, studied psychological suffering in the staff of an electrical services company. This chapter, immersed in the world of mental health, focuses on some variables using the authors' own standardized instrument. This study continues a previous one, studying psychological stress in those employees who must meet the demands of customers. These employees, in some cases, must deal with customers' annoyance regarding services or costs. The study results showed important levels of psychological distress and suffering. This chapter is highly relevant to work conditions.

The fourth section of the book is dedicated to the study of the neuroscientific evidence of psychiatric illnesses, *psychoses* in the sense of Kurt Schneider; that is, mental illnesses of the body or somatic base, caused by somatic alterations. The somatic illnesses described here cause behavioral disorders. The way to study them is to "explain" ("*erklaren*").

The first study in this section, the twenty-fifth chapter, initiates approaches to the etiology of schizophrenia. Written by Ane Murueta-Goyena-Larrañaga, Harkaitz Bengoetxea Odriozola, Pascual Ángel Gargiulo, Naiara Ortuzar, and José Vicente Lafuente Sanchez, the chapter describes the neuropathological findings related to Dizocilpine (MK-801) murine models of schizophrenia. The relevance of neuropathology in schizophrenia is clearly established in this chapter, and the interactions of glutamate and gamma aminobutyric acid (GABA) are described and related to schizophrenia. The involved brain circuitries are described in a detailed manner. Additionally, the neurophysiological phenomena present in schizophrenia are here related to modifications in some neuron populations. The authors also note altered gamma-band oscillations in schizophrenic patients, drawing attention to a possible deficit in fast-spiking parvalbumin-expressing interneurons related to the illness. This chapter constitutes an effort to link basic and clinical evidence, and structural and functional facts.

In the twenty-sixth chapter, presented by Alyssa Sbisa, Maarten Van Den Buuse, and Andrea Gogos, the effect of estradiol and its analogues on cognition is reviewed, with the effect evaluated in preclinical and clinical studies. The relevance of these findings to schizophrenia is raised. The authors start from epidemiological and clinical evidence, suggesting a relevant role of estrogen in schizophrenia. They also mention a growing body of literature suggesting the possibility of estrogen therapy in this illness. The roles of the sex steroid hormone 17β-estradiol and selective estrogen receptor modulators (SERMs) are mentioned and developed. This is really an advanced and interesting article by this solid team.

The twenty-seventh chapter constitutes a very interesting review of neuropsychiatric symptoms related to cholinergic deficits in Parkinson's disease (PD). The authors, Santiago Pérez-Lloret, María Cecilia Peralta, and Francisco Barrantes, point out that in this illness not only dopaminergic neurons die. Recent findings have shown that neurodegeneration is also present in norad-

renergic, serotoninergic, cholinergic and other monoaminergic neuronal populations. In this chapter, the role of cholinergic deficits underlying cognitive dysfunction, psychosis and sleep disturbances in PD and the corresponding treatment, are widely explained. Studies of different drugs in related fields are also reported. This is a chapter written by a research group who have been generating recent interesting findings in the field.

The authors of twenty-eighth chapter, Philipp Singer and Benjamin Yee, analyze a possible new approach for schizophrenia treatment. Following the glutamate evidence, they study here the inhibition of glycine transporters. The role of treatment with glycine reuptake inhibitors (GRIs) is reviewed, with its goals and failures. The rationale and potential of GRIs to treat other neuropsychiatric conditions beyond schizophrenia, such as obsessive compulsive disorder, depression, anxiety disorders, alcohol dependence, epilepsy and pain is discussed. The dual action of glycine in the nervous system is described, evaluated and discussed as a problem in the development of related drugs. These authors are, again, generators of recent information.

The twenty-ninth chapter, written by Francisco Ciruela, Víctor Fernández-Dueñas, Xavier Altafaj, Fernando Contreras, Antoni Vallano, José Manuel Menchón, and Marta Valle León, opens new windows to schizophrenia treatment. The authors, researchers involved in the area, review the role of the adenosinergic system in the neurobiology of schizophrenia. Starting from this point, they suggest possible new methods of schizophrenia treatment. The reasoning is that, since adenosine plays an important role in dopaminergic and glutamatergic transmission, it may be a tool with which to manage schizophrenia treatment. An "adenosine hypothesis of schizophrenia" is also proposed, establishing a connection between the disruption of adenosine homeostasis within certain brain areas and corresponding behavioral consequences, with interesting homologies with schizophrenia symptoms.

The authors of the thirtieth chapter are members of a clinical research group working in neuroimaging. They are Nicolás Fayed, Javier García-Campayo, Eduardo González-Toledo, and Laura Viguera. In this chapter, recent neuroimaging findings in chronic pain, fibromyalgia, somatization and coping in somatoform pain disorders are interestingly summarized. The authors propose magnetic resonance imaging (MRI) as the investigative method of choice for standard use in clinical practice. The study of metabolism, using magnetic resonance spectroscopy and structural information obtained through voxel-based morphometry offers, in the opinion of these authors, interesting alternatives for the study of the brain and its related illnesses. Additional imaging parameters, such as vascularity (perfusion) and cellularity (diffusion-weighted imaging) are proposed here. These authors suggest interesting new lines of neuroscience research.

In the thirty-first chapter, Eduardo González-Toledo, Nicolás Fayed, Laura Viguera, Kanika Sharma, Piyush Kalakoti, Navdeep Samra, Anil Nanda, and Hai Sun propose the utility of magnetic resonance findings as a tool for registering structural and functional brain impairments in patients with traumatic brain injury (TBI). This problem is an important cause of death and disability in the United States, with significant morbidity (impaired thinking or memory, movement disorders, perception troubles, emotional changes, personality

changes, depressive disorders, anger, insomnia, and social disturbances). The authors maintain that patients with mild TBI present with brain iron/mineral deposits, abnormal cortical thickness, abnormal metabolites, disruption of white matter tracts, and decreased or lost connectivity in brain networks. All these findings led the authors to propose magnetic resonance findings for damage detection. Once again, this group generates the information in the field.

The thirty-second chapter concerns liaison psychiatry. The authors, María Soledad Barboza, Julia Cittadini, Milagros de Hertelendy, Mauricio Sebastián Farías and Natacha Loiácono, have developed interesting hospital statistics regarding interconsultations. They found that a significant percentage (10%) of interconsultations were due to delirium detected in all medical inpatients, and a significant group of patients (30%) in general medical settings had consultations for a psychiatric disorder. In this study, high users of medical care presented with psychiatric disturbances (depressive disorders, anxiety, and somatization). Underdiagnosis is also considered and discussed. This chapter is written by a group with wide clinical experience in an important hospital in Argentina.

The thirty-third chapter, written by María Andrea Delgado, Adriana Fochesato, Luis Isaías Juncos, and Pascual Ángel Gargiulo, reviews evidence regarding a proposed metabolic association between a gut-brain axis and autism spectrum disorders (ASD). The role of such factors in these neurodevelopmental disorders is widely analyzed. A complex interaction between genetic and environmental factors is postulated here. Some nutritional findings, such as food intolerances, allergies, altered intestinal permeability (leaky gut), immune dysregulation, neuroinflammation, and oxidative stress may be triggering ASD symptoms. Patients with ASD have shown increased urinary levels of β-casomorphin and gliadorphin peptides, both compounds produced by the incomplete digestion of compounds such as gluten and casein. The possible role of opioid peptides in ASD is suggested here. Possible factors involved in these pathologies are analyzed by this group.

In the thirty-fourth chapter, written by Rose Emily Nina-Estrella, an interesting update on dementia is presented. Diagnoses, pathophysiology, and treatment are discussed by this psychiatrist. An interesting comparison is drawn between the diagnostic criteria for dementia in recent manuals and the diagnostic criteria for Alzheimer disease (AD), vascular dementia (VaD), Lewy-body dementia (LBD), frontotemporal dementia (FTD). All them are analyzed and discussed. Different kinds of dementia are here presented and evaluated. An important comment is made on differential diagnoses. The fourth and fifth editions of the Diagnostic and Statistical Manual of Mental Disorders (DSM) are compared at this point. The author's wide knowledge and experience is evidenced here.

An interesting review regarding the action of nutritional factors on the brain is offered in the thirty-fifth chapter Silvina Álvarez, Nidia Gómez, Lorena Navigatore Fonzo, Emilse Sánchez, and María Sofía Giménez wrote this interesting update. The role of impaired brain function and cognitive performance in depressive disorders is discussed, as are micronutrient status at all ages, vitamin deficiencies, and mineral deficiencies that are related to psychiatric symptoms. Dietary schedules are proposed for preventing some

chronic diseases. Proposed biochemical and molecular mechanisms of nutritional factors in the brain are described. This chapter, written by an experienced group, may have important preventive value.

Finally, the thirty-sixth chapter addresses cognitive problems in patients with chronic renal disease. This review is performed by Luis Augusto Juncos, Kiran Chandrashekar, and Luis Isaías Juncos. Its main axis is the cognitive impairment and eventual dementia that occurs in patients with chronic kidney disease (CKD). Cognitive impairment and dementia (CI/D) are related here to CKD and end-stage renal disease (ESRD). Comorbidities that increase the risk of cognitive impairment are widely revised. The authors maintain that almost every stage of CKD is associated with an increased risk of CI/D. This risk appears to be increased in a parallel manner with increases in the severity of CKD. Mechanisms of vascular dementia appear to be facilitated by vascular disease in CKD/ESRD. An interesting overview of the epidemiology, pathogenesis/pathophysiology, diagnostic approaches, and therapeutic considerations of CI/D in patients with CKD/ESRD is presented here.

To conclude, I again express my gratitude to my wife, Adriana, for our long life together, and our seven sons and daughters. I also thank all my family, my parents, grandparents and brothers, who are also coauthors, in some sense, of the present book. I thank again Prof. Dr. Mesones Arroyo for our long friendship and for all our shared experiences in the clinic and in our academic and teaching life.

On behalf of the authors, I take this opportunity to thank the Springer editorial team, headed by Mr. Gabriel Pires, Mrs. Lorraine Coffey, Mr. Dinesh Vinayagam, and Ms. Saanthi Shankhararaman. I would like to thank them for their patience, dedication and invaluable and efficient work. And, finally, I express my profound gratitude to all my colleagues, who made this book posible with their invaluable contributions.

Mendoza, Argentina
November 13, 2016

Pascual Ángel Gargiulo

Contents

Part II From Basic Neurosciences to Human Brain

Contributors

Osvaldo Agamennoni, PhD Commission of Scientific Research, Department of Electronic Engineering and Computers, National University of the South (Universidad Nacional del Sur), Bahía Blanca Buenos Aires, Argentina

Xavier Altafaj Pharmacology Unity, Department of Pathology and Experimental Therapy, Faculty of Medicine, IDIBELL, University of Barcelona. Llobregat Hospital, Barcelona, Spain

Neurosciences Institute, University of Barcelona, Barcelona, Spain

Silvina Mónica Alvarez, PhD Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis. Laboratory of Cronobiology. Multidisciplinary Institute of Biological Research (IMIBIO). Argentine National Council of Scientific and Technical Research (CONICET), San Luis, Argentina

Ivana Anton Mlinar, PhD Department of Philosophy. Faculty of Philosophy and Literature, National University of Cuyo, Mendoza, Argentina

Argentine National Council of Scientific and Technical Research (CONICET), Mendoza, Argentina

Jorge B. Aquino, PhD Faculty of Medicine, Austral University. Argentine National Council of Scientific and Technical Research (CONICET), Buenos Aires, Argentina

Miriam Dolly Arancibia de Calmels, PhD Faculty of Social Sciences, National University of San Juan, San Juan, Argentina

Ricardo Aranovich, MD Academic Council, ACTA Foundation, Buenos Aires, Argentina

Gonzalo Arrondo Mind-Brain Group, Institute for Culture and Society (ICS), University of Navarra, Pamplona, Navarra, Spain

Department of Philosophy, University of Navarra, Pamplona, Navarra, Spain

Emilce Artur de la Villarmois, Pharm. D Department of Pharmacology, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

Institute of Experimental Pharmacology (IFEC), Argentine National Council of Scientific and Technical Research (CONICET), Córdoba, Argentina

Gustavo Baiardi, PhD Faculty of Chemical Sciences, Catholic University of Córdoba, Córdoba, Argentina

Laboratory of Neuropharmacology, Institute of Biologic and Technological Resarch (IIBYT). Argentine National Council of Scientific and Technical Research (CONICET). National University of Córdoba, Córdoba, Argentina

María Soledad Barboza, MD Department of Psychiatry, Faculty of Medicine, National University of Rosario (UNR), Rosario, Santa Fe, Argentina

Francisco J. Barrantes, MD, PhD Laboratory of Molecular Neurobiology, Institute of Biomedical Research, Faculty of Medical Sciences, Catholic University of Argentina (UCA). Argentine National Council of Scientific and Technical Research (CONICET), Buenos Aires, Argentina

Jorge Martínez Barrera, PhD Faculty of Philosophy, Pontifical Catholic University of Chile, Santiago, Chile

Marilia Barros Department of Pharmaceutical Sciences, School of Health Sciences, University of Brasilia, Brasilia, DF, Brazil

Primate Center and Department of Physiological Sciences, Institute of Biology, University of Brasilia, Brasilia, DF, Brazil

Osvaldo Martin Basmadjian Institute of Experimental Pharmacology of Córdoba (IFEC). Argentine National Council of Scientific and Technical Research (CONICET). Department of Pharmacology, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

Christian Luis Bender, PhD Institute of Experimental Pharmacology of Córdoba (IFEC). Argentine National Council of Scientific and Technical Research (CONICET). Department of Pharmacology, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

Arkaitz Bengoetxea Odrioizola Laboratory of Clinical and Experimental Neuroscience, Department of Neuroscience, Faculty of Medicine and Nursery, University of the Basque Country (UPV/EHU), Leioa, Bizkaia, Spain

Javier Bernacer Mind-Brain Group, Institute for Culture and Society (ICS), University of Navarra, Pamplona, Navarra, Spain

Esther Berrocoso, PhD Department of Psychology, Area of Psychobiology, Neuropsychopharmacology and Psychobiology Research Group, University of Cádiz, CIBER for Mental Health (CIBERSAM), Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz (INiBICA), Cádiz, Spain

Carlos Bonantini, PhD Faculty of Psychology, National University of Rosario (UNR), Rosario, Argentina

Aline Caron Borges Department of Pharmaceutical Sciences, School of Health Sciences, University of Brasilia, Brasilia, DF, Brazil

Claudia Bregonzio, PhD Institute of Experimental Pharmacology of Córdoba (IFEC). Argentine National Council of Scientific and Technical Research (CONICET). Department of Pharmacology, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

Paola Buedo Laboratory of Cognitive Neurosciences Development, Institute of Electric Engineering Research, National University of the South (Universidad Nacional del Sur), Argentina National Scientific and Technological Research (IIIE-CONICET-UNS), Bahía Blanca, Argentina

Claudia Patricia Calderón Cathedra of Pharmacology. Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, San Luis, Argentina

Gastón Diego Calfa, PhD Institute of Experimental Pharmacology of Córdoba (IFEC). Argentine National Council of Scientific and Technical Research (CONICET). Department of Pharmacology, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

Maria Romina Cattaneo Faculty of Health Sciences (ES Faculty of Psychology), National University of Rosario (UNR), National Council of Scientific and Technical Research (CONICET), Rosario, Santa Fe, Argentina

Kiran Chandrashekar, MD Department of Medicine. Division of Nephrology, University of Mississippi Medical Center, Jackson, MS, USA

Francisco Ciruela Pharmacology Unit. Department of Pharmacology. Faculty of Medicine, University of Barcelona. Government Pavilion. Llobregat Hospital, Barcelona, Spain

Julia Cittadini, MD Department of Psychiatry, Faculty of Medicine, National University of Rosario, Rosario, Santa Fe, Argentina

Fernando Contreras Service of Psychiatry, Bellvitge Universitary Hospital (IDIBELL), University of Barcelona, Llobegat Hospital (CIBERSAML), Barcelona, Spain

Ricardo F. Crespo, PhD Faculty of Philosophy and Literature, National University of Cuyo. Argentine National Council of Scientific and Technical Research (CONICET). Austral University, Pilar, Buenos Aires, Argentina

Pilar de Castro-Manglano Department of Psychiatry, University Hospital of Navarra, Pamplona, Navarra, Spain

Milagros de Hertelendy Department of Psychiatry, Faculty of Medicine, National University of Rosario, Rosario, Argentina

María Andrea Delgado, PhD J Robert Cade Foundation, Córdoba, Argentina

Renata B.M. Duarte Department of Pharmaceutical Sciences, School of Health Sciences, University of Brasilia, Brasilia, DF, Brazil

Primate Center and Department of Physiological Sciences, Institute of Biology, University of Brasilia, Brasilia, DF, Brazil

Luis E. Echarte, MD, PhD Institute for Culture and Society, School of Medicine, University of Navarra, Pamplona, Navarra, Spain

Mauricio Sebastián Farías, MD Department of Psychiatry, Faculty of Medicine, National University of Rosario, Rosario, Santa Fe, Argentina

Nicolás Fayed Miguel, MD, PhD Department of Neuroradiology, Quirónsalud Hospital, Zaragoza, Spain

Gerardo Fernández Commission of Scientific Research, Department of Electronic Engineering and Computers, Institute of Research in Electric Engineering (IIIE), National University of the South (Universidad Nacional del Sur), Bahía Blanca, Buenos Aires, Argentina

Víctor Fernández-Dueñas Pharmacology Unit, Department of Pathology and Experimental Therapy, Faculty of Medicine, Llobregat Hospital (IDIBELL). Neurosciences Institute, University of Barcelona, Barcelona, Spain

Adriana Fochesato J Robert Cade Foundation, Córdoba, Argentina

Miguel Gallegos, PhD Faculty of Health Sciences, National University of Rosario (UNR). National Council of Scientific and Technical Research (CONICET), Rosario, Argentina

Gilberto A. Gamboa-Bernal, MD, MSc, PhD Department of Bioethics. Faculty of Medicine, University of the Sabana, Medellin, Colombia

Pablo Emanuel García, BA Universidad Católica San Pablo, Arequipa, Perú

Javier Garcia-Campayo, PhD Miguel Servet Hospital, University of Zaragoza, Zaragoza, Spain

Pascual Ángel Gargiulo, MD, PhD Laboratory of Neurosciences and Experimental Psychology, Area of Pharmacology. Department of Pathology, Faculty of Medical Sciences, National University of Cuyo (UNC). Catholic University of Argentina (UCA). Argentine National Council of Scientific and Technical Research (CONICET), Mendoza, Argentina

Marisa Hilda Garraza Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, San Luis, Argentina

Antonella Gasbarri Department of Applied Clinical Science and Biotechnology, University of L'Aquila, Coppito, Italy

Marcelo Giachero, PhD Laboratory of Behavioral Neurobiology, Center of Biological Sciences, Federal University of Santa Catarina, Florianopolis, Santa Catarina, Brazil

María Sofía Giménez, PhD Multidisciplinary Institute of Biological Research (IMIBIOSL). Argentine National Council of Scientific and Technical Research (CONICET). Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, San Luis, Argentina

Emiliano Giraudo Department of Pharmacology, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, San Luis, Argentina

Andrea Gogos, PhD Florey Institute of Neuroscience and Mental Health, Parkville, Melbourne, VIC, Australia

Nidia N. Gómez, PhD Cathedra of Biochemistry. Department of Biochemistry and Biological Sciences. Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, San Luis, Argentina

Eduardo González-Toledo, PhD Department of Radiology, Louisiana State University Health Sciences Center, Shreveport, LA, USA

Francisco Güell Mind-Brain Group, Institute for Culture and Society (ICS), University of Navarra, Pamplona, Navarra, Spain

Jens R. Helmert Department of Psychology, Technische Universität Dresden, Dresden, Saxony, Germany

Mirta Susana Ison, PhD Social and Environmental Sciences Institute (INCIHUSA), Argentine National Council of Scientific and Technical Research (CONICET), Technological Scientific Center (CCT Mendoza - CONICET). Faculty of Psychology, Aconcagua University, Mendoza, Argentina

Luis Augusto Juncos, MD Departments of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, USA

Luis Ignacio Juncos, PhD, MD J Robert Cade Foundation, Córdoba, Argentina

Piyush Kalakoti, MD Department of Neurosurgery, Louisiana State University Health Sciences Center, Shreveport, LA, USA

Celina Korzeniowski, PhD Argentine National Council of Scientific and Technical Research (CONICET), Technological Scientific Centre (CCT Mendoza - CONICET). Consultant of the Research Institute, Faculty of Psychology, Aconcagua University, Mendoza, Argentina

José Vicente Lafuente Sánchez, PhD, MD Laboratory of Clinical and Experimental Neuroscience, Department of Neuroscience, Faculty of Medicine and Nursery, University of the Basque Country (UPV/EHU), Leioa, Bizkaia, Spain

Natacha Loiacono, MD Department of Psychiatry, Faculty of Medicine, National University of Rosario (UNR), Rosario, Santa Fe, Argentina

Melisa Mandolesi Faculty of Psychology, National University of Rosario, Rosario, Santa Fe, Argentina

Walter Manucha, PhD Area of Pharmacology, Department of Pathology, Faculty of Medical Sciences, National University of Cuyo. Argentine National Council of Scientific and Technical Research (CONICET), Mendoza, Argentina

Natalia Andrea Marchese Institute of Experimental Pharmacology of Córdoba (IFEC). Argentine National Council of Scientific and Technical Research (CONICET). Department of Pharmacology, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

José Manuel Menchón Service of Psychiatry, University Hospital of Bellvitge (IDIBELL), University of Barcelona. Llobregat Hospital (CIBERSAML), Barcelona, Spain

Juan Antonio Mico, MD, PhD Department of Neuroscience, Pharmacology and Psychiatry, Neuropsychopharmacology and Psychobiology Research Group, CIBER for Mental Health (CIBERSAM), Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz (INiBICA), Cádiz, Spain

Víctor Alejandro Molina, PhD Institute of Experimental Pharmacology of Córdoba (IFEC). Argentine National Council of Scientific and Technical Research (CONICET). Department of Pharmacology, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

Jose Ignacio Murillo, PhD Mind-Brain Group, Institute for Culture and Society (ICS), University of Navarra, Pamplona, Navarra, Spain

Department of Philosophy, University of Navarra, Pamplona, Navarra, Spain

Ane Murueta-Goyena Larrañaga, MSc Laboratory of Clinical and Experimental Neuroscience, Department of Neuroscience, Faculty of Medicine and Nursery, University of the Basque Country (UPV/EHU), Leioa, Bizkaia, Spain

Anil Nanda, MD, MPH Department of Neurosurgery, Louisiana State University Health Sciences Center, Shreveport, LA, USA

Lorena Navigatore Fonzo, PhD Cathedra of Biochemistry. Department of Biochemistry and Biological Sciences. Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, San Luis, Argentina

Rose E. Nina-Estrella, MD, PhD Faculty of Health Sciences, Department of Pharmacology, School of Physiological Sciences, Autonomous University of Santo Domingo, (Universidad Autónoma de Santo Domingo), Santo Domingo, Dominican Republic

Hisao Nishijo System Emotional Science, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan

Victoria Belén Occhieppo Institute of Experimental Pharmacology of Córdoba (IFEC). Argentine National Council of Scientific and Technical Research (CONICET). Department of Pharmacology, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

David Orozco Commission of Scientific Research, Department of Electronic Enginering and Computers. National University of the South (Universidad Nacional del Sur), Bahía Blanca, Buenos Aires, Argentina

Alicia del Carmen Panini Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, San Luis, Argentina

Sebastian Pannasch Department of Psychology, Technische Universität Dresden, Dresden, Saxony, Germany

Enrico Patrono Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama System Emotional Science, Sugitani, Toyama, Japan

Maria Constanza Paz, PhD Institute of Experimental Pharmacology of Córdoba (IFEC). Argentine National Council of Scientific and Technical Research (CONICET). Department of Pharmacology, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

María Cecilia Peralta, MD Coordinator Parkinson's Disease and Movement Disorders Clinic, Department of Neurology, CEMIC University Hospital, Buenos Aires, Argentina

Mariela Fernanda Pérez, Pharm. D, PhD Institute of Experimental Pharmacology of Córdoba (IFEC). Argentine National Council of Scientific and Technical Research (CONICET). Department of Pharmacology, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

Laura Perez-Caballero Department of Psychology, Area of Psychobiology, Neuropsychopharmacology and Psychobiology Research Group, University of Cádiz, CIBER for Mental Health (CIBERSAM), Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz (INiBICA), Cádiz, Spain

Santiago Perez-Lloret, MD, PhD CPI Institute of Cardiology Research Marcelo T. de Alvear, Buenos Aires, Argentina

Assunta Pompili Department of Applied Clinical Science and Biotechnology, University of L'Aquila, Coppito, Italy

Víctor Fabián Quiroga Calegari Faculty of Psychology. National University of Rosario (UNR,), Rosario, Argentina

Navdeep Samra, MD Trauma and Surgical Critical Care, Louisiana State University Health Sciences Center, Shreveport, LA, USA

Emilse S. Sánchez, PhD Faculty of Chemistry, Biochemistry and Pharmacy. National University of San Luis. Laboratory of Cronobiology. Multidisciplinary Institute of Biological Research. Argentine National Council of Scientific and Technical Research (CONICET), San Luis, Argentina

Alyssa M. Sbisa, BAppSc. (Hons) Florey Institute of Neuroscience and Mental Health, The University of Melbourne Parkville, Melbourne, VIC, Australia

Kanika Sharma, MD Department of Neurosurgery, Health Sciences Center, Louisiana State University, Shreveport, LA, USA

Philipp Singer, PhD Roche Diagnostics International Ltd., GCS Product Documentation, Rotkreuz, Zug, Switzerland

Hai Sun, MD Department of Neurosurgery, Health Sciences Center, Louisiana State University, Shreveport, LA, USA

Gustavo E. Tafet, MD, PhD Department of Psychiatry and Neurosciences, Maimonides University, Buenos Aires, Argentina

Mauricio Roberto Teves Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, San Luis, Argentina

Carlos Tomaz, PhD Neuroscience Research Group, CEUMA University, UNICEUMA, São Luis, MA, Brazil

Sonia Torres-Sanchez Neuropsychopharmacology and Psychobiology Research Group, CIBER for Mental Health (CIBERSAM), Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz (INiBICA), Cádiz, Spain

Antoni Vallano Pharmacology Unit, Department of Pathology and Experimental Therapy, Faculty of Medicine. Llobregat Hospital (IDIBELL). Neurosciences Institute, University of Barcelona, Barcelona, Spain

Marta Valle-León Pharmacology Unit, Department of Pathology and Experimental Therapy, Faculty of Medicine. Llobregat Hospital (IDIBELL). Neurosciences Institute, University of Barcelona, Barcelona, Spain

Maarten Van Den Buuse, PhD La Trobe University, School of Psychology and Public Health, Melbourne, VIC, Australia

Laura Viguera, MD Department of Anesthesiology, Miguel Servet Hospital, Zaragoza, Spain

Benjamin K. Yee, DPhil (Oxon) Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong, China

Part I

Epistemological Considerations About the Study of Normal and Abnormal Human Behaviors

Phenomenology of the Encounter According to Józef Tischner

1

Miriam Dolly Arancibia de Calmels

Abstract

The general crisis of individualism is reflected in the conflicts generated by human beings themselves in different parts of the planet: fundamentalism, xenophobia, gender violence, wars, genocide, and exploitation of human beings. Politically, individualism is presented under the form of extreme Manichean positions: left or right, capitalism or socialism, liberalism or communism. However, human society is much more complex; these simplifications are no longer possible. Neither the left nor the right will provide paradise, even less if their exponents think themselves to be possessors of absolute truth. On the other hand, respect for the diversity of the other view is not exhausted in the development of critical thinking. The purpose of this review is to schematically describe the viewpoint of Tischner, based on the notion of encounter to refer to the original experience with another human being where the other takes the highest level of persuasion. The encounter with another is the *agathologic* horizon of the interpersonal experience, and opens up the possibility of meeting with oneself in a new way. By impotence or ignorance many tragedies are possible, and in fact they have taken place in the darkest periods of mankind, when evil apparently killed good, resulting in the most sadistic forms of selfishness. Finally, the present review tends to demonstrate that on the agathologic horizon, the manifestations of the other and oneself are developed in a true sense of the good and the bad, the different logos that fit the drama or tragedy in interpersonal relationships.

Keywords

Encounter • Agathologic • Phenomenology • Complexity

M.D.A. de Calmels, PhD
Universidad Nacional de San Juan,
Avda. Ignacio de la Roza y Meglioli,
C.P. 5400 San Juan, Argentina
e-mail: mdacalmels@gmail.com

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_1

Introduction

The philosophy of encounter is one of the most original accomplishments of Józef Tischner, and constitutes a challenge for contemporary philosophy. The general crisis of individualism is reflected in the conflicts generated by human beings themselves in different parts of the world: fundamentalism, xenophobia, gender violence, wars, genocide, and exploitation of human beings. Politically, individualism is presented under the form of extreme Manichean positions: left or right, capitalism or socialism, liberalism or communism. However, human society is much more complex; these simplifications are no longer possible. It follows that the encounter with another as the *agathologic* horizon of the interpersonal experience opens new ways for understanding people.

The philosophy of Tischner has been defined as connected with phenomenology and close to the thought of Emmanuel Lévinas, although Tischner himself preferred the term "philosopher without labels." He was a thinker who could hardly be confined to any philosophical school.

We can divide the work of the Polish philosopher into two periods. The first involves *The World of Human Hope* and *Thinking in Values*, in which Tischner developed a philosophy of man connected with axiological problematics. In the middle of the 1970s, the theme of the other emerges in *The Ethic of Values and Hope* (1976), and in the article *Phenomenology of the Encounter* (1978).

In the 1970s, Tischner became increasingly influential in Poland's intellectual life. While sticking to the phenomenological tradition, he also followed the leads he found in the philosophy of drama and the metaphysics of the good, blazing his own path. At the end of the 1970s, he also took issue with Marxism in the book *The Polish Shape of Dialogue*.

In the second period of his work, which started in the late 70s and the early 80s, he published *The Philosophy of Drama* and *The Controversy over the Existence of Man*, which place the other at the heart of their philosophical reflections. Tischner arrives at metaphysics of the good embedded within the Platonic tradition [1].

The problematic of encounter was taken up by Husserl, who influenced many Polish thinkers through Roman Ingarden. One of the main themes of transcendental phenomenology is intersubjectivity. According to Husserl, intersubjective experience plays a fundamental role in our constitution of objectively existing subjects, other experiencing subjects, and the objective spatial–temporal world [2].

Tischner adds that to have a primary experience of another human being is to have an encounter, and he proposes recasting Husserl's old catchphrase "back to things themselves" into "back to other human beings." Intersubjective experience is empathic experience; it occurs in the course of our conscious attribution of intentional acts to other subjects, in the course of which we put ourselves into the other's shoes [3].

An encounter is to be "face to face" with someone. It enables us to gain an intuition of another human being's face, which reveals a truth about the other. The face is not the same as a veil or a mask. A veil covers the face, a mask aims to create an illusion of a face, and thus they do not reveal who that human being is. In contrast, a face can tell us that.

Therefore, Tischner analyzes what a face is. He applies a phenomenological method proposed like a plan: first, a discussion about the essence of the encounter; next, a description of the face by comparing it to a veil and a mask; and finally the challenge of seeing or reading the face.

The first question is, what is an encounter with another beyond particularities? It means, beyond circumstantial facts, not only Marxists of Tischner's time, not only refugees of the twentieth century, not only Marxists of the 2010s in Latin America, but beyond all of these; so, the question is about the essence of encounter.

We will meditate deeply in the following part of this chapter. We will analyze reciprocity, *aretetic* function, wonder, and admiration according to Tischner's proposal. It is important to underline that those reflections emerged in constrained situations when Soviet power dominated Poland. In consequence, to deeply understand the relevance of these thoughts it is necessary to remind ourselves of this context.

The Context of Tischner's Meditations

Just as Gandhi had fought against the British monopoly, so the social movement *"Solidarity"* and the Catholic Church in the mid-1980s challenged the government's monopoly over information, history, and cultural life [4]."*Solidarity* was born, and its power lay in the fact that for the first time in the post-war years all social groups gathered under one banner: workers, intellectuals, artists, farmers, and the youth. We walked shoulder to shoulder, and we knew where we were heading". Lech Walesa, Gdansk, 2006 [5].

In fact, Solidarity was a movement which emerged from the industrial working class with the support of the Catholic Church; therefore, it is not strange that the theoretical basis came from the priests, and what is more, from the sermons of some priests, such as Józef Tischner. In his sermon at the Wawel Castle in Kraków on October 1980 during a Mass for Solidarity, leaders initiated a series of texts published in the weekly *Tygodnkik Powscechny* (The Universal Weekly). Later, those texts were published as a collection entitled *Etyka Solidarności* (The Ethics of Solidarity, 1981). Ever since then, he has been generally regarded as the chaplain of Solidarity. In a homily preached at Zaspa in Gdańsk in 1987, Pope John Paul II cited Fr. Tischner's texts as the ones which best rendered the truth about "Solidarity" [6].

In *Perspectives of the New Ethos of Work* [7], Tischner puts in context the ethical aspects of the social revolution, which is not a fratricidal war; on the contrary, it means a leap of history to something new. Therefore, revolution has some ethical sense; it is the step on the road of progress. The Polish events of the year 1980 were described using the word "revolution," although not one person was killed, nor did any basic change in the structure of government take place during these events—the Communist Party continued in power.

Adam Michnik ([9], p. 66) wrote: "August 1980 designates the date of the drafting of a new social contract: what had been only vivid black and white; what had not been more than shared became something organized. And it was our luck. Luck is not easy to achieve. What is at stake is not only the commitment between the sovereign society and power deprived of its sovereignty, it is what society should negotiate with that power".

The workers' protest in August referred not only to the economic conditions characterizing the state, but primarily to the ever more unbearable relationships between people ([7], p. 29). Economic and strictly political issues were only a part of more fundamental matters. The solidarity ethos is a synthesis of rebellion and hope, a protest against some evil and a project for a better organization of social life.

After World War II, Marxist-Socialism became the official ideology of the government ruling Poland. The nation entered the period of "socialist building," which was to cover gradually all spheres of social life, from economy to culture. However, the sense of the socialist ideology was never and nowhere defined unambiguously, it was outlined only by the government's actual policy.

They spoke about "the socialization of the means of production" but it was never known what range of things was denoted by the concept of "the means of production," nor was the expression "the dictatorship of the proletariat" clear.

Another example noted by Tischner about the ambiguity was the approach of socialism's ideology to religion: at first, religion was treated as an anachronism from the past, but later this claim was attenuated.

Another important notion ([7], p. 30) is truth, which is a kind of bond that links person with person, people with people. Trust means that a man can rely on another man not only in ordinary situations, but also during the extreme situations of life. The one who trusts another man does not have to subject him to incessant supervision, since one knows in advance what the other will do. The crisis of truth occurs when the elementary bond between people begins to crack. The place of faith and faithfulness is taken by distrust and suspicion. Social life is permeated by gloom and uneasiness, and fears are awakened.

The crisis of trust can take various forms: a simple distrust evoked by the fact of not keeping

an obligation, a suspicion caused by making a sham promise, or a formal betrayal which makes one's friend prey to their enemies.

The solidarity ethos found sympathetic grounds in Christian ethics, and especially in the whole of John Paul II's teachings focused around three values: human dignity, every person's right to the truth, and the obligation of faithfulness towards the fundamental values of Polish culture.

With regard to the notion of solidarity, Tischner considers that this virtue is born all by itself, spontaneously, from the heart; it is born of goodwill and awakens goodwill in people, it is born of the pages and the spirit of the Gospels, and does not need an enemy or an opponent to consolidate and develop. It is directed toward everyone and not against anyone. The dignity of man is founded on his conscience; the deepest solidarity is the solidarity of consciences.

The solidarity about which Tischner is speaking is neither a concept nor a complete ethical theory, it is an idea. It is something to imitate, which defines itself in the course of realization and requires understanding. It is impossible to be in solidarity with people who have no conscience. Not every "Us," not every "together" is yet solidarity. Tischner insists that authentic solidarity is solidarity of consciences.

The ethics of solidarity develops and becomes manifest in a particular social system, in a particular time and place. This place permits dialogue to emerge, when people have come out from their underground places, have come closer to each other, have started exchanging words ([7], p. 41).

Not every conversation between people is a reliable dialogue. Reliable dialogue brings about true revolutions in the life of people and societies. Solidarity is always solidarity resulting from some dialogue, it means:

1. A reliable dialogue grows out of a certain assumption that must be accepted by both sides; it is not possible to learn the truth about each other if one remains distanced from one another, closed inside walls of fear. The first condition of dialogue is the ability to sympathize with the other's point of view. It is not only compassion, but is about something more, a recognition that the other, from his point of view, is always to some extent right. If the other can be right, then it is possible that I am not entirely right. Dialogue is the building of reciprocity.
2. Solidarity is suffering, a suffering that was inflicted on a man by another man. The man has enough suffering: diseases, weaknesses, and death—another man should not bring additional pain, he should rather strive to ease the burden of the cross which his neighbor carries. Solidarity is born among working people in order to liberate man's work from superfluous pains. The word exploitation must be applied to the reality which is suffering; a working man's suffering at the hands of his neighbor.
3. Finally, a reliable dialogue is always a dialogue of awakened consciences, is concerned with the truth about the unnecessary suffering of working people. The suffering of the working man gives high moral standing to the speech of solidarity which is above all, the speech of testimony. One thing more is particularly important: hope—a hope that awakens, a hope that matters, and that things are capable of being changed. People of dialogue-in-solidarity must guard this hope closely.

Tischner arrives in this way at the notion about work, which is the axis of solidarity. Work is a particular form of a person-to-person conversation, which serves to sustain and develop human life, is a conversation in the service of life.

The dialogue of work goes further than an ordinary conversation. It embraces ever greater circles of people who often do not know each other's faces. Each conversation conceals within itself some kind of wisdom. Work has a particular, *sui generis* inner wisdom. This wisdom imposes demands on people; it defines for them suitable standards. Each person must know what he should do, so that an organic whole can grow out of fragments of work. The wisdom of work determines the natural wisdom of working people. Today, its beginnings must be learned at school, but eventually one reaches this wisdom when, through real work, it begins to run in the blood.

On the other hand, the ethical aspect of work cannot be considered in isolation from the value which life itself has for man. Life is the basic value, and work serves life both when it sustains life and guarantees its development and when it gives a deeper meaning to life. Thanks to the value of life which work serves, work gains value and dignity.

The product of human work grows out of communication and serves communication. The fruit of work is like a word which journeys through time and space.

According to Tischner, education creates bonds between teacher and pupil which are analogous to those of fatherhood, which can be understood in many ways, superficially or deeply. When looking more deeply, fatherhood is not only passing on life; in addition, fatherhood means also passing on hope. The father is a guardian of the child's hope, he is the support and strength of this hope.

Maybe only by considering the context in which Tischner wrote his reflections is it possible to understand his words "only those who have hope can teach and nurture; they teach by shaping the hopes of pupils" ([7], p. 46).

Education and upbringing are work upon the spirit—work according to hope, only after hope does love come: "we walked through life, not knowing what life was about, as if we were half-asleep. The voice of our teacher has roused us out of this slumber. The rest had to be done by ourselves" ([7], p. 47).

In this way, the ethics of solidarity becomes an ethics of awakening to fatherhood following the principles of hope. One must get through the world of illusions to what is fundamental.

However, there are betrayals in abandoning ideals. Tischner is very clear when he defines what betrayal means; it is the cardinal sin against the solidarity of consciences. It is the breaking of the bond established in faithfulness which is an integral element of hope. He insists on the notion of hope, because he considers that it directs us toward a certain future; through hope, the future becomes our value. As a result of directing us to future values, hope enables us to overcome present difficulties; it refers us to people, because this means that there is someone to whom we entrust our hope.

The faces of betrayal are:

1. The most tragic symbol among betrayals is the betrayal of Judas. It is a betrayal by a co-worker which reached the point of delivering somebody to death; this betrayal came as an abuse of trust.

   One who participates in a community can betray in two ways: open and concealed.

   Denunciation is an open form of betrayal, and it consists in giving someone's secret away to another man who, knowing the secret, will strive to enslave the person betrayed. It is unethical. This is what Judas did.

   Refusal to work together is a concealed form of betrayal. Like Peter, he did not pick up the rope thrown to him. The one who carried out his part of the work imprecisely as he did "denied" others, betrayed them, and failed the hopes which were entrusted to him.
2. Work also requires trust between those of us who work and those for whom we work. Betrayal takes the form of an "abuse" of good intentions, an abuse of someone else's work. An evil use is made of good work and its good fruit.
3. Last, there is a third dimension to the confiding of hope: a bond between those who comprehensively organize work in a given society and those who carry it out. Betrayal on this plane is manifested as a crisis of work, and leads the work to be doomed to senselessness.
4. In conclusion, Tischner defines the solidarity of consciences as an ethical movement whose basic principle is faithfulness. Like an appeal, he says: "let us be faithful to each other. Let us be faithful in spite of denunciation, in spite of the denying of each other, the waste of people's work by other people, the abuse of work, let us depart from work which is senseless" [7] (p. 51).

This call to faithfulness is a form of struggle against the exploitation of a man by another man on the level of work. Now we will understand better why Tischner affirms that the face is born from the tragic, and that encounter is to experience a face that is the starting point of the phenomenology of encounter.

The Encounter

Tischner ([3], p. 41) considers that an encounter with another human being is an event, and through it the experience of another can reach the highest peak of intuition. The encounter marks a breach in the space of being with another, but the encountered appears not at as an opponent to be fought; on the contrary, it appears as a possibility to start anew.

The encountered other is transcendent, existing radically beyond me. In the encounter the face becomes perceptible, and it is given to us within a particular horizon which illuminates and let us sees the face. The encountered other is different, because the horizon that makes the experience of the other possible is different. "The difference becomes palpable when we realize that the presence of another's face demands that I also make my own face present to him" ([3], p. 43). In consequence, the horizon of encounter is a horizon for encountering another and also oneself. This horizon shows projected possibilities and opens up new ones. It is the occasion to encounter oneself in a new way.

Tischner ([3], p. 44) returns to the Greek term *agathon* and arrives at a full definition of encounter. It is an opening up of the agathological horizon of interpersonal experience.

The agathological horizon is where all the manifestations of the other and of myself are ruled by a certain logos: the logos of good and evil, of what is better and what is worse, of rises and falls, victory and defeat, salvation and damnation.

The presence of another is the presence of the intuition of existence, which is a continual event where the other carries with him not a neutral existence but one which is problematized, contestable, and contested. In encountering another, not even the ethical horizon appears, but metaphysics. The basic function of the agathological is to reveal, to show existence as a problem. The agathological is thought-provoking: the axiological reveals paths for action. Then, the axiological shows how to salvage the goodness of existence, and the face emerges not from the axiological but the agathological horizon.

The human face reveals the truth of the human being: "everything we know of the other we see through the prism of his face" ([3], p. 49). The face should not be identified with a veil and nor with a mask, it is a place where another human being's truth is made visible to us. A veil only hides the view, whereas a mask proceeds to introduce illusions, deformation, games.

Tischner explains the effects of the hidden through the example of shame. He considers the intentionality of shame quite paradoxical ([3], p. 51); it is the visibility of the invisibility of something else, a mystery.

Shame would be impossible without others, and permits us to differentiate between emotional expressions characterized by monological and dialogical intentionality. Dialogical emotions require another who feels ashamed on account of our presence; we learn that the other wants us to cover or veil him from our look. Therefore, there is interpersonal dialogue even before words are used.

The expression of shame is a form of dialogue playing a role of a veil. However, a veil is not the same as a mask, which intends to create a false illusion, not simply to hide as with shame.

Tischner uses the example of shame as an instrument to explain that it serves as a reminder that my knowing another ought to be preceded by my accepting his value. He emphasizes the phenomenon of shame due to the subject of shame, who is the axiological Self. A personal Self, as the subject of all the experiences of values in the world, is a value that defines a sort of human dignity.

In consequence, the difference between veil and face is not quantitative but qualitative. The veil makes sense only on an axiological level; the face reaches the level of agathology. By the shame we can enter the horizon of the face. The veil is something a human being has, while the face is something a human being is.

Similarly, the other is; his existence is problematic, and like the veil offers a chance to pass from the axiological to the agathological sphere, we have a chance to be found in the experience of the other's freedom. The first freedom ever experienced is the freedom of another. The veil is a

manifestation of this freedom. Tischner considers that the other exists through me, and his sense of dignity can come about thanks to me.

From the objective side of the experience of encounter, we consider the horizon on which the face is revealed and the opposites: mask and veil. But a mask is neither a veil nor even a face. A veil only hides the face, a mask lies. They both appear alongside another human being; a mask is a mask through the fault of others, and the veil appears simply on their account. The intentionality of the mask is quite twisted; it tries to create an illusion completely unlike the real state of affairs. The dishonest man tries to put on a mask of honesty, the lazy man pretends to be hardworking, the unjust one wants to seem just, and the unfaithful one pretends to be faithful. There is a relationship of axiological opposition established between the mask and the truth; a negative value wants to look like its positive counterpart.

The mask emerges from the person, but at the same time the mask is about others. The intentionality of the mask branches out in many directions. The mask also contains a reference to other people and it is different from a face. This difference is much greater than that between a face and a veil; they both appear within the axiological horizon, but the veil does not introduce lies to this horizon in the way that the mask does.

At this point Tischner introduces the notion of truth and freedom. The discovery of a mask forces us to interrogate the truth of the other. The face appears on the agathological horizon where the basic ruling factors are a nameless good and evil opposing it. The other is free and the other is the bearer of a truth. "Another's face is a freedom and a truth living in the light of goodness and in the darkness of evil" ([3], p. 59).

The face expresses the existential movement by means of which and through which man gives a radical justification of his existence. Therefore, the other is not a "being for me" and we are not a "being for him," we are free. The face resists possession. Tischner does not arrive at the Leibnizian monads; on the contrary, he affirms that we are open onto each other. "We encounter, we long for encounter, we carry memories of encounters with us. This means, not being "for others" and not being "in ourselves," we can be "through each other."

From the subjective aspect of this experience, we consider the ways of experiencing and living the tragedy of the other and of opening and closing ourselves to it, internal and intimate aspects in an encounter. The objective and subjective sides of an encounter cannot be separated. From the subjective side we will consider the implications of notions: "aretetic," wonder and astonishment.

Tischner defines "aretetic" as the special function of self-aware freedom constituting the face. In this way, Tischner reminds us of the Greek word *areté* in order to underline that the face is the external appearance of man's truth and it is generated by the participation of *areté* in the good.

Areté is not a virtue among virtues but the basis of them and permeates each one of them. It is the condition of any virtue being possible. It is the reason that Tischner considers areté as sensitivity to the agathological horizon of a human being. "It is a valor in the deepest sense of the word" ([3], p. 67). Valor suggests a participation in what can be gained by a victory, a free fulfillment without external coercion.

Therefore, the face is not only a manifestation of truth but also of freedom. To encounter another must experience his face and *areté* becomes *tetic* as it constitutes the face. Constituting presupposes choosing, where there is no choice there is no face. "The aretetic function of constituting a face is the deepest core of the dialogue between men" ([3], p. 68).

In fact, the encounter leads man into the depths of the great mysteries of existence, where questions are born about the meaning of everything that is. In describing the aspects of the mystery, wonder is the most obvious word.

Tischner defines wonder as something containing admiration and familiarity with a bit of suffering. Plato and Aristot*l*e considered that wonder is the birthplace of philosophy. Wonder has a very special existential status, it is neither an act of the consciousness such an act of attention, nor it is a mood. Conscious acts are necessarily acts of the conscious subject. Wonder is a

way of opening human existence to the dramatic dimension of every being and especially of a human being.

Wonder reveals the ambivalence of human existence, the ambiguity of the axiological. Tischner emphasizes that wonder brings man to participate in the drama of the meaning of existence, and thus facilitates man's existential responsibility for him. Man sees that his life confronts possible evil and possible perdition, as well as possible good and possible salvation. "Wonder places man at a crossroads" ([3], p. 72).

There is a close relationship between suffering and an immediate awareness of tragedy and thought ([3], p. 32). It is not only a relationship between Act and subject, permitting influences thereby into thinking that this is its expression, that it is a free answer. By inciting wonder, an object or another person can bring salvation. Tischner asserts the spontaneous reaction to the perspective of salvation is fascination, admiration, enchantment. The way to admiration is paved by astonishment.

Astonishment, admiration, and enchantment taken together in all their variants share the fact that they can be incited both by experiences of people and by experiences of things. These experiences are intentional ([3], p. 73).

According to Tischner, the object of astonishment is not something fully determined. It is different in relation to what was before, but we still do not know what it is in itself. This inspires curiosity, and for this reason astonishment and admiration are the sources of radical thinking.

However, we can note the difference between astonishment and admiration**.** Astonishment is an event which fulfills itself in one and the same present moment, while admiration and its manifestations are states of consciousness which endure in time. Abandoning a state of astonishment or a state of admiration is disenchantment.

Conclusion

In summary, Tischner theorizes in a realistic and understandable way from the cultural fund from which emanates his thinking. Thinking means to overcome a radical uncertainty. Thought is a spiritual power by which pains are not only overcome, but which also encourages one to support them. The dignity of thinking promotes courage as much as hope ([10], p. 60).

Tischner adds to Kant by completing the definition of "the encounter": nothing in the world could be called good without qualification except goodwill. An encounter is an event and it constitutes a certain a posteriori, it starts from experience. At the same time, an encounter is possible thanks to an ideal a priori that secretly governs the course of the encounter and is prior to it. The presence of this a priori disposes us to repeat encounters ([10], p. 25).

There is a deep connection between thought and values; when interest doesn't develop into axiology, nihilism and dialogue are refuted. This is the case with totalitarian systems. Totalitarianism is more than just tyranny or absolutism; the difference between them is in the way it seeks to legitimate its power which is conceived as the expression of an absolute force which rules over everything in the territory in which it prevails [10].

For this reason, it is essential to remember that radical thinking combines in itself the agathological and the axiological. Consequently, this thinking provides a basis not only for every science, for ontology, but also for a possible politics. And politics in the radical sense of the term is the science of projects, reasonable projects for sacrifices by human beings at this stage in history.

Nowadays, politics needs to remember the deep sense of dialogue in order to achieve understanding beyond differences. In the existence of a dialogical relation, the other person appears as a subject not an object. It is openness toward the other.

Tischner applies the phenomenological method for explaining the essence of the dialogical relation that is ruled a priori by the category of space. The objects, things ([3], p. 113), and people are always somewhere,

somehow tied to places where they remain motionless. The objects and people located in space are ruled by the principle of one-next-to-the-other. Agreeing with Husserl**,** Tischner indicates that in the intentional consciousness directed at another person, a consciousness that objectifies man, the experience of bodies are of particular significance.

The other person is not the object; he is participant in a dialogue. Thus, the relation of man to man is not intentional but something more, it is dialogical. In an encounter we always encounter someone, and the phenomenon of the tragic emerges there. The tragic occurs where some good, some value can be destroyed by some evil, some antivalue [6].

We can say that we have experienced an encounter when we have experienced concrete good and evil, tragic freedom. For this reason, the key to axiology is an encounter with another, as such axiological experience presupposes hope and reveals the values that can be realized here and now.

It is evident how much Husserl influenced Tischner, who found in phenomenology the theoretical basis for Solidarity and resistance to the communist regime and to confront the crisis of hope of modernity.

In the same way, it is also evident how very important are his thoughts for actual crisis. It is a crisis of values, a crisis of hope, and they become the big challenge for us today. In the same way as Tischner, we need to recover encounter, dialogue, and thinking in values as the way to nurture the deepest meaning of humanity.

References

1. Drwięga M. The place of the other in Józef Tischner's philosophical anthropology. In: Thinking in values: the Tischner Institute journal of philosophy. N° 3. Krakow: The Tischner Institute; 2011, p. 11–33.
2. Beyer C. "Edmund Husserl", The Stanford encyclopedia of philosophy (Summer 2015 Edition), Edward N. Zalta (ed). URL: http://plato.stanford.edu/archives/sum2015/entries/husserl/
3. Tischner J. Phenomenology of the encounter. In: Thinking in values: the Tischner Institute journal of philosophy. N° 3. Krakow: The Tischner Institute; 2011, p. 39–62.
4. Weigel G. Final revolution: the resistance church and the collapse of communism. Oxford: Oxford University Press; 2003.
5. Paczkowski A, Byrne M, editors. From solidarity to martial law: the polish crisis of 1980–1981. A documentary history. Budapest/New York: Central European University Press.
6. Stawrowski Z. Solidarity means a bond. In: Thinking in Values: The Tischner Institute journal of philosophy. 2007;N° 1:159–71.
7. Tischner J. Thinking in Values: The Tischner Institute journal of philosophy. 2007;N° 1.
8. Tischner J. Perspectives of the New Ethos of Work, in Thinking in Values: The Tischner Institute journal of philosophy. 2007;N° 1: 28–36.
9. Michnik, A., Penser la Pologne. Morale et politique de la résistance, Paris: La Découverte/Maspero, 1983. Our translation.
10. Tischner J. The challenge of totalitarianism. InstytutJTischnera: Kraków; 2005.

2 Psychopathology and Corporality: The Possibilities of Intersubjectivity for Restoring Experience. The Cases of Schizophrenia and Autism

Ivana Anton Mlinar

Abstract

Starting from a phenomenological analysis of the lived body [Leib], and then coming to the phenomenological genesis of verbalization, it can be shown that the self and intersubjectivity have their original sense and constituent possibility in pre-linguistic experience, essentially tied to corporality, and not in reflexive structures of superior strata. Moreover, the phenomenological approach points out that psychic pathologies cannot be understood as mere brain illnesses, but that they have their own "place" in the lived relation between subject and world, which is mediated by corporality [Leiblichkeit]. When the latter loses its transparency, experience is affected and the intersubjective world, able to be experienced, gets lost. On the basis of these phenomenological principles contrasted with some fruitful therapies, I intend to show that there is evidence to maintain that a minimal self and intersubjectivity do not get lost even in extreme psychic illnesses (such as schizophrenia and autism), in which precisely personality and intersubjectivity—including linguistic and communication capacities—as such are disrupted. And consequently, these assumptions should become not only premises to overcome the prevailing medical and even psychiatric dichotomy between bodily functions and mental states—which disregards the person in its unitary, concrete and bodily [leiblich] existence—but also the condition of the possibility of effectiveness of a therapy.

Keywords

Phenomenology of corporality • Intersubjectivity • Minimal self • Schizophrenia • Autism

I. Anton Mlinar, PhD
Department of Philosophy, National University of Cuyo, Centro Universitario – M5502JMA, Mendoza, Argentina

CONICET (National Council of Scientific and Technical Investigations), Buenos Aires, Argentina
e-mail: mlinariv@yahoo.es

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_2

Introduction

In the presence of a person, in its figure, its behavior, its gestures—the corporal becomes the base for interpersonality: this body, this corporal being in front of me is *the* other, and not its cover or support. One of the big contributions of phenomenology from Husserl on is the acknowledgement of the body and corporality [Leiblichkeit] in its subjective character [1, 2]. The body is certainly not conscious as "something" mine, rather as the unnoticed background of my turning toward [Zuwendung] the world and the others. That is why, in turn, corporality in a broad sense transcends the body as such and refers to the relationship anchored in it between the person, the world, and all their relational circumstances. Hence, psychic illness seems to have its own "place" in this lived relation between subject and world, which, in that sense, is mediated by corporality [Leiblichkeit].

This paper intends to show, first, the phenomenological genesis of verbalization, with its origin in lived experience. Second, genetic analysis brings to light that both the self and intersubjectivity have their original sense and constituent possibility in pre-linguistic experience—essentially tied to corporality— which, in turn, could be confirmed in psychopathologies such as schizophrenia and autism, in which personality and intersubjectivity as such are affected. Finally, this phenomenological analysis seems to support the principle that both ipseity—or a minimal self—and a minimal intersubjectivity never get lost, this way not only making a consequent practice of therapies possible, but, moreover, offering a unitary perspective of the psychiatric patient as person and not as a "piece of nature"—whether a brain or a mere body to heal.

Corporality and the Phenomenological Genesis of Language

Things and the world exhibit a variable orientation in relation to the absolute here instituted by the body. Our experience of them is adjusted to our bodily movements, which motivate the multiplicity of their appearances. Consequently, every perspectival appearance presupposes in turn that the experiencing subject is himself given in space, that is (a bit paradoxically phrased), perceptual intentionality presupposes an embodied subject [3] (p. 176), [4] (p. 284). But this does not mean that the subject should be a kind of presence or activity *in* a spatial object [5] (p. 240). In other words: the body as center of orientation and movement and, correspondingly, the kinesthetic system (my potentiality of mobility) should not be identified with the position and the movement attributed to our objectified body, since this occurs in an objective space already constituted as being independent of my orientation and movement. Quite the contrary: my original body-consciousness, my subjectivity, my concrete self-awareness implies that the function (movement, action) of the body is originally experienced as a spontaneous field of activity, as the activity of the ego [6] (p. 540), as an "I can" [7] (p. 14).

In psychiatry and well as in medicine, however, a perspective prevails of investigation of the body [Körper] as substrate (and not of corporality [Leiblichkeit] in a phenomenological sense) in which material conditions of alteration of psychic experience are sought. The dichotomy between bodily functions and mental states consequently disregards the person in its unitary, concrete, and bodily [leiblich] existence [8, 9].

This remark is essential in order to understand that every sense—that is, to have a world and to relate to others, phenomenologically expressed: to mean (noetic, or intentionally understood) and that what is meant (noematic)—has its origin in this experience, in the lived relationship between the subject and the world that is conveyed through body and space. *On* this originary articulated experience, *on* this sphere of sense, ride predication and judgment, that is, linguistic acts, language [10]. Thus, it becomes clear that while not every noema actually is the linguistic meaning of an expression, none is excluded from becoming one. Therefore, pre-linguistic experience is not a featureless blur, but is pre-organized into rough types, which afford a toehold for further development [10, 11]. That is why this pre-linguistic stratum of

experience becomes so important, then, as the cradle of sense; it is the origin of the constitution of the things, of the word to me, and, therefore, here that can be traced every pathology and, consequently, every therapy should be anchored.

Corporality and Intersubjectivity

My possible and effective experience of the world intertwines with the information I receive from the other one's experience, and that has for me the character of an appresentation. The appresentation transcends the given so that it intends in an empty way something that could be given in a subsequent moment. For example, the perception of the side of a cube also appresents the other sides because it refers us to them. Now, what it is in the world for me results from the convergence of the presentations and the appresentations of the foreign experience that are given to me with the expression of the foreign body or the language.

How is it possible to perceive an other as other and not as an object? A physical body is apprehended as lived body [Leib] through a motivated association because of its similarity to my own lived body, on the ground that it moves and is affected in an analogous way. Without any active intervention of the "I", the sense originarily instituted with respect to my own body is transferred to the analogous physical body. We win in this way the foreign lived body of an "I" that animates it, as another "I". This means that perceiving the foreign body as an other is to experience his body as a zero point of appearances. That is why the foreign body is the first intersubjective, then it is the most primitive step to objectivation: it constitutes the first—indeed still imperfect—object, the first intersubjectively identified of the experience of different subjects. What is in front of me is thus also a subject, in front of whom I myself am and who has my own body in an external way of appearance. As we see, the person, an other, is constituted in the experience before all conceptual and general thinking [6] (p. 110), before any language. Intersubjectivity emerges as an interpersonal space, as a synthesis of the centered and an off-center perspective. The importance of this genetic analysis lies in the fact that it makes it possible to understand subjectivity and self-awareness as a structure that always implicitly includes the other. Additionally, this structure does not appear as a fixed "I", kind of an "acquired property", but rather as a continual movement and an intentional production of the adjustment of perspectives.

In this interpersonal space, *the* experience is generated, and it should allow both the arrival to language and the off-center perspective, that is, the constitution both of the "I" and also of the other and the objective world. The interpersonal space is also ontogenetically the first in the process of the development of subjectivity.

It can be seen why the phenomenological distinction between body [Körper] and lived body [Leib] becomes crucial to understand psychic illness, because it is precisely in the extent to which the lived body [Leib] emerges disturbing as physical body [Körper] that it can complicate and distort the experience of the world and the others. In other words, the more the body becomes independent, the less free becomes the person in her relationships with the world. Fuchs [8], for instance, presents and analyzes these principles in some mental illnesses such as melancholy, schizophrenia, old age depression and aging paranoia. Therefore, at the same time, corporality—in this phenomenological sense—is the field where one should go to meet the patient, to try to *restore* his experience, as we will see.

Psychopathology, Corporality, and Person

If psychic illness seems to have its proper "place" in the lived relation between subject and world, which is mediated by corporality [*Leiblichkeit*], it is because the specific human faculties of objectivation and of self-relativization, as well as of free will and of commitment are precisely associated with the retracement of the body to its mediating function, with the transparency of corporality in front of the world. When corporality loses this transparency, experience becomes affected, and

submerges the person in her own world because of having lost the intersubjectively experienceable world. We consider the expressions of the sick person no more as free, sense-directed intentions, but we look for mental or organic causes for them. As a result, corporality obtains, however, a decisive meaning for a treatment full of understanding of the psychotically-changed person. It becomes possible to grasp the structural changes in the body–environment–relationship of the sick person, and then its lack of freedom, its inability, turn out to be understandable, together with the fact that this sick person can see the world and react differently. We follow then, departing from the body, the particular way in which the world builds up and reality is constituted.

If the patient were determined only causally in his manifestations, this effort of reconstruction would be certainly in vain and illusory. But it is not. The changed corporality presents only another kind of basis of experience for the configuration of the world. The patient still keeps therefore "freedom in the lack of freedom", but it is more difficult to recognize it in its restriction.

We would like to set out briefly how this alteration of corporality—in the phenomenological sense exposed—occurs in two mental illnesses, taken as paradigmatic examples: schizophrenia and autism. We would like also then to present possible explanatory principles of the effectiveness of some treatments from a phenomenological perspective.

Schizophrenia

Schizophrenia [8, 12–14] affects the person in its own personality. Phenomenologically, its central disorder consists of the breakdown of the intentionality, that is, the ability to head for the world perceiving, thinking, feeling, and acting and at the same time to be conscious of it. The disorder expresses itself in an intentional depersonalization that takes all fields of experience. As "inversion of the intentionality" [8], the patient faces his own performances as foreign, made from outside.

The intentional alienation of perceiving manifests itself in the delusion-atmosphere as apophenic appearance-like and self-referring relevance of all that is perceived. In this way, the intentional alienation of thinking leads to the experience of inspiration of thoughts or verbal hallucinations, because the individual's own thoughts appear as not intended and the verbal hallucinations are interpreted as an intentional alienation of the "inner speech". In a similar way, the influence of the will is interpreted as alienation of the bodily performances of actions. And then it comes further to the alienation of bodily experience itself, which results in a decoupling of the bodily resonance of the expression and in the release of a primary, physiognomic–ecstatic relationship of the body and the environment. At the height of this dissolution of boundaries, phenomena of embeddedness and of magic space appear. The central, intentional disorder of schizophrenia is the absence of the assumption of perspective: that is, in alternation with the perspective of the other the schizophrenic loses his personal center, embedded in his own body. He cannot assert his own perspective opposite to the foreign one. The schizophrenic delusion could be understood as an autistic communication that consequently inhibits the danger of the foreign perspective to protect the subject from the loss of self.

Autism

The DSM-5 includes autism within ASD (autistic spectrum disorders), together with other neurodevelopmental disorders characterized by impaired social interaction, verbal and non-verbal communication, imaginative and symbolic activity, and restricted and repetitive behavior.

Autistic patients [14–19] lack adequate emotional responses, which does not mean that they do not have any feelings or capacity to express them, because they show them, but rather that they do not adjust to social expectations. Autistic persons can be insensible to some auditory stimuli as the human voice, but at the same time hypersensible to other sounds as the friction of a paper. Most individuals with ASD have various learning difficulties.

All mental disorders imply more or less profound disturbances of intersubjectivity, that is, a restricted freedom of behaving and interacting with others in the common life-world. However, the concepts of intersubjectivity currently prevailing in clinical psychology and psychopathology are mainly based on a mentalistic approach that locates the disorder causally inside the patient. That is why they offer inadequate accounts of these disorders because of their disembodied ontology. On this view, disorders of intersubjectivity, such as autism or schizophrenia, are derived from a faulty development or functioning of "Theory of Mind"-modules (ToM) [20–22], that is, a presumable impossibility to conceive the other in terms of mental states different from one's own [9, 14]. There are, as well, some approaches that even see an excessive reliance on a theory of mind rather than a lack of such [14] (p. 68).

From a phenomenological point of view, however, intersubjectivity is primarily based on a prereflective, immediate relationship of self and other in an emergent bi-personal field, as exposed above. From a phenomenological approach, autism should rather be conceived as a disorder of primary or embodied intersubjectivity. Instead of a theory deficit, autistic and schizophrenic patients rather suffer from a basic disturbance of being-with-others which they try to compensate by a 'morbid rationalism', i.e., precisely by hypothetical constructs and assumptions about the world of the others.

The "inappropriate" or "abnormal" affections and behaviors in the experience of these patients may actually be inherently meaningful. They strive to make sense of some basic disturbances and re-establish some form of coherence with the world, though this may only be possible in the form of delusions or autistic withdrawals.

That is why the phenomenological analysis opens a more comprehensive and personal approach, pointing to using as therapy the search for a language of bodily experience that may help to understand the patient in his bodily-spatial existence, and to finding common words for his experience, in order to recover or reconstitute in some way the world and the selfhood from there.

Therapies

In the case of schizophrenia, if it expresses itself as disembodiment, simple and concrete exercises in body- and direction-space should be promoted in order to restore the constitution of the self and of reality. This therapy oriented to body [23] aims at the anchoring of the patient in his bodily center (through conscious breathing, for example) and at the reinforcement of the I–vitality or –activity through easy exercises of position, movement, and perception. The tactile sense acquires a special meaning, because it promotes the experience of the limits of the self and the not-self. Touching the patient's own body is also part of it, considering the close connection between the arising double sensations and the self-experience.

In the case of autism, it has been demonstrated that there are a variety of basic sensory-motor deficits. The sensory-motor system is especially important for motor learning, including sitting, walking, mouth-shaping, and writing. Some authors [24] propose that the world seems to move too fast for people with autism, and that they need to slow it down by exploring it in a particular way. Therefore, the disruption of the sensory-motor system not only limits the type of learning or relationship in which they can engage, but also reconfigures their whole way of learning and being related.

A controversial method called "facilitated communication" has enthusiastic defenders and determined opponents [15, 17]. It has, nevertheless, shown its benefits. Considering the importance that motor disorders (apraxias or dyspraxias) may have on these types of ailment, this approach uses attempts to help autistic individuals to communicate by means of writing using a computer or a similar device and using, only initially, physical support (holding the hand of the subject whom one want to assist, so that he can initiate the action, and control impulsivity and/or perseverations due to disorders in the elaboration of complex voluntary motor actions). One starts out by pointing to figures, to later move on to copying words, completing blank spaces in a sentence (predictable and unpredictable) and at the highest expected level

achieving open independently written conversations. What has even been observed in some cases is that the writing modified some actions, enabling some organization of behavior and development of language [17]. In patients who presented aimless wandering and racing, turning on and off of lights, hair-pulling, repetition of numbers and insults, marked impulsivity, etc., such types of conduct were reduced after initiating the writing process, thus confirming how language modulates and organizes conducts which depend on language itself. Being that these characteristics are present in the most severe cases, i.e., the ones who lack language or present severely disturbed language, it is likely that the development of language (in the referenced cases) was what allowed for regulation of behavior in semiotic terms.

Defenders of this method are unable to explain this phenomenon, and tend to look for answers in possible connections between the systems of muscles engaged in speaking and writing. We are skeptical about this suggested foundation; it explains neither if nor why writing could, in some way, reconfigurate behavior and confer new configurations of experience.

After having explained at the beginning how senses acquired through experience could settle and become articulated as language, one cannot overlook the fact that writing itself is an experience, in this case precisely facilitated, and that means that an other becomes a sort of supplement which permits the own body to gain an experience that initially is not strictly linguistic; it starts by using images and because writing requires not only a representation and symbolic system in action, but a fine motor-system and sense coordination as well. But slowly an ascending way to symbolization can occur and enable the access to linguistic senses, not necessarily to oral language. Through linguistic senses the intersubjective space becomes a wider common horizon, since a new interaction, a new communication takes place. Experience becomes in some way objectified through writing, and thus another way of self-transcendence is found, which makes the resonance of the own lived senses possible and their consequent modulation or compatibilization with others. That is why the experience, the behavior, the senses, that is, the world of the autistic person (but not less the one of a not-autistic) can be modified.

Final Considerations: Restoring Experience Through Intersubjectivity

Some premises could be pointed out that seem to be confirmed by what has been exposed above.

First: The phenomenological analysis shows that it is not exaggerated to assert that ipseity, that is, a *minimal* or *core self* [9, 25] gets never lost, even in pathologies such as schizophrenia and autism, characterized as ailments of the basic structure of the person and her self-apprehension. Fuchs [8] (p. 182) asserts that "schizophrenia is … the illness of the person as such, the disorder of its intentional ability and her self-apprehension in the movement of eccentricity. Only the person can become schizophrenic, because the illness affects the core of what distinguishes her from the animal. But through it the person does not become an animal. She suffers, she is afraid, she feels alienated and overwhelmed; precisely thereby she testifies that she is still a person. In the extreme depersonalization and distortion appears still the person of the sick being self."

The minimal self is the tacit, pre-reflective self-awareness that is present in every experience without reflection, because every perception and action involves an implicit self-awareness which is "immediately, non-inferentially" given self-ownership [9] (p. 549). Pre-reflective consciousness is in fact un-thematic, tacit, non-linguistic, immediate [14, 26], therefore it does not exist apart from the experience, as an additional mental act. As the basic form of selfhood it is bound to the body, as lived body, as constituting moment of the being-in-the-world. However, it is necessary to make a distinction between two aspects of the minimal sense of self: the sense of self-ownership and the sense of self-agency [27]. Precisely in the case

of schizophrenic experiences, such as thought insertion for example, the patient might claim that he is not the one who is thinking a particular thought, when in fact he is the one who is thinking the thought. In such cases the person misidentifies the source of the thought. Considering this phenomenon in the context of motor actions makes the distinction clearer between the sense that my body is moving—self-ownership—and the sense that it is me who initiates the action, that I am the source of it—self-agency. Nevertheless, in the case of involuntary action (e.g., someone pushes me) I can acknowledge with consistency that a movement is mine but I am not the cause or the origin of it. Normal experience of voluntary actions brings these two aspects together in an indistinguishable unit. Phenomena such as delusions of control, auditory hallucinations, and thought insertion suggest that the sense of agency rather than the sense of ownership is affected. We can notice, in fact, that self-ownership must even be acknowledged to experience thought insertion; the patient finds himself indeed having a thought—though perceiving it as alien, ignoring its source.

What some authors have called 'non-conceptual first-person content' and 'ecological self' [28–30], consisting of the self-specifying information attained in perceptual experience, is precisely what phenomenology points out through the notion of lived body as an originary and founding moment of the self. Perceiving objects or movement goes hand in hand with gaining information about me, information that is pre-linguistic and non-conceptual.

Second: if there is a minimal self, there should be also a minimal intersubjectivity. Our understanding of other or our assuming another as other subject and not as an object is not primarily inferential or a matter of capacity of prediction or explanation of the behavior of others (as is assumed by the ToM). We understand each other well enough through our shared engagement in the common world, simplified or distorted as it could be, because bodily behavior is meaningful, it is intentional, and as such it is neither internal nor external, but rather beyond this abstract and artificial distinction. Some approaches develop this idea as the second-person perspective [16, 31]. When perceiving the actions and expressive movements of other persons, one sees them as meaningful and goal-directed. No inference to a hidden set of mental states is required. In the face-to-face encounter, we are neither confronted with a mere body, nor with a hidden psyche, but with a unified whole.

Third: It is precisely this minimal self and this minimal intersubjectivity which should be recognized, and on which every therapy should confidently be based, to come in contact with the patient not as a "piece of disturbed nature" but as a suffering person whose horizon and world originarily include mine and vice versa. Thus, a human encounter becomes not only possible but necessary.

References

1. Husserl E. Ideen zu einer reinen Phänomenologie und phänomenologischen Philosophie [Ideas pertaining to a pure phenomenology and to a phenomenological philosophy]. Phänomenologische Untersuchungen zur Konstitution [Studies in the phenomenologyof constitution]. (Husserliana IV). Den Haag: Martinus Nijhoff; 1952.
2. Merleau-Ponty M. Phénoménologie de la perception. Paris: Gallimard; 1945.
3. Husserl E. Ding und Raum [Thing and space]. Vorlesungen 1907 [Lessons 1907]. (Husserliana XVI). Den Haag: Martinus Nijhoff; 1973.
4. Merleau-Ponty M. Le visible et l'invisible [The visible and the invisible]. Paris: Editions Gallimard; 1964.
5. Husserl E. Zur Phänomenologie der Intersubjektivität [Phenomenology of intersubjectivity]. Texte aus dem Nachlass [Texts from the estate]. Erster Teil [Part 1]. 1905–1920. (Husserliana XIII). Den Haag: Martinus Nijhoff; 1973.
6. Husserl E. Zur Phänomenologie der Intersubjektivität [Phenomenology of intersubjectivity]. Texte aus dem Nachlass [Texts from the estate]. Zweiter Teil [Part 2]. 1921–28. Den Haag: Martinus Nijhoff; 1973.
7. Husserl E. Analysen zur passiven Synthesis. Aus Vorlesungs- und Forschungsmanuskripten, 1918–1926. Den Haag: Martinus Nijhoff; 1966.
8. Fuchs T. Psychopathologie von Leib und Raum. Phänomenologisch-empirische Untersuchungen zu

depressiven und paranoiden Erkrankungen [Psychopathology of body and space. Phenomenological–empirical investigations about depressive and paranoid illnesses]. Steinkopf: Darmstadt; 2000.
9. Fuchs T. Phenomenology and psychopathology. In: Gallagher SH, Schmicking D, editors. Handbook of phenomenology and cognitive science. Dordrecht/New York/Heidelberg/London: Springer; 2010.
10. Husserl E. Erfahrung und Urteil. Untersuchungen zur Genealogie der Logik [Experience and judgement. Investigations about the genealogy of logic]. Hamburg: Meiner; 1999.
11. Simons P. Meaning and language. In: Smith B, Smith DW, editors. The Cambridge companion to Husserl. Cambridge: Cambridge University Press; 2006.
12. Fuchs T, Schlimme J. Embodiment and psychopathology: a phenomenological perspective. Curr Opin Psychiatry. 2009;22:570–5.
13. May R, Angel E, Ellenberger E, editors. Existence. A new dimension in psychiatry and psychology. New York: Basic Books; 1958.
14. Zahavi D, Parnas J. Conceptual problems in infantile autism research. Why cognitive science needs phenomenology. J Conscious Stud. 2003;10(9–10):53–71.
15. Calzetta JJ, Orlievsky GD. Trastornos severos del desarrollo: De la escritura a la representación [Severe developmental disorders: from writing to representation]. Anu Investig. 2005;12:317–25.
16. Schilbach L, Timmermans B, Reddy V. Toward a second-person neuroscience. Behav Brain Sci. 2013;36:393–462.
17. Orlievsky D, Cukier S. Language, writing, and activity disorder in the autistic spectrum. Front Integr Neurosci. 2013;7(42) doi:10.3389/fnint.2013.00042.
18. De Jaegher H. Embodiment and sense-making in autism. Front Integr Neurosci. 2013;7(15) doi:10.3389/fnint.2013.00015.
19. He J, Jespersen E. The embodied nature of autistic learning: implications for physical education. Phys Cult Sport Stud Res Spec Issue Disabil Sport Adapt Phys Activity. 2015;65:63–73.
20. Leslie AM. Pretense and representation: the origins of "theory of mind". Psychol Rev. 1987;94:412–26.
21. Autism FU. Explaining the enigma. Oxford: Basic Blackwell; 1989.
22. Baron-Cohen S. Mindblindness. London: MIT Press; 1996.
23. Scharfetter C, Benedetti G. Leiborientierte Therapie schizophrener Ich-Störungen. Schweiz Arch Neurol Neurochir Psychiatr. 1978;123:239–55.
24. Gepner B, Mestre D. Rapid visual-motion integration deficit in autism. Trends Cogn Sci. 2002;6:455.
25. Zahavi D. Subjectivity and selfhood: investigating the first-person perspective. Cambridge: MIT; 2005.
26. Zahavi D. Self-awareness and alterity. A phenomenological investigation. Evanstone: Northwestern University Press; 1999.
27. Gallagher S. Philosophical conceptions of the self: implications for cognitive science. Trends Cogn Sci. 2000;4:14–21.
28. Gibson JJ. The ecological approach to visual perception. Boston: Houghton Mifflin; 1979.
29. Neisser U. Five kinds of self-knowledge. Philos Psychol. 1988;1:35–59.
30. Bermúdez J. The paradox of self-consciousness. Cambridge: MIT Press; 1998.
31. De Jaegher H, Di Paolo E, Gallagher S. Can social interaction constitute social cognition? Trends Cogn Sci. 2010;14:441–7.

3 The Relationship Between Philosophy and Neuroscience from Dan Zahavi's Phenomenology of Mind

Pablo Emanuel García

Abstract

The bridge between psychiatry and neuroscience is not the only one we have to build; it is also necessary to narrow the gap between neuroscience and philosophy. This does not imply reducing the latter to the former or vice versa, but rather linking the two without eliminating their individual characteristics. Taking that into account, Dan Zahavi's phenomenology of mind can make a great contribution by presenting itself as a different option within philosophy of mind, which up until the last few years was dominated by the analytic tradition. In this chapter, I present Zahavi's proposal in four steps. First, I clarify the term *phenomenology*. This choice is not accidental, because nowadays this concept is used by diverse traditions and with different meanings. Second, I make the fundamental distinction between *first-person perspective*—which corresponds to phenomenology—and *third-person perspective*—compatible with neuroscience. Third, I explain the methodological stages assumed by Zahavi from the Husserlian tradition. These stages enable him to study from the *first-person perspective* rigorously: *epoché*, phenomenological reduction, eidetic variation, and intersubjective verification. Finally, I develop the issue of *naturalization of phenomenology* in order to establish a dialogue between science and philosophy. For Zahavi that *naturalization* does not necessarily imply reductionism, but can be understood as something necessary for a fruitful exchange between those disciplines.

Keywords

Philosophy of mind • Phenomenology • Neuroscience • Dan Zahavi • First-person perspective • Naturalization

P.E. García, BA
Universidad Católica San Pablo,
Urb. Campiña Paisajista s/n, Quinta Vivanco, Barrio
de San Lazaro, Arequipa 04001, Perú
e-mail: pgarcia@ucsp.edu.pe

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_3

Introduction

The bridge between psychiatry and neuroscience is not the only one that we have to build; it is also necessary to narrow the gap between neuroscience and philosophy. This does not imply reducing the latter to the former or vice versa, but rather linking the two without eliminating their individual characteristics. Taking that into account, the phenomenology of mind of Dan Zahavi (1967–) can make a great contribution by presenting itself as a different option within philosophy of mind, which until recently was dominated by the analytic tradition.

At present, the current of thought begun by Edmund Husserl (1859–1938) is gaining more space in this context thanks to three facts that favored its incorporation [1] (pp. 26–27): the growing interest within the philosophy of mind in the *hard* issue of the consciousness, the desire to develop a proposal of cognition that would be embodied, and the necessity of referring to the experience from neuroscientific studies.

All these questions necessarily imply a way of understanding the dialogue between philosophy and science, which in recent years has been specified in terms of the philosophy and neuroscience relationship, given the great advances in the latter discipline. The effort to link these areas is not new, but without doubt the context of philosophy of mind provides a more concrete space to pose the question from the philosophical perspective, since it has a look that is clearly interdisciplinary.

In broad terms, in philosophy of mind we find two broad tendencies with respect to the link between philosophy and neuroscience. On the one hand are those who insist on reducing the first to the second, as does Paul Churchland [2], or those who do not eliminate philosophy but reduce it to an area dedicated only to resolving semantic themes and not as a path that can offer knowledge about reality and human beings (for example, Maxwell Bennett and Peter Hacker [3]). On the other hand are those who think that it is possible to affirm the distinction of knowledge, with the peculiar characteristics that belong to both disciplines, and at the same time support the necessity of dialogue to advance knowledge. This second tendency is being developed in a variety of philosophic traditions: the analytic, where John Searle [4–8] can be considered (even with some difficulties [9, 10]); the thomistic, one of whose major exponents in the context of philosophy of mind is Juan José Sanguineti [11–19]; and the phenomenological, within which the proposal of Dan Zahavi is classified. He is a concrete example of how this philosophical line can intervene in fundamental themes for philosophy of mind, seeking an integration, without eliminating their own characteristics, between phenomenology and neuroscience, and simultaneously in dialogue with analytic philosophy.

In this chapter, I develop the relationship between phenomenology and neuroscience (and the positive science in general) based on his proposal. For this I explain, first, what the Danish philosopher understands by *phenomenology*. Second, I make the fundamental distinction between *first-person perspective*—which corresponds to phenomenology—and *third-person perspective*—compatible with neuroscience. Third, I explain the methodological stages assumed by Zahavi from the Husserlian tradition. These stages enable him to study from the *first-person perspective* rigorously: *epoché*, phenomenological reduction, eidetic variation, and intersubjective verification. Finally, I develop the issue of *naturalization of phenomenology* in order to establish a dialogue between science and philosophy.

Phenomenology as Explored by Dan Zahavi

To analyze the relationship between philosophy and neuroscience within the proposal of Zahavi, it is convenient to begin with a quick clarification of the term *phenomenology*. This choice is not accidental, because how the concept is considered determines how the relationship with positive science is established. Moreover, nowadays this notion is used by diverse traditions and with different meanings, often without considering the reference to the philosophical tradition

that constituted it in a specific way of doing philosophy [20] (p. 76). Some speak of *phenomenology* as if it were synonymous with *description*, others as akin to *subjective experience* (understood as the view of each), and there are those who identify it with *introspective method*. Even within the phenomenological trend itself we find differences [21] in the way this concept is understood.

For Dan Zahavi [22], the concept of phenomenology refers to a *philosophical tradition* founded by Edmund Husserl (1859–1938), which includes different thinkers such as Martin Heidegger (1889–1976), Jean-Paul Sartre (1905–1980), Maurice Merleau-Ponty (1908–1961), Michel Henry (1922–2002), and Edith Stein (1891–1942), among others. Without denying the particular nature of each of these philosophers, and the critiques that were made, Zahavi shows that in all of them there are some common assumptions that make it possible to speak of a specific philosophical tradition, such as the interest in subjectivity, intentionality, consciousness, and the self, among other things.

Because of the important representatives included in this current of thought, the influence it has had on so many others (such as Adorno, Gadamer, Habermas, Derrida, and Foucault), and the constant reference it has been in the dialogue between different traditions of the twentieth century (such as existentialism, hermeneutics, structuralism, deconstructionism, and post-structuralism), phenomenology "can be regarded as the cornerstone of what is often (but somewhat misleadingly) called 'continental philosophy'" [23] (p. 68).

The name of this philosophy has to do with the fact that the interest is in the study of the phenomenon, that is, in what appears to the consciousness. Following Heidegger, Zahavi understands the phenomenon "as that which shows itself, as beings' own manifestation" [22] (p. 675), or referring to Husserl "as the manifestation of the thing itself" [24] (p. 55). This leads him to consider phenomenology as "a philosophical analysis of the different modes of givenness, and in connection with this as a reflective investigation of those structures of understanding that permits different types of beings to show themselves as what they are" [22] (p. 675), [24] (p. 55).

This can lead to a false distinction between what appears (broadly defined) and reality as if they were two different things, as if the object were beyond the phenomenon or the phenomenon concealed the object. By contrast, Zahavi thinks that the *phenomenon* manifests the reality, is the reality as given—that, after all, is the only way we can know. *Appearance* is not the synonym of *phenomenon*, but is the object as it appears to a "superficial glance" [25] (p. 22); *reality*, instead, refers to the object but in the way that it can "appear in the best of circumstances" [25] (p. 22). Appearance and reality is "a distinction internal to the phenomenon" [25] (p. 22).

"If we wish to grasp the true nature of the object, we had better pay close attention to how it manifests and reveals itself, be it in sensuous perception or in scientific analyses" [23] (p. 55). As for the latter aspect, Zahavi—referring to Husserl—affirms that the objective of phenomenology "is not to obtain new empirical knowledge about different areas in the world, but rather to comprehend the basic relationship to the world that is presupposed in any such empirical investigation" [22] (p. 665). For him [26], this can lead to understand Husserl's proposal as being aligned with internalism, which maintains that our access to the world is conditioned and is done through internal representations. In Zahavi's interpretation, the link between consciousness and world is, rather, constitutive. Precisely, his interest in consciousness lies in the fact that consciousness reveals the world, because it is through it that reality is presented to the subject.

It is also important to note that for Zahavi, phenomenology "is basically […] a transcendental philosophical endeavor" [27] (p. 340) although different to the Kantian transcendental philosophy which, because of the distinction between phenomenon and *noumeno*, is unable to leave skepticism [22, 26] (p. 663, 680). As well as being a transcendental proposal, phenomenology is idealistic in that it "is committed to the view that the world is necessarily correlated to an intersubjective community of embodied subjects" [28] (p. 84).

Husserlian transcendental idealism can be understood as "anti-representationalist criticism of metaphysical realism" [26] (p. 364). The metaphysic realism which he opposes states that knowledge is a mere copy of a reality that exists independently of the knower. For Zahavi, this proposal pretends to study a world that would exist beyond the presentation to the consciousness, tries to access to the world *in itself* eliminating any subjective element. In this way, it would differ from the common conception of the world, where subjective and objective characteristics take place together. Here, it would seek a pure *third-person perspective*, as in the case of the scientific proposal which argues that the researcher could look at the reality "from nowhere" [28] (p. 85) to reflect the characteristics and classifications that already exist in nature.

According to Zahavi, a conception of this kind—which could be characterized as an attempt of a *neutral* knowledge of the reality—is impossible. There is no world in itself and others which appear, there exists only one real world that is given to consciousness. Thus for Husserl—as interpreted by Zahavi—the true realism is phenomenological idealism [24] (p. 70), [28] (p. 87).

What was said above does not mean that *transcendental idealism* can be identified with *metaphysical idealism*. The latter maintains that there is a metaphysical dependence of reality from the subject, misinterpreting the notion of absolute consciousness that Husserl proposes as being like a divine consciousness. In contrast, in the phenomenological proposal the existence of the real is not denied but asserts a "constitutive link between mind and world", where reality is not a brut datum but "a system of validity and meaning" that calls for a subjectivity, "that is, experiential and conceptual perspectives" [28] (p. 80).

Despite taking away the metaphysical realism and idealism (so in some sense Husserl's position could be characterized as *beyond* realism and idealism [24] (p. 72), [29]), Zahavi [28] shows that in the Husserlian proposal there is no opposition to metaphysics itself; on the contrary, phenomenology necessarily implies it. The opposition is to a certain metaphysics (as mentioned) which is not compatible with the phenomenological proposal.

To complete the characterization of phenomenology proposed by Zahavi, it is finally important to mention that in this current of thought there is a clear concern about the method—to which I will refer in this chapter—but without reducing their interests only to this. In addition, phenomenology proposes an understanding of reality as a whole with reference to the subject who knows it. In this sense it shares, in some respects, the object of study with the science, and this is one of the reasons why we should be open to dialogue with it, without renouncing our own characteristics. Phenomenology and neuroscience are disciplines that relate and differ. And to start this understanding accurately, I explain below how Zahavi understands the *first-person perspective* and the *third-person perspective*.

First-Person-Perspective and Third-Person-Perspective

Dan Zahavi makes the distinction between *first-person perspective* and *third-person perspective* as a way to explain the relationship between phenomenology and neuroscience. This terminology is also used by other philosophers and is a key to understand the theme of this chapter in the context of contemporary philosophy, but it does not have the same meaning for all thinkers.

Absolutism of the Third-Person-Perspective

In some philosophers, an opposition exists between the two perspectives which ends in favoring one of them: the *third-person perspective*. According to this approach, to understand the issue only an approximation of this type should be taken into account if we want to achieve *objective* knowledge. Therefore, only natural science—which uses this perspective—would be valuable, because it could provide a reliable and universal knowledge of reality. Here, the reality

studied would be in front of the scientist and could be evaluated by anyone with full objectivity and transparency, avoiding the biases which are inherent in subjectivity. In contrast, the *first-person perspective* would be *subjective*, providing mere opinions; this would reveal only the particular state of each individual's inability to achieve certain knowledge applicable to all individuals.

For Zahavi, this erroneous opposition between the two perspectives is based on another pair of false contrapositions corresponding to their objects of study. First, it states that the *objects of sciences*—corresponding to the *third-person perspective*—have nothing to do with the knowledge and everyday actions that are carried out without any rigorous reflection on the *world of life*—examined by the *first-person-perspective* [24] (p. 126). In addition to this, secondly, Zahavi argues that there is an opposition between *primary sensitive qualities* and *secondary sensitive qualities* [24] (p. 127). The *third-person-perspective* would put us in contact with the primary sensitive qualities (weight, size, shape, etc.), i.e., those aspects of reality that can be expressed quantitatively and would therefore be independent of the particularities of each knower subject. This would allow a universal, reliable, and objective knowledge. In contrast, the *first-person perspective* would provide access to the secondary sensitive qualities (such as color, taste, texture, etc.), i.e. features of the world that depend inherently on the specific subject who knows them, providing a biased knowledge of reality.

Although at first sight this would seem a reasonable position, nevertheless it is not entirely clear. As Gallagher and Zahavi [25] (p.18) say, the opposition between first- and third-person perspective can be misleading because the terms *objective* and *subjective* are ambiguous [25] (p. 19). This aspect was also highlighted in analytic philosophy by John Searle [7, 8], who demonstrates the difference of meanings by referring to the distinction between *ontological* subjectivity and objectivity and *epistemic* subjectivity and objectivity (although there are some differences, not less important, between the mode of understanding *first-person perspective* in the analytical tradition and phenomenology [1] (p. 51–52)).

Objectivity and Subjectivity

Because of the ambiguity of the terms, in one sense we can say that both perspectives are objective and subjective, but in another sense not. They are *objective* in that they both seek to avoid prejudices, limitations, and insights that may depend on the passing liking or feeling of the person who knows. That is, seek to understand some aspect of reality, including man, and that knowledge could be shared with others, i.e., it would be universal. In this sense, not only science seeks objectivity; phenomenology also has the same interest because it does not seek to provide a simple description of circumstantial facts or individual preferences, as when someone says he prefers coffee to tea, or summer to winter. Such inquiries are not on the horizon of phenomenological study.

On the other hand, the two perspectives are *subjective* in that they are an act of knowledge, and all knowledge is realized by a particular subject. The same designations indicate that we are talking about a *perspective*, and this can only be attributed to a subjectivity, because things cannot have a perspective on something; they are not those *who know*, but in all cases, *the known*. This implies that a pure *third-person perspective*, conceived as a cognitive act that has no influence of subjectivity, is illusory. As Zahavi says, "[t]here is no pure third-person perspective, just as there is no view from nowhere. To believe in the existence of such a pure third-person perspective is to succumb to an objectivist illusion" [25] (p. 40). Therefore, the *third-person perspective* always implies inevitably the experience of a particular subject.

If the two perspectives are objective and subjective in the sense explained above, they are not if one considers other meanings of these adjectives, which make it possible to differentiate these approaches. Without denying the above, we

can say now that the *third-person perspective* is *objective*—and not subjective—in that it studies the reality heading straight to it and considers it as a thing, without seeking to scrutinize to what extent there is a subjective dimension present in that knowledge. And this is valid in two ways. First, in that when studying reality, this is conceived without considering how subjectivity affects that cognitive act, and second, in that when studying human beings we do not consider them as subjects, that is, we do not pay attention to their capacity of feeling, but know them as a substance that lacks subjectivity. Moreover, the *first-person perspective* is *subjective*—and not objective—mainly because in this approach the reality is considered as presented to consciousness, as experienced. As with any conscious act, knowledge has a subjective, experiential dimension that neuroscience with its own method cannot grasp. Only from a first-person perspective is it possible to know that dimension, and this aspect is unavoidable if one wants to properly understand what any conscious act is.

Considering this latter sense, it is possible to affirm that neuroscience exists in a broader context, the experience of the world, upon which it performs its own task. This makes it possible to eliminate the aforementioned contraposition between *objects of science* and the *world of life*. If the world of life and of science is understood ontologically, one notes that there is something in common between the two areas of study, since the two "belong within the natural attitude and … consequently, don't presuppose the effectuation of the transcendental reduction" [24] (p. 132). If it is considered transcendentally, it is evident that the two "are constituted by transcendental (inter) subjectivity, for which reason both objectivism and scientism must be rejected" [24] (p. 132).

As Zahavi [22] (p. 664) states following Merleau-Ponty, what the scientist knows, from a *third-person perspective*, presupposes a *first-person perspective*, that is a determined pre-scientific experience of the world, and not only presupposes the experience itself but the existence of the world that is given in a certain way. The latter is the so-called *natural attitude*, that the phenomenologist—unlike the scientist—should not presuppose but research. Science begins with some propositions that are not justified—and do not have to be—but that should be clarified to fully understand how we know; and here lies one of the fundamental tasks of phenomenology. The phenomenologist questions the way in which things are presented. He does not ask about their measurable characteristics but about the ways in which a certain reality is given to the subject. This makes it possible to understand that in a broader conception of the world of life, science would fall within it [24] (p. 130).

The emphasis placed on the *first-person perspective* by phenomenology is not only about considering that we should include this approach if we want to study mental phenomena (as in the analytical proposal of John Searle, mentioned above). The concern is transcendental [22] (p. 674). The world can only be given to consciousness, that is to say only in the first-person experience is reality presented [22] (p. 674), both in the order of ordinary knowledge and the sciences in general. Reality can be known because is presented somehow to our consciousness, that is because it is a *phenomenon*.

The reason why phenomenologists have been so preoccupied with describing and analyzing the fundamental features of subjectivity, be it its structures of intentionality, of embodiment, of temporality, of historicity, of intersubjective embeddedness, is that they have been convinced that a thorough philosophical understanding of the structures of knowledge, truth, meaning, and reference must include an investigation of the first-person perspective. If we wish to understand how physical objects, mathematical models, chemical processes, social relations, cultural products, can appear as they do and with the meaning they have, then we will also have to examine the subject to whom they appear. If we wish to understand the world that we experience and live in, we also have to investigate subjectivity. Truth, meaning, reality are always a truth, meaning, and reality *for* somebody [22] (p. 674).

One can say that the *first-person perspective*—present in all conscious mental states—has three fundamental characteristics that phenomenology seeks to study rigorously. First, it is clear that things appear in a different way depending on the kind of reality; for example, it is not the same to perceive the color red as to perceive green.

Second, it also includes the distinction of the various ways in which the same reality can presented, i.e., the same red color can be seen as perceived, imagined, desired, etc. And the third aspect, which is essential to understand the *first-person perspective*, is that all conscious mental states always occurs *to me*, always brings a sense of ownership for the subject who experiences the act [20] (pp. 66–68). And the latter also applies to scientific knowledge, because science is not just a set of abstract knowledge that does not belong to anyone—which is impossible—but it is realized by specific subjects, "it is a specific theoretical stance towards the world" [22] (p. 679).

Epistemic Asymmetry

The first- and third-person perspectives can be ways of knowing any reality: things, other persons, or the same subject who knows. However that knowledge is not acquired in these different cases in the same way and this entails an "epistemic asymmetry" [20] (p. 67) between them, depending on the perspective one takes as point of departure. From the first-person perspective there is a predominance of the self-awareness, because in this modality one gets a knowledge that is impossible to obtain from an other perspective. The primacy does not mean that the self-knowledge is manifested first clearly in ordinary life, on the contrary, usually persons are turned towards things and the self-consciousness goes unnoticed. But if we have a critical position with this *natural attitude* we realize that there is something that occur in the base of all knowledge: the awareness of ourselves. The preeminence of this knowledge means that the subject is *always* presented to itself and do this in a *unique* way, as I explain below.

I cannot access things as they are in themselves, but I know them as presented in my consciousness, that is, from my subjectivity. This does not cause a skeptical position, as if it were impossible to access the real thing; rather, I affirm the subjective dimension of the knowledge and the difference of self-knowledge with respect to things and others.

I understand nothing in the same way that I know myself, because here the knower and the known coincide. This feature cannot be compared with the difference between experiencing two things, for example when I say that chocolate or coffee taste differently; nor with the difference between two acts of consciousness aimed at the same object, as when one speaks of the distinction between actually sipping coffee and imagining it. On the contrary, when I talk about the consciousness that the subject has of himself, there is a fundamental difference based on the fact that I am naturally what I know, which does not happen when I know something which is different from me, otherwise I would become the known when I experience it.

The asymmetry in knowledge refers not only to the difference between knowing something and knowing myself, but also to the way in which we experience other people. As for the latter, Zahavi says that the expressions of others are significant, are part of their subjectivity, and therefore reveal, not conceal. This is the issue of *second-person perspective* or *empathy*, defined as "a form of intentionality in which one is directed towards the other's live experiences" [20] (p. 73). This philosopher has written several texts to expand on this issue [25, 30–34], avoiding behaviorism and the position which argues an inferential knowledge of the others. Mental states and behavior differ (hence deception is possible), but both are part of subjectivity, and in ordinary life, in a certain context, behavior speaks of the mind. But this contact with the mental aspects of the other is not inferential, as if I started from his behavior and later supposed, without much confidence, that the other is in a specific subjective state. The key here is to understand that the subjectivity of the person is embodied, exists in the world and can also be known directly.

From the *third-person perspective* I can also know myself, but as yet another object of reality. I can also comprehend what is different from me, a subject or a thing, but always considering them as something without subjectivity. I think that from this perspective, the preeminence of one kind of knowledge over another is different from that stated in the *first-person perspective*. Here, the primacy is of the knowledge of the non- personal things, in that they are simpler and therefore easier to understand from this point of view.

The difference between *first-person perspective* and *third* is not that one is individual and the other universal, that one is merely descriptive of private situations and the other explains features available to any subject. In both perspectives there are knowledge, that is "an identification or synthesis between that which is intended and that which is given […], and truth as an identity between the meant and the given" [24] (p. 31). What makes it possible to distinguish one perspective from another has to do with the starting point, and the viewpoint from which we consider the person or object studied.

Methodological Steps to the Study from the First-Person Perspective

First-Person Perspective as a Method

Considering what was established earlier, you might think that the phenomenological method is a process of introspection, by which we enter from outside into the consciousness to look for something. However, this is just an image that does not help us to understand the proper way to proceed in phenomenology. This misunderstanding can lead to mistakenly understanding this tradition as immanent, closed to others, and therefore in line with metaphysical idealism.

If introspection is "a mental operation that enables us to report about our own current mental states" [24] (p. 54), the distinction in relation to the phenomenological method is triple. First, introspection is a *kind* of experience that does not account for the experience itself, but presupposes it, and this is precisely what is of interest to phenomenology. Second, introspection only provides specific knowledge, relating to a single subject; however, phenomenology wants to achieve structures that occur in all human beings, and in this sense is a universal knowledge. Because of the transcendental character, this philosophy asks about the conditions of possibility of the phenomenon, and is one step before inside–outside distinction, which is assumed to speak of introspection (or internalism and externalism) [26]. And finally, the third difference is that by introspection the subject looks inwards at itself, closing the possibility to an opening to reality. In contrast, the phenomenological methodology considers the experience, in which is given the reality.

As the phenomenologist seeks to deepen the experience—broadly considered, not as is understood by empiricism—I think that the approach taken by Zahavi could be considered as a *method of first person with some concrete steps for a rigorous study*: *epoché*, *transcendental or phenomenological reduction*, *eidetic variation*, and *intersubjective verification*. While the Danish philosopher does not speaks explicitly of the *first-person perspective* as a methodology, although he seems to suggest this in some cases [23] (p. 70), I think that considering it in that way can help to understand this issue.

Steps to Study from the First-Person Perspective

Epoché is the first concrete step of this method, and consists in suspending our *natural attitude* toward the world, i.e., putting our beliefs or theories of reality as given in parenthesis [23] (p. 70). When scientist studies his own object, it is presented as indisputable, as happens in everyday life where people are directed toward reality presupposing its existence. For this reason, Husserl [35] (§ 30) calls this tendency *natural attitude*, because it is not only typical of the sciences but also of the everyday knowledge that we have of reality, by which we take for granted that things exist and are in a certain way and not another. Here, the involved does not notice that the real is given to the conscience, and it is precisely this aspect that phenomenology seeks to understand, highlighting the contribution of subjectivity.

With *epoché* there is no intention to eliminate the *natural attitude* but to explain it, nor to deny the world; this "entails a change of attitude towards reality, and not an exclusion of reality" [22] (p. 670). Here it seeks to leave aside "a certain naivety, the naivety of simply taking the world for granted, thereby ignoring the contribution of consciousness" [20] (p. 82, note 3).

This attitude does not necessarily lead to immanentism or skepticism, but rather allows us to be open to capture the sense of the real, to *return to the things themselves*, because the interest is in the act of knowledge since it is linked with the object [23] (p. 71).

Although natural inclination, corresponding to science and everyday knowledge, is suspended here, I think there is an element in this attitude that must be present in neuroscientific research. It should be guided only by the studied reality and not by theories preconceived as true. And as in phenomenological reflection, also in science *epoché* is not a step that takes place the first time and then is then left in order to move to the next; rather, it is an attitude that must be maintained throughout the process of knowledge.

Epoché has to be understood in conjunction with *transcendental reduction*, second element of the phenomenological method, "the purpose of which is to liberate us from a natural(istic) dogmatism and to make us aware of our own constitutive (that is, cognitive, meaning-giving) contribution" [24] (p. 46). Transcendental or phenomenological reduction specifically seeks "to analyse the correlational interdependence between specific structures of subjectivity and specific modes of appearance or givenness" [25] (p. 25), [22] (p. 669), [1] (p. 53). Here, *reduction* should not be understood as the elimination of one to another but as a *redirection*, in the sense of reflexively turning our attention to how the world appears to us [24] (p. 46). It seeks to explore the ways in which reality and structures of subjectivity (the latter permits things to present themselves in the way they do) appear. Because "only through a radical turn toward that which in a strict sense is given from a first-person perspective, can transcendental analysis commence" [24] (p. 50). This is what manifests the principle governing the whole methodology: "to regard every originary intuition as the legitimizing source of cognition" [29] (p. 46).

This transcendental reflection does not imply a loss, as if the world is excluded from our interest and we focus only on our subjectivity. Rather, by this process occurs "an *expansion* of our field of research" [24] (p. 46). Transcendental reduction is also studying the world, but as given to consciousness, i.e., as a phenomenon. Phenomenologists seek to study "the very dimension of appearance or givenness, and to disclose its structure and conditions of possibility" [22] (p. 671), which implies a transcendental perspective.

This reduction allows us to access the transcendental subject, i.e., the subject "as the subjective condition of possibility for manifestation" [24] (p. 46). Commenting on Husserl, Zahavi presents two major ways to approach it, the Cartesian and the ontological. Using the first, it is possible to understand that things are given to consciousness in a different way to how it gives to itself; consequently, he says that a study of consciousness from the *third-person perspective* is incomplete, it is necessary to know it from the *first-person perspective*. Also, in this Cartesian way, it is possible to note the priority of the subjectivity, because "[t]he world is not something that simply exists. The world appears, and the structure of this appearance is conditioned and made possible by subjectivity" [24] (p. 52). This is not idealism, but affirms that it is impossible to understand "the nature of meaning, truth, and reality" [24] (p. 52) without some reference to subjectivity. The "reality is not simply a brute fact detached from every context of experience and from every conceptual framework, but is a system of validity and meaning that needs subjectivity, that is, experiential and conceptual perspectives if it is to manifest and articulate itself" [24] (p. 69). For this, it needs subjectivity, not in the sense that the latter creates the world, but rather in that an objectivistic interpretation does not capture the ontological status adequately.

To be real, to be an objectively existing object, is to have a specific regulated structure of appearance, it is to be given for a subject in a certain way, with a certain meaning and validity, not in the sense that the object can exist only when it actually appears, but in the sense that its existence is connected to the possibility of such an appearance [24] (p. 70).

This line of thought has the risk of interpreting phenomenology as immanent, so it is necessary to complement it with the *ontological* method,

where one starts with the giving of some regions of reality. If I analyze how these areas are presented, I have to question using subjective structures that allow this. "We are led to the acts of presentation, perception, judgment, and valuation, and thereby to the subject (or subjects) that the object as appearing must necessarily be understood in relation to" [24] (p. 50). Here, access to subjectivity is indirect, through the things that arise [29] (p. 46). This transcendental subjectivity differs from and is assumed in the analysis of the empirical subject, i.e., the studies of man by the positive sciences. It is the same subject but studied from different perspectives.

For Zahavi [22] (p. 668), in Husserlian analysis, transcendental reduction is key, because with it it is possible to differentiate the phenomenological proposal and the point of view of the natural sciences [36] (p. 6), [27] (p. 337), [22] (p. 685), and also show its own contribution to the knowledge of the subject. This philosophical tradition has a transcendental preoccupation, i.e., to seek *the constituting dimension of subjectivity*, to which natural science cannot access [22] (p. 667). As transcendental, Husserl's proposal is *foundationalist*, which does not mean that phenomenology is a discipline from which the empirical sciences must infer its contents. It studies the conditions of possibility for something to be done, conceiving this task as work that never ends but must be continually improved. For Zahavi, Husserl's foundationalism has to be understood as "a way of emphasizing and maintaining the difference between the empirical and the philosophical stance, between the mundane and the transcendental attitude" [22] (p. 673). In this sense, phenomenology clarifies "the framework within which all other sciences take place" [24] (p. 66).

Eidetic variation makes it possible to capture the essential features of the thing. This act is not passive; it implies that the subject makes changes using his imagination to the different aspects of the experienced object, so that he captures the elements that cannot be changed without the reality ceasing to be what it is. Thereby, it is possible to differentiate the accidental from the essential characteristics [24] (pp. 38–39) and to know the invariant elements of the various ontological regions. And though the access to these structures is from the *first-person perspective*, to the extent that it refers to the essential, is possible to say that the phenomenological knowledge is not particular but *universal*, so when I know some features of human beings, I capture aspects presented in *all* persons.

The last component of the phenomenological methodology followed by Zahavi is *intersubjective verification*, i.e., confrontation with the point of view of others with respect to the experience of the structures. This implies the recognition of the fallibility of every human and, at the same time, the possibility that everyone has to grasp universal subjective structures. This aspect helps to confirm that the phenomenological method is not introspective. If this were the case, phenomenology would only make it possible to know private aspects that would occur only in the particular subject, and therefore confrontation with others would be impossible. On the contrary, based on the experience that each individual has of reality and of itself, invariant structures can be achieved by various subjects, and therefore they can be corrected by the contribution of others.

Naturalization of Phenomenology

The characterization of the *first-person perspective* and *third-person perspective*, as a mode of relating phenomenology and neuroscience, shows in particular various aspects that make it possible to differentiate these ways of knowing human beings. Now, it is necessary to look even more deeply into how these disciplines can be linked. In the work of Zahavi, the mode of carrying out this task can be understood by referring to the issue of the *naturalization of phenomenology*.

Naturalization as Mathematization

Some philosophers, such as Roy et al. [37], understand that the naturalization of phenomenology would solve the difficulty that Joseph Levine

called "the explanatory gap" [38], i.e., the (apparent) impossibility to close the gap between subjective experience and the explanation given by neuroscience. The point is that for them *to naturalize* means to align with the proposals of natural science. This would involve a process of mathematization of phenomenology, i.e., translating their findings to a mathematical language in order to have something in common with the natural sciences. These authors are aware of the explicit opposition of Husserl to it, but according to their interpretations, this fact was due to the conception of science of the founder of phenomenology, which today is already obsolete, so the contradiction would become irrelevant and the naturalization of phenomenology would be possible without difficulty.

But the proposal of Roy et al. has at least three difficulties. First, naturalization of phenomenology proposed by them really contrasts with the view of Husserl. Zahavi says that Husserl's critique of naturalism is not based on a conception of science in particular but "on a number of transcendental or philosophical reasons" [27] (p. 335), expressed in opposition to objectivism and representationalism and in affirmation of transcendental subjectivity. The notion of the transcendental subject should not be understood here as if it were an external reality to the world, as a different subject from the empirical subject. Empirical and transcendental subject refer to the same subject but from different aspects, "being aware of oneself as an object in the world, and being aware of oneself as a subject for the world" [27] (p. 335). This is the difference between consciousness taken as a reality of the world—studied by psychology—and consciousness considered from a transcendental perspective, neither as a thing nor as a psychic reality—studied by phenomenology.

Zahavi thinks that Roy et al. seek to naturalize phenomenology by eliminating its transcendental character, which in the end leads to the abandonment of phenomenology as a philosophical discipline, by reducing it to a phenomenological psychology. Husserl distinguished between phenomenological psychology and transcendental phenomenology. The former is "a form of descriptive, eidetic, and intentional psychology which takes the first-person perspective seriously, but which […] remains within the natural attitude", is "a local regional–ontological investigation" [27] (p. 339). The latter discipline studies the consciousness too, but as a "condition of possibility for meaning, truth, validity, and appearance" [27] (p. 339), i.e., it searches "the constitutive dimension of subjectivity" [27] (p. 339).

For the Danish philosopher [36] (p. 3), this discussion describes what has happened in the twentieth century in the philosophical field, which can be characterized as a process that goes from anti-naturalism to naturalism. Here, *naturalism* is understood as the proposal of the natural sciences with a specific methodological and metaphysical design. It argues that reality is of only one type, "namely things with natural properties" [36] (p. 5); therefore, the only criterion to justify an affirmation is that provided by natural science. As a result, many argue that the method that should be used in every study is that of these disciplines.

This proposal, for Zahavi, is so committed to metaphysical realism, in the manner that I explained above. Using this line of thought, consciousness is conceived as a subject in the world and not as "a subject for the world, that is, a necessary condition of possibility for any entity to appear as an object in the way it does and with the meaning it has" [36] (p. 5). In this form of naturalization, they forget that science itself is not just a set of arguments made in the abstract, but is always performed by someone; "it is a specific theoretical stance towards the world" [36] (p. 10). Any scientific approach is based on the discussion of conscious experiences that have scientists involved. In this sense, consciousness is not a barrier to objectivity but a necessary requirement, more than "microscopes and scanners" [36] (p. 6).

The phenomenological investigation of consciousness is not motivated by the wish to find a place for consciousness within an already well-established materialistic or naturalistic framework. In fact, the very attempt to do so, assuming that consciousness is merely yet

another object in the world, would prevent one from discovering and clarifying some of the most interesting aspects of consciousness, including the true epistemic and ontological significance of the first-person perspective [25] (p. 25).

Second, the mathematization of phenomenology has another difficulty, since mathematical language expresses only the quantitative aspects of reality. There are other dimensions that cannot be expressed in this way. A proper understanding of this solves the opposition mentioned in the second point between primary qualities and secondary qualities. In the naturalization of Roy et al., primacy is given to the primary qualities to the detriment of the secondary. But if we understand naturalization differently, not as a process that leads to doing phenomenology as an extension of neuroscience or natural sciences in general but as a different discipline, it would be possible to account for the qualities that cannot be quantified.

And the third problem in this proposal of naturalization is that it does not resolve the *explanatory gap* but eliminate it (apparently) by reducing the findings of one discipline (phenomenology) to another (neuroscience). An attempt to create a relationship between brain and consciousness necessarily demands knowing what is going to correlate, and therefore not only involves the study of brain function but also of what you mean by conscious acts. Even a position that claims to be reductionist must admit this; one must first know what it is that one wants to reduce [20] (p. 69).

Naturalization Without Reductionism

Zahavi notes that the link between phenomenology and empirical sciences was not always understood in the same way by the representatives of this philosophical tradition. In Husserl, it is conceived as a fruitful relationship where science can provide data that then could be deepened by phenomenology, as specifically happened in the study of *embodiment* and *intersubjectivity* that led him to consider issues addressed by science [27] (p. 341). Against this, in Heidegger, for example, the link becomes very difficult [27] (p. 340), but in Merleau-Ponty the relationship is radicalized even more than in Husserl, recognizing explicitly that phenomenology should to talk to sciences to advance, this—for Zahavi—without abandoning its transcendental character [27] (p. 342).

In the face of the reductionist naturalization in the texts of Zahavi, there exists another way to naturalize phenomenology, inspired primarily by Husserl, conceived as the *necessary exchange between this branch of philosophy and natural science* [36] (pp. 14–15). For him, cooperation must be applied without abandoning the differences between disciplines [27] (pp. 344–345), in order to achieve a mutual benefit. Science and philosophy need each other, so the link should be bidirectional, not unidirectional. There can be neither reduction of phenomenology to science, nor can science be a deduction from the philosophical conclusions [36] (p. 9). The link between them should consist of a "*mutual enlightenment*" [1] (p. 63, my translation).

Thus, concretely, with regard to the contribution that phenomenology can make to science it can be said, first, that phenomenologists have very rich descriptions of various experiences that can help neuroscientists to better understand that the subject studied is living in the first person. This is not irrelevant, since this dimension is present in all reports made by individuals who are part of their own experiments in neuroscience. Many neuroscientific data have a complete sense when they can be linked to these experiences, as in psychiatric studies. This is a meeting point where a correlation is possible between the experience described by phenomenology and the information regarding the nervous system process, where the evaluated subject has such conscious acts.

In addition to this aspect of linkage data, the contribution of phenomenology to neuroscience is concerned, secondly, with what the positive sciences have lost sight of because of the predominant objectivism, and which causes—according to Husserl—a sharp division between the scientific world and the *world of life* [24] (p. 126). Phenomenology can provide an epistemological and ethical foundation for science.

When the neuroscientist performs his task, he assumes several things, such as that reality exists

and does so with a certain way of being, that the subject studied has some experience, as well as the scientist who analyzes the data, assumes that objectivity is possible, as well as the fact that we are able to know the truth, etc. Rightly, phenomenology seeks to understand those issues that are presented as evident to the neuroscientists and which they do not extend. Therefore, this philosophical tradition provides epistemological foundations for neuroscience, through studies regarding how it is possible to know, to what extent reality is presented to our consciousness, about the contribution of subjectivity in this issue, how to reach the truth, etc. Based on this, phenomenology can even question science about its presuppositions.

In addition, achieving the mode of being of the conscious subject, it is possible to present a higher horizon to science by referring to other values that allow for a man's full development. Considering this, it can also lead the scientist to ask about the limits to be established in the experiments—since not everything possible is ethically valid—and about the concrete actions that are performed with the information provided by science, because such events may turn against man himself.

There is a third aspect which phenomenology can bring to neuroscience and which refers to the specific task of the scientist, because it can help to develop other ways of experimentation. Both the description of experiences, such as the establishment of certain structural elements of all conscious acts and the subject himself, as well as the fact of providing an ethical horizon for scientific activity, can give elements to the neuroscientists in order to reformulate their experiments or create new approaches to the subject.

With regard to what science can contribute to phenomenology, it should be noted that there are two major types of contribution: discoveries at a personal level and for the subpersonal order [1] (pp. 62–63), in matters of normal psychological and pathological states. These data should encourage phenomenology to deepen, or even review, the proposals developed so far. As far as information at the personal level, science may propose, for example, descriptions of perception of the own body or the impossibility to do it, also the understanding that the subject has of himself and others. It can offer descriptions of the experience of freedom or the lack of it in actions, or the way in which children relate with other people or things, also of some emotions in different cultures, such as explanations of the different types of memory, etc. All this information helps the phenomenologist to expand the knowledge of these aspects that he has from immediate experience.

With regard to personal data, taken from study of the nervous system, the activation of certain brain areas considered relevant to a specific conscious task can indicate to phenomenologists that facts analyzed from their perspective have dimensions that may not have been considered. This does not imply that the philosopher must take the interpretations made by scientists about the studied facts [36] (p. 14), but the proposals should *motivate* him—not force him—to deepen their answers [39] (p. 163). That depth may then even show that he was wrong and that he should revisit some formulations.

Conclusion

As has been clear throughout this chapter, reducing the gap between neuroscience and philosophy does not mean eliminating the distinction of knowledge to redirect all of them to a single discipline. Nor does it imply shaping a new more general knowledge in which the specificities of each field are dissolved.

Neuroscience and phenomenology share the material object of study, to create a classical language, so they cannot ignore what each discipline has achieved to know about it. But it is also necessary to note that they possess different formal objects, studying different aspects of the same reality that presents itself as complex. And it is this characteristic which ultimately establishes the requirement of multiple perspectives if one wants to truly know the human being [39] (p. 181).

The notions of *first-person perspective* and *third-person perspective* serve to understand the differences and possible links between phenomenology and neuroscience. They express two different ways of understanding

the human person, irreducible one to another but also complementary to achieve a comprehensive view. The *third-person perspective* is not the only one who can provide reliable data, the *first-person* can bring the experience that the subject has of himself and from it the structural characteristics of all conscious actions. This information is essential for neuroscience, if the scientific makes even the claim—as it should do—to understand the specific people that are presented.

On the other hand, the process of naturalization of phenomenology involves abandoning the reductionist claim to lead to a broader vision of reality, and with it rigorous knowledge and truth. It makes it possible to develop bridges to link the two disciplines on specific issues that will lead to mutual enrichment. Therefore, neither opposition nor elimination, but *collaboration* in order to a better knowledge of the person should be encouraged.

References

1. Gallagher S, Zahavi D. La mente fenomenológica. 2nd ed. Madrid: Alianza Editorial; 2013.
2. Churchland PM. Materia y conciencia. 2nd ed. Barcelona: Gedisa; 1999.
3. Bennett M, Dennett D, Hacker P, Searle J. La naturaleza de la conciencia. Cerebro, mente y lenguaje. Pidós: Barcelona; 2008.
4. Searle JR. Mentes, cerebros y ciencia. Madrid: Cátedra; 1985.
5. Searle JR. El redescubrimiento de la mente. Barcelona: Crítica; 1996.
6. Searle JR. El misterio de la conciencia. Barcelona: Paidós; 2000.
7. Searle JR. Libertad y neurobiología. Barcelona: Paidós; 2005.
8. Searle JR. La mente: una breve introducción. Bogotá: Norma; 2006.
9. García PE. La libertad en la filosofía de la mente de John Rogers Searle. Eikasia Revista de Filosofía. 2014;32:203–6.
10. García PE. La perspectiva de primera persona como metodología de estudio en filosofía de la mente desde John R. Searle. Revista de Investigación de la UCSP. 2014;5(5):29–46.
11. Sanguineti JJ. La interacción entre ciencias y filosofía. Paper presented at: XXIX Semana Tomista; 6–10 Sept 2004; Buenos Aires.
12. Sanguineti JJ. Filosofía de la mente. Un enfoque ontológico y antropológico. Madrid: Palabra; 2007.
13. Sanguineti JJ. Libertad y cerebro. Paper presented at: Workshop of the Pontifical University of the Holy Cross; Nov 2008; Roma.
14. Sanguineti JJ. La scelta razionale: un problema di filosofía della mente e della neuroscienza. Acta Philosophica. 2008;17:247–72.
15. Sanguineti JJ. Libertad y base cerebral. Papeer presented at: Pontificia Universidad Católica Argentina; 17 Sept 2008; Buenos Aires
16. Sanguineti JJ. El yo como causa. Sapientia. 2010; 66:23–39.
17. Sanguineti JJ. I compiti delle neuroscienze e l'antropologia filosofica. Un discorso sul metodo. In: Renna L, editor. Neuroscienze e persona: interrogativi e percorsi etici. Bologna: EDB; 2010.
18. Sanguineti JJ. The ontological account of self-consciousness in Aristotle and Aquinas. Rev Metaphys. 2013;67:311–44.
19. Sanguineti JJ. Neuroscienza e filosofia dell'uomo. Roma: EDUSC; 2014.
20. Zahavi D. Subjectivity and the first-person perspective. Southern J Philos 2007(45, Spindel Supplement):66–84.
21. Zirión QA. La situación actual de la fenomenología y sus tareas pendientes. In: Sandoval E, editor. Fenomenología y hermenéutica convergencias y divergencias. Saarbrücken: Editorial Académica Española; 2012.
22. Zahavi D. Phenomenology. In: Moran D, editor. Routledge companion to twentieth-century philosophy. London: Routledge; 2008.
23. Thompson E, Zahavi D. Philosophical issues: phenomenology. In: Zelazo PD, Moscovitch M, Thompson E, editors. The Cambridge handbook of consciousness. Cambridge: Cambridge University Press; 2007.
24. Zahavi D. Husserl's phenomenology. Stanford: Stanford University Press; 2003.
25. Gallagher S, Zahavi D. The phenomenological mind: an introduction to philosophy of mind and cognitive science. London: Routledge; 2008.
26. Zahavi D. Internalism, externalism, and transcendental idealism. Synthese. 2008;160(3):355–74.
27. Zahavi D. Phenomenology and the project of naturalization. Phenomenol Cognit Sci. 2004;3(4):331–47.
28. Zahavi D. Husserl and the 'absolute'. In: Ierna C, Jacobs H, Mattens F, editors. Philosophy, phenomenology, sciences: essays in commemoration of Husserl. Phaenomenologica. Dordrecht: Springer; 2010.
29. Zahavi D. Beyond realism and idealism. Husserl's late concept of constitution. Danish Yearbook Philos. 1994;29:44–62.
30. Zahavi D. Intersubjectivity. In: Luft S, Overgaard S, editors. The Routledge companion to phenomenology. London: Routledge; 2011.
31. Zahavi D. Self and other: exploring subjectivity, empathy and shame. Oxford: Oxford University Press; 2014.

32. Zahavi D. Subjectivity and Selfhood: investigating the first-person perspective A Bradford Book. London: MIT Press; 2005.
33. Zahavi D. Empathy and mirroring: Husserl and Gallese. In: Breeur R, Melle U, editors. Life, subjectivity & art: essays in honor of Rudolf Bernet. Dordrecht: Springer; 2012.
34. Zahavi D. Self, consciousness, and shame. In: Zahavi D, editor. The Oxford handbook of contemporary phenomenology. Oxford: Oxford University Press; 2012.
35. Husserl E. Ideas relativas a una fenomenología pura y una filosofía fenomenológica. 2nd ed. México-Buenos Aires: Fondo de Cultura Económica; 1962.
36. Zahavi D. Naturalized phenomenology. In: Gallagher S, Schmicking D, editors. Handbook of phenomenology and cognitive science. Dordrecht: Springer; 2010.
37. Roy J-M, Petitot J, Pachoud B, Varela FJ. Beyond the gap, an introduction to naturalizing phenomenology. In: Roy J-M, Petitot J, Pachoud B, Varela FJ, editors. Naturalizing phenomenology. Stanford: Stanford University Press; 1999.
38. Levine J. Materialism and qualia: the explanatory gap. Pac Philos Q. 1983;64:354–61.
39. Zahavi D, Gallagher S. Forum on "The phenomenological mind" by S. Gallagher and D. Zahavi. Leitmotiv. 2010:155–83.

4 Aristotelian Hylomorphism: A Framework for Non-physicalist Philosophers About Philosophy of Mind

Ricardo F. Crespo

Abstract

The results of a recent survey on the philosophical views of contemporary, mainly Anglo-Saxon professional philosophers have shown that a supposed predominance of physicalist reductionist positions in the philosophy of neurosciences is far from unanimous. This paper explores one possible philosophical position rooting a non-physicalist reductionist conception of mind. It suggests and argues that a classical philosophical frame, Aristotelian hylomorphism, provides adequate non-reductionist answers that do not fall into dualisms. Finally, it offers the corresponding conclusions.

Keywords

Philosophy of neurosciences • Non-reductionist positions • Hylomorphism

The results of a recent survey on the philosophical views of contemporary, mainly Anglo-Saxon professional philosophers, have surprised me (see Bourget and Chalmers [1]). While I believe that we are witnessing an increasing prevalence of materialist reductionist positions in the philosophy of neurosciences, this survey shows that this conception is not undisputed. Only 12.2% of philosophers surveyed deny free will, and only 16.9% hold a biological view of personal identity. In addition, 56.5% uphold a physicalist position about mind; consequently, 43.5% maintain a non-physicalist position.

Some philosophers distinguish physicalism from materialism for specific reasons (see Stoljar [2], p. 1). Some physical entities do not seem to be material: waves, energies, and so on. However, "while 'physicalism' is no doubt related to 'physics', it is also related to 'physical object' and this in turn is very closely connected with 'material object', and via that, with 'matter'(ibid.). In fact, today, these terms are regularly used interchangeably" (ibid.). Consequently, 43.5% of philosophers hold a non-materialist position about mind. What does this mean? It could be that they believe that the mind is not physical, and they are dualists. Another, more likely possibility is that they

R.F. Crespo, PhD
Universidad Austral, Universidad Nacional de Cuyo, CONICET, Mariano Acosta s/n y Ruta Nacional 8 Pilar, Buenos Aires B1629WWA, Argentina
e-mail: rcrespo@iae.edu.ar

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II,*
DOI 10.1007/978-3-319-53126-7_4

are non-reductive physicalists with regard to the mind. There are different non-reductivist positions, such as the "multiple realizability" thesis proposed by Hilary Putnam [3], "Supervenience" (see Davidson [4], p. 218, and McLaughlin and Bennett [5]), epistemological and ontological emergentism (see Timothy O'Connor and Hong Yu Wong [6]), and dualism (for example, Hasker [7]). Other non-reductivist positions are developed by authors belonging to a "family" of neuro-phenomenological currents: "autopoiesis", the "enactive approach", "organizational teleology", and top-down causation conceptions, stressing the teleological and self-organizational character of living organisms. See, for example, Varela and Thompson [8], Thompson [9], Di Paolo [10], Mossio, Saborido, and Moreno, [11], and Auletta et al. [12]. Here I do not discuss these theories, but I introduce an alternative classical position: Aristotelian hylomorphism. Finally, I offer the corresponding conclusions.

The Hylomorphic Aristotelian Conception of the Human Being

Pre-Socratic philosophers, or *physicoi*, as Aristotle called them, searched for the principles or origins of natural phenomena, that is, the fundamental constituents of all natural objects. In *Metaphysics*, Aristotle reviews the different pre-Socratic proposals about the nature of these principles and develops his own position with regard to them (which he also developed in his *Physics*). A thing may be said to be in different senses, but the primary sense is the substance (book Z [VII], 1 [13]). In *Physics*, Aristotle deals with the movements and changes happening in substances. These are of different types: qualitative, quantitative, and local—all three inhering in the substance—and a fourth type, the generation or corruption of the very substance. This fourth type calls for a different subject (from the substance). He concludes:

"… If, then, we grant that the things of Nature have ultimate determinants and principles which constitute them, and also that we can speak of them 'coming to be' not in an incidental but in an essential sense—[…]—then it is obvious that they are composed, in every case, of the underlying subject and the 'form' which their defining properties give to it" (*Physics* I, 7, 190b 18–21 [13]).

He calls this underlying subject of substantial changes, *próte hýle*, ultimate underlying or prime matter (*Physics* II, 1, 193a 29 [13]). (On Aristotle's metaphysical conceptions, see, e.g., S. Marc Cohen [14], Aquinas is clear in sustaining the essentially pure potentiality, formlessness, and absolute inseparability (from form) of prime matter. See Lang [15] on prime matter for Aquinas.) For Aristotle, this substantial composition does not imply that matter and form are parts of the substance: they "cannot even exist if severed from the whole" (*Metaphysics* VII, 10, 1035b 24–25 [13]). He also affirms: "the final matter and the form are one and the same thing, the one potentially, and the other actually" (*Metaphysics* VIII, 6, 1045b 18–19 [13]). The form is the primary cause, the actuality of being, the unifier of the substance, and it is not an element but a principle (cf. *Metaphysics* VII, 17, 1041b 25–31 [13]). As Anna Marmodoro synthesizes, "the substance is a composite of matter and form, and yet *one*" ([16], p. 20).

Aristotle applies his theory of hylomorphism to living beings. For him, the *psyche* (soul) "is the first actuality of a natural organic body" (*On the Soul* II, 1, 412b 4 [13, 17]), namely, the form. It provides actuality, identity, and persistence to the living being (cf. Irwin, [18], p. 288). In Stephen Brock's expression ([19], p. 342), it is its "ontological energy". The body, according to Irwin's interpretation of Aristotle ([19], pp. 285; see also p. 294), is not the "remote" matter—the chemical stuffs, i.e., what Descartes considers the body—but the "proximate" matter, which is the ensouled body: the body does not exist outside an ensouled body. Body and soul only exist as a living being—an ensouled body—which is not body or soul or a composite of both, as if they were different entities, but an ontological unity. They are not united accidentally. Soul and body are one as form and matter are one, and the actions are from the unity:

> … It is not necessary to ask whether soul and body are one, just as it is not necessary to ask whether the wax and its shape are one, nor generally whether the matter of each thing and that of which it is matter are one. For even if one and being are spoken of in several ways, what is properly so spoken of is the actuality (*On the Soul* II, 1, 412b 6–9 [13, 17]).

They are one at the level of the substance which is the living being, and they are two at the level of substantial principles, but they do not outlive if separated (cf. *On the Soul* I, 1, 413a 5 [13, 17]). In a sense, we have a dualism, but it is not the Cartesian (and probably the emergentist) dualism in which two different substances are accidentally united.

Consequently, the activities of living beings—including human beings—are not of the soul or of the body, they are activities of the whole: "human beings are psychophysical wholes", as Jaworski expresses it ([20], p. 307). David Bostock ([21], p. 97) maintains "the soul is not 'the ghost in the machine' that *makes* the machine work as it does, but is rather the working of the 'machine' itself." Even to think cannot be performed without the body (*On the Soul* I, 1, 403a 10 and 403b 19 [13, 17]). Aristotle affirms: "still to say that the soul gets angry is as if one were to say that the soul weaves or builds a house. Probably it is better not to say that the soul pities, or learns, or thinks, but to say rather that the man with its soul does this things" (*On the Soul* I, 4, 408b 13–15 [13, 17]). Michael Frede explains: "there is just one subject, the animate object, which in virtue of the particular kind of form or soul it has, is capable of all these things [actions]" ([22], p. 97). This conception avoids the problem of mental causation. Frede clarifies: "I do not mean to commit Aristotle to the view that there is no way in which a useful distinction might be drawn which extensionally comes reasonably close to the distinction between mental doings and physical doings" ([22], ibid.).

While avoiding a dualistic view of the human being, Aristotle's hylomorphic conception of the soul as the form of the body allows for two compatible explanations (*On the Soul* 403a 39 -403b 2 [13, 17]):

> … The natural philosopher [the scientist] and the logician [philosopher, psychologist] will in every case offer different definitions, e.g., in answer to the question what is anger. The latter will call it a craving for retaliation, or something of the sort; the former will describe it as a surging of the blood and heat around the heart. The one is describing the matter, the other the form or formula of the essence.

The form is closely related with Aristotle's "cause for the sake of which" (later called, final cause). The final cause is the most excellent perfection to which the form tends for the very fact of being this form. Aristotle emphasizes the role of the form and the final cause because they provide an ontological root to the unity of the human being.

We could be tempted to consider the soul (only) as the organization of the material constituents of the human being. However, as Frede remarks ([22], p. 98), Aristotle's proposal goes beyond this. Aristotle himself argues this point (*On the Soul* I, 4 [13, 17]). Frede indicates: "we do not try to understand the configuration in terms of the material constituents and their properties, but rather the other way round; we try to understand the material constituents and their properties in terms of the form or organization" ([22], p. 99). The body is not simply matter, it is ensouled matter, "we understand why parts, organs, structure, and other bodily processes of animals are as they are when we understand them in psychic terms" (Irwin [18], p. 290): they are teleologically oriented by the form–soul. At the same time, "we correctly believe that psychic states are not reducible to purely material states, because psychic states have a teleological role that resists reduction" (Irwin [18], pp. 291–2).

According to Aristotle's conception, soul is a natural and non-material reality (Irwin speaks of "non-material"). It opens up the door to non-physicalist naturalism. Consequently, it obviously fits into the non-physicalist view of the mind–body question. However, things are not so easy. Aristotle takes a further step in book III 4 of *On the Soul* that introduces conflict into this explanation. This step concerns the intellect or mind that apprehends forms. Aristotle states:

"the mind (*noûs*) is separable" (429b 5) from the body; "as objects [by abstraction] are separable from their matter so also are the corresponding faculties of the mind" (429b 18–23) [13, 17]. The intellect has to be receptive of the form of an object, and thus it has to be immaterial and separable from matter as it is the form from its object. The final consequence is that "when isolated it is its true self and nothing more, and this alone is immortal and everlasting" (*On the Soul*, III, 5, 430b 23–24 [13, 17]). These are some of the most debated passages of Aristotle's work (see Shields [23]). If the correct interpretation is that the argument is deeply flawed or that for one or other reasons we can disregard it (as most Aristotelians think), hylomorphism as a non-dualist and non-physicalist position can still be sustained. But if we take Aristotle's words literally into account we are in problems, for if this part of the soul remains after death, aren't we being strongly dualists when it comes to human beings?

Aristotle's possible proposal of an immortal mind implies a lot of problems. When the human being is alive, the soul is united with the body, constituting an inseparable substance. But then, when the human being passes away, that part of the soul remains immortal, implying that it is separable. However, given its mentioned dependence on the body, it is difficult to conceive its condition after death. To know might be a not exclusively material activity, but it also depends on matter (external senses, imagination); it is an activity of the person. The separable mind cannot interact with the external world separated from the body.

Taking immortality as given implies that "non-materiality" of mind is of a special kind, because it is also separable from matter. There are other non-material realities, like form and accidents, which are non-material but cannot exist without matter. Accidents inhere in substances. Aristotle asserts that "*to on polachos legetai*" (*Metaphysics* IV, 2, 1003a 33 [13])—"being" has several meanings—of which the primary one is substance, but which also includes "weaker" kinds of being. For example, though supported by matter, structures, forms, actions, and thoughts are non-material things, but they are natural. However, they do not exist without matter. Soul as a form falls under this condition but, additionally, it seems that for Aristotle, the human soul includes a non-material subsistent substance. This position looks like an extremely ad-hoc explanation which implies a sort of strong dualism: if this substance which is mind was previously part of the whole, weren't there actually two substances? As Norman Kretzmann ([24], p. 128) remarks, human beings have a "uniquely problematic status among creatures in virtue of the peculiar character of the human soul."

Hylomorphism Re-examined

According to Aristotle, the living human being, like all living beings, is oriented towards ends. This orientation is rooted in the form, and concerns the whole living being. Some of these ends are sought spontaneously, and others more or less consciously. This consciousness is somehow present in animals through their senses, feelings, and desires, while human beings have high levels of consciousness on account of their superior faculties, i.e., mind and will. This teleological characteristic of animals and human beings stemming from their forms (their souls) unifies and identifies them.

Aristotle identifies form with nature and essence (*Physics* II, 1; *Metaphysics* VII, 10 [13]), essence with form (e.g., *Metaphysics* VII, 13 [13]), and form with actuality (e.g., *Metaphysics* VII, 17[13]). As Irwin explicates,

> … In identifying substance with form and form with actuality, Aristotle explains his view of the basic subjects. Their continuity is determined by continuity of form, not by continuity of matter; and form is continuous when the organization, structure and modifications of the matter are explained by the same teleological laws ([18], p. 237).

What is the teleological law governing human life? Aristotle answers this question with the famous *ergon* argument, "the human good proves to be activity of the soul in accord with virtue, and indeed with the best and most complete virtue […] Moreover in a complete life" (*Nicomachean Ethics* I, 7, 1098a 17–19 [13]). In the final chapter

of *Nicomachean Ethics* (X, 7, 1177a 13–23 [13]), he pinpoints this virtue:

> … If happiness is activity in accordance with virtue, it is reasonable that it should be in accordance with the highest virtue; and this will be that of the best thing in us. Whether it be reason (*noûs*) or something else that is this element which is thought to be our natural ruler and guide and to take thought of things noble and divine, whether it be itself also divine or only the most divine element in us, the activity of this in accordance with its proper virtue will be perfect happiness. That this activity is contemplative (*theoretiké*) we have already said.
>
> Now this would seem to be in agreement both with what we have said before and with the truth. For, first, this activity is the best (since not only is reason the best thing in us, but the objects of reason are the best of knowable objects), and second, it is the most continuous, since we can contemplate truth more continuously than we can do anything.

As we have seen, Aristotle could have claimed that the soul—or a part of the soul, i.e., the mind—is immortal because it possesses this contemplative faculty (*On the Soul* III, 4 [13, 17]). This notion would be consistent with the teleological orientation of the human being; the soul is immortal and contemplation remains after death. This is not the case of animals because their soul is not immortal. Aristotle nicely explains:

> … But such a life would be too high for man; for it is not in so far as he is man that he will live so, but in so far as something divine is present in him; and by so much as this is superior to our composite nature is its activity superior to that which is the exercise of the other kind of virtue. If reason (*noûs*) is divine, then, in comparison with man, the life according to it is divine in comparison with human life. But we must not follow those who advise us, being men, to think of human things, and, being mortal, of mortal things, but must, so far as we can, make ourselves immortal, and strain every nerve to live in accordance with the best thing in us; for even if it be small in bulk, much more does it in power and worth surpass everything. […] And what we said before will apply now; that which is proper to each thing is by nature best and most pleasant for each thing; for man, therefore, the life according to reason (*kata tòn noun bíos*) is best and pleasantest, since reason more than anything else is man. This life therefore is also the happiest (*Nicomachean Ethics* X, 7, 1177b 27–1178a 8 [13]).

However, is this activity of a separate kind? Yes and no. It is the activity of this part of the soul of the human being who has now departed from the remote matter, which is no more human remote matter but only dead bones and flesh. Consequently, it is the activity of what remains of the human being after death. There is a suggestive phrase in *Metaphysics* (VII, 10, 1035a 17–18 [13]):

> … For even if the line when divided passes away into its halves, or the man into bones and flesh, it does not follow that they are composed of these as parts of their essence, but rather as matter (*hyles*): and these are parts of the concrete thing, but not also of the form, i.e., of that to which the formula refers.

That is, bones and flesh as only remote matter are not part of the essence: they do not exist outside the ensouled body. Regarding the soul, Aristotle perhaps conceives the part of it that thinks (*nouns)* as separable from matter (*hyle*) (*On The Soul* III, 4, 429b 23 [13, 17]). He wonders why we only think intermittently (III, 4, 430a 6–7 and III, 5, 430a 18 [13, 17]). Mind has potential and active dimensions. Referring to the latter, Aristotle states: "Mind in this sense is separable, impassive and unmixed, since it is essentially an activity" (*On the Soul*, III, 4, 430a 12–18 [13, 17]).

What would happen when we pass away? Mind would remain immortal, detached from matter. What really passes away is the remote matter. The consequence stemming from this isolation from remote matter is that the soul will not continue acquiring knowledge—at least in a knowable way—and will continue possessing only the feelings and the forms that it had previously acquired. Perhaps for Aristotle, in this way—isolated from the potentiality of matter—the remaining part of the human being will be able to accomplish its teleological function: continuously contemplating the forms that it had contemplated (and loved) during its life. This is not clear because, on the one hand it seems that for Aristotle thinking is independent of organs, but on the other hand Aristotle fluctuates about this:

> … In most cases it seems that none of the affections, whether active or passive, can exist apart from the body. This applies to anger, courage, desire and sensation generally, though possibly thinking is an exception. But if this is too a kind of imagination, or at least is dependent upon imagination, even this cannot exist apart from the body (*On the Soul*, I, 1, 403a 6–10 [13, 17]).

On the supposition that the thesis of immortality were truly Aristotelian, we may add that it would not undervalue the relevance of human beings' remote matter, the stuff out of which the ensouled body is built. Death implies a big change for the human being. But "vita mutatur, non tollitur" ("life is changed, not taken away"): though in a different way, the life of the human being continues, following the same teleological orientation. This orientation is what provides the unity that prevents falling into dualism despite death.

Is this *post-mortem* scenario better than the former state? Aristotle would possibly believe that it might be, to a certain extent, because the human being would be now accomplishing its end of contemplating, avoiding the interferences stemming from perception of the external world.

However, one might hypothesize—something that would have been impossible without a Christian inspiration—that once the soul has lived this new life, it could be better for it to readopt its natural or original state as an ensouled body or a soul embodied. In this respect, some interesting passages by Aquinas may prove thought-provoking (on the advantages of Aquinas' version of hylomorphism see Edward Feser [25], specially pages 183–188). These passages will also help discard the strong dualist doubt about the human being, shedding light on the root of its unity:

> …The soul communicates that existence (*esse*) in which it subsists to the corporeal matter, out of which together with the intellectual soul there results unity of existence; so that the existence of the whole composite is also the soul's existence. This is not the case with other non-subsistent forms. For this reason the human soul retains its own existence after the dissolution of the body; whereas this is not the case with other forms (*Summa Theologiae –ST–,* I, q. 76, a 1, ad 5 [26]).

> … To be united to the body belongs to the soul by reason of itself, as it belongs to a light body by reason of itself to be raised up. And as a light body remains light when removed from its proper place, retaining meanwhile an aptitude and an inclination for its proper place, so the human soul retains its proper existence (*esse*) when separated from the body, having an aptitude and a natural inclination to be united to the body (*ST*, I, q. 76, a 1, ad 6 [26]).

> … To solve this difficulty we must consider that as nothing acts except so far as it is actual, the mode of action in every agent follows from its mode of existence (*modum essendi*). Now the soul has one mode of being when in the body, and another when apart from it, its nature remaining always the same; but this does not mean that its union with the body is an accidental thing for, on the contrary, such union belongs to its very nature, just as the nature of a light object is not changed, when it is in its proper place, which is natural to it, and outside its proper place, which is beside its nature (*ST*, I, q. 89, a. 1, corpus [26]).

Aquinas sustains that "form gives existence (esse) to matter" ("forma dates se materiae"; *De ente et essentia*, 4 [27] and *De principiis naturae* 1, 4 [28]); "form is that according to which the thing has being" ("forma sit secundum quam res habet esse", *Summa contra gentiles,* III [29], also see *De Potentia* 7.2 obj. 10 and *ad* 10 [30], *De Anima*, a.6c [31]; on form, *esse*, actuality and the soul, see Dewan [32, 33]): that is, *esse* "comes into" the form–matter composite by way of the form. It is being (existence, *esse*) that grounds the unity of the human being, and *esse* remains in the separated soul, though tending to recover its former state. Like Aristotle, Aquinas believes the soul is not only the form but also the end of the body, which is an organized body. He distinguishes between the matter and the body, which is spiritualized matter, ensouled body. The phenomenological (Scheler, Husserl, Merleau-Ponty) concept of *Leib* (living body), different from *Körper* (physical body), may be very well explained and understood using this metaphysical frame.

For Aquinas, *esse* means the actuality of being, which correlates to the Aristotelian notion of *energeia*. Form, then, brings actuality to the substance. In Aquinas' *Commentary on Aristotle's Physics* (I, 15 [34]), he asserts that "each thing just to this extent is in act, inasmuch as it has form." Besides, in *ST*, he states: "Being belongs to form, which is act" ("*Esse autem per se convenit formae, quae est actus*"; I, 75, 6, corpus [26]), and "the substantial form causes being in act in its subject" ("Forma substantialis causa esse in actu in suo subiecto" *ST* 1.77.6, Corpus [26]). Aquinas states in *De Principiis Naturae*: "forma facit esse in actu, ideo forma dicitur esse

actus" (n. 4 [28]), "id per quod fit actu, scilicet forma" (n. 8), and "materia non habet esse completum nisi per formam" (n. 32 [28]).

Specifically referring to the soul, he clarifies that "the soul is a substantial form; hence, it is necessary that it be the form and the 'act,' not merely of the whole but of every part" ("unde oportet quod sit forma et actus" *ST* 1.76.8, corpus [26]). And on his *Commentary on Aristotle's De Anima* (II, l. VII, 319 [35]), he affirms:

> … The soul is the cause of the living body as the form. (…) [It] is the cause of anything, as the substance, i.e., as the form which is the cause of being. For each being is actual (*est actu*) through the form. But the soul is the cause of being in living things; for they live as means of the soul, and the act of living itself is their existence.

This notion (and reality) of *esse* or *actus essendi* and actuality strengthens the unity expressed by the hylomorphic conception of the ensouled body. The individuality in the case of the human person comes from the form which is an "already" subsistent incomplete substance that provides the *actus essendi* (root of the individuality and identity) to the whole. Stephen Brock ([19], pp. 344–5) explains:

> … Matter *is*—is *in act*—through form; form is in act through itself. This is how they can make for a true ontological unit, one substance: they have one *esse*. Together they constitute the whole, proportionate subject of this *esse*. Nonetheless, the *esse* "reaches" the matter only through the form. And so it is *conceivable* that, prior [ontologically] to its reaching and actualizing the matter, the *esse* even be "seated" in the form, as in a partial subject. In that case, the matter may condition many of the form's effects, but it will not condition the form just in itself, in its own *being in act*.

Additionally, the intellectual character of the human soul brings about its immortality and leaves the door open for a hypothetical reunification with remote matter by way of an external agent. This is also explained by Aquinas. This intellectual character shows that the form which is the human soul is not only a form providing existence to the human being but has existence in itself: it is *habens esse* (*De Anima* a.18c [31], and see also *ST* I, q. 76, a.1 ad 5 [26]) and is consequently incorruptible. It is a form composed of *esse* and *essentia*, acting respectively as act and potency, but simply form without matter (*De Anima* a.6c [31]): it is incomplete as form, but subsistent.

Eleonore Stump's [questionable, see, e.g. Pasnau [36], p. 363] interpretation of Aquinas's thought about the *post-mortem* human situation clearly evokes what Aristotle seems to hint about this (see above). She explains that for Aquinas constitution is not identity, and thus, a human being is not identical to the metaphysical parts that constitute him. She concludes: "On Aquinas's view, a human being can survive even the loss of his entire body, when the substantial form [the soul] remains" ([37], p. 461). This does not mean that the person is the soul but "although a person is not identical to his soul, the existence of the soul is sufficient for the existence of a person" ([37], p. 463).

Conclusion: Linking Aristotle's Hylomorphic Conception of the Human Being with Contemporary Mind–Body Theories

Some Aristotelian scholars have tried to identify links between Aristotle's psychology and contemporary mind–body positions. One cannot but agree with Irwin when he states that "the extreme, eliminative materialist believes that there are no genuine formal causes" ([18], p. 293). Consequently, for an eliminative materialist position, mind as conceived by Aristotle would not exist. Irwin adds that a less extreme, reductive materialist approach would consider formal causes but identify them with matter. Aristotle, on his part, conceives the soul as the ensouled body—because the living being is a unity—but he distinguishes it from the remote body. Living beings cannot be reduced to their remote matter: they are more than that.

Hence, can we conclude that Aristotle is a "non-reductive materialist"? He is clearly non-reductive, but he is materialist in only one sense: when considering the human being as a united whole. This is the central Aristotelian conception

of the living being. However, we should bear in mind that one Aristotelian principle (*archai*)—form—is non-material because by its definition it "informs" matter.

Furthermore, Aristotle's position is close but not compatible with emergentism, because in his opinion, *psyche*, though more than simple materials, is not emergent, in the sense of being generated by them; moreover, it is the principle that determines what the living being is, thus configuring matter. As Jaworski asserts, "unlike classic emergentism, hylomorphism denies that emergent properties are generated or produced by lower-level processes or states. Higher-level phenomena are instead ways in which lower-level occurrences are structured, and structures in general are not generated or produced by the things they structure" ([20], p. 160). The human being is born a complete human being from the very beginning of its conception, with all its properties that have not emerged. In addition, putting apart the idiosyncratic case of the human being, life does not emerge from inert stuff: the minimum original unit for the development of life is already organic. Thomas Nagel ([38], p. 6) states:

> ... It is prima facie highly implausible that life as we know it is the result of a sequence of physical accidents together with the mechanism of natural selection. We are expected to abandon this näive response, not in favour of a fully worked out physical/chemical explanation but in favour of an alternative that is really a schema for explanation.

For Nagel, "a teleological explanation comes to seem more eligible" ([38], p. 118). We need "a cosmic predisposition for the formation of life" ([38], p. 123). He cites scientists such as Francis Crick, Jacques Monod, and Ernest Mayr, for whom the origin of life from chemical evolution from a dead environment would be a miracle. For Nagel, natural teleology would be a plausible alternative ([38], p. 124).

In addition, as Derek Jeffreys [39] notes quoting Aquinas, emergentism falls into a metaphysical mistake concerning the notion of causality: "nothing can act beyond its species, since the cause must always be more powerful than its effect" (*ST* I-II, q. 112, Ic [26]). Finally, it is Madden's argument: "thoughts cannot emerge from neurophysiological processes, because they have universal content" ([40], p. 267). Pasnau ([36], pp. 355–357) shows the difficulties of this argument being used for trying to prove the independence of the mind concerning thinking.

My conclusion is that Aristotle's hylomorphism provides an explanation of the mind–body relationship that does not fall into reductionism or a Cartesian strong dualism (see Jaworski [21], pp. 309 and 353). We can argue for it as an inference for the best explanation ([21], p. 270). I cannot refrain from quoting a suggestive passage of Pasnau ([36], p. 364):

> ... That we still think of this as a problem [the "problem" of mind–body interaction] is rather curious, since it is a historical artifact of a few decades in the seventeenth century when philosophers like Descartes formed the conviction that all causation at the material level occurs through mechanical impact, and at the same time wanted to treat the mind as immaterial and still interacting with bodies. Once Cartesian mechanism was abandoned in favor of a broader conception of forces as the causal agents in nature, the mind can be seen as just one among many forces in the natural world. And this is the older Aristotelian perspective as well, inasmuch as our intellectual powers are just forms—powers of the soul—that can act in nature just as other forms, accidental and substantial, act in nature.

Hylomorphism applies clearly and not problematically in the case of non-human living beings. However, it is not undoubted when it comes to the human being. The immortality of its soul stemming from its intellectual capacity poses a question mark: isn't this a dualist position? After death, two different substantial entities seem to remain: the soul and an accidental ensemble of corrupted substances, while before death, this plurality constitutes a unity. The thesis in this paper has been that, indeed, two different substantial entities remain after death: one is the remote matter, which is no longer human, and the other is the soul of the same human being with its proper being and identity, though incomplete.

I cannot tell if Aristotle would have agreed with this. But I have done my best in this thesis to maintain his position against dualism while sustaining soul's immortality. Aquinas has certainly assisted in this task. In addition, regardless of his

view on the soul's immortality, I conclude that Aristotle's position constitutes a satisfactory non-physicalist explanation of the mind, where the soul is a form which is non-material and natural.

Acknowledgments I express my gratitude to William Jaworski, Juan José Sanguineti, Stephen Brock, Ivana Anton Mlinar, Miguel Verstraete, Ceferino Muñoz, Santiago Gelonch and Juan Francisco Franck for useful comments on previous versions of this paper.

References

1. Bourget D, Chalmers DJ. What do philosophers believe? Philos Stud. 2018;170:465–500.
2. Stoljar D. Physicalism. Stanford encyclopedia of philosophy, 2015. http://plato.stanford.edu/entries/physicalism/. Accessed 29 Jan 2015.
3. Putnam H. The nature of mental states. In: Mind, language and reality: philosophical papers, vol. II. Cambridge: Cambridge University Press; 1975. p. 429–40.
4. Davidson D. Mental events. Reprinted in Davidson, editor. Essays on actions and events. Oxford: Clarendon Press; 1980, p. 207–85.
5. McLaughlin B, Bennett K. Supervenience, Stanford encyclopedia of philosophy, 2011. http://plato.stanford.edu/entries/supervenience/. Accesed 29 Jan 2015.
6. O'Connor T, Hong Yu Wong. Emergent properties, Stanford encyclopedia of philosophy; 2012. On line. http://plato.stanford.edu/entries/properties-emergent/. Accesed 30 Jan 2015.
7. Hasker W. The emergent self. Ithaca: Cornell University Press; 1999.
8. Varela F, Thompson E. The embodied mind. Cambridge: MIT Press; 1991.
9. Thompson E. Life and mind: from autopoiesis to neurophenomenology. A tribute to Francisco Varela. Phenomenol Cogn Sci. 2004;3:381–98.
10. Di Paolo EA. Autopoiesis, adaptability, teleology, agency. Phenomenol Cogn Sci. 2005;4:429–52.
11. Mossio M, Saborido C, Moreno A. An organizational account of biological functions. Br J Philos Sci. 2009;60(4):813–41.
12. Auletta G, Ellis GFR, Jaeger L. Top-down causation by information control: from a philosophical problem to a scientific research programme. J R Soc Interface. 2008;5:1159–72.
13. Aristotle, The complete works of Aristotle. The Revised Oxford Translation, Barnes J, editor. Princeton University Press, 6th printing with corrections; 1995.
14. Cohen SM. Aristotle's metaphysics. Stanford encyclopedia of philosophy; 2012. http://plato.stanford.edu/entries/aristotle-metaphysics/#SubMatSub. Accessed 2 Feb 2015.
15. Lang D. The Thomistic doctrine of prime matter. Laval théologique et philosophique. 1998;54(2): 367–85.
16. Marmodoro A. Aristotle's Hylomorphism without reconditioning. Philos Inquiry. 2013;36(1–2):5–18.
17. Aristotle, On the soul, trans. Hett WS, Loeb Classical. Cambridge: Harvard University Press; 1957.
18. Irwin TW. Aristotle's first principles. Oxford: Clarendon Press; 1990.
19. Brock S. Spirituality and hylemorphism. In: Doctor Communis, Proceedings of the XII Plenary Session, Vatican City; 2013, p. 340–45.
20. Jaworski W. Philosophy of mind. In: A comprehensive introduction. Malden/Oxford: Willey-Blackwell; 2011.
21. Bostock D. Space, time, matter and form. Essays on Aristotle's physics. Oxford: Clarendon Press; 2006.
22. Frede M. On Aristotle's conception of the soul. In: Nussbaum M, Rorty AO, editors. Essays on Aristotle's De Anima. Oxford: Oxford University Press; 1995. p. 93–107.
23. Shields C. The active mind of De Anima iii 5. Stanford encyclopedia of philosophy. 2010. http://plato.stanford.edu/entries/aristotle-psychology/active-mind.html. Accessed 29 Jan 2015.
24. Kretzmann N. Philosophy of mind. In: Kretzmann N, Stump E, editors. The Cambridge companion to Aquinas. Cambridge: Cambridge University Press; 1993. p. 128–59.
25. Feser E. Philosophy of mind. London: One World Publications; 2006.
26. Aquinas T. Summa Theologiae. Torino: Marietti; 1950.
27. Aquinas T. De ente et essentia. http://legacy.fordham.edu/halsall/basis/aquinas-esse.asp. Accessed 23 Mar 2015.
28. Aquinas T. De Principiis Naturae. http://dhspriory.org/thomas/DePrincNaturae.htm. Accessed 23 Mar 2015.
29. Aquinas T. Summa contra gentiles. Torino: Marietti; 1946.
30. Aquinas T. De Potentia. In: Quaestiones Disputatae, vol. II. Torino: Marietti; 1949. p. 7–83.
31. Aquinas T. De Anima. In: Quaestiones Disputatae, vol. II. Torino: Marietti; 1949. p. 276–362.
32. Dewan L. St. Thomas, form, and incorruptibility. In: Dewan L, editor. Form and being. Studies in Thomistic metaphysics. Washington, D.C.: The Catholic University of America Press; 2006. p. 175–87.
33. Dewan L. Saint Thomas and form as something divine in things. Milwaukee: Marquette University Press; 2007.
34. Aquinas T. In octo libros Physicorum Aristotelis expositio. Torino: Marietti; 1950.
35. Aquinas T. The Commentary of St. Thomas Aquinas on Aristotle's treatise on the soul, translated by Kocourek RA. St. Paul: The College of Saint Thomas; 1946. On line. https://ia802704.us.archive.org/11/items/CommentaryOfSt.ThomasAquinasOnAristotlesTreatiseOnTheSoul.Tanslated/kocourek.pdf. Accessed 4 Apr 2015.

36. Pasnau R. Philosophy of mind and human nature. In: Davis B, Stump E, editors. The Oxford handbook of Aquinas. Oxford/New York: Oxford University Press; 2012. p. 348–68.
37. Stump E. Resurrection and the separated soul. In: Davis B, Stump E, editors. The Oxford handbook of Aquinas. Oxford/New York: Oxford University Press; 2012. p. 458–66.
38. Nagel T. Mind and cosmos. Oxford/New York: Oxford University Press; 2012.
39. Jeffreys DS. The soul is alive and well: non-reductive physicalism and emergent mental properties. Theol Sci. 2004;2(2):205–85.
40. Madden JD. Mind, matter and nature. Washington D.C.: The Catholic University of America Press; 2013.

5 Aristotle's Concept of the Soul and the Link Between Mind and Body in Contemporary Philosophy

Jorge Martínez Barrera

Abstract

The purpose of this paper is to examine the true extent of neurological explanations of mental events and human actions. There is no doubt that advances in neuroscience are posing challenges that go beyond the fields of biology and physiology. The increasingly thorough knowledge of brain topography is very close to identifying the areas involved in the decision-making processes that precede human actions. This has led to the assumption, by some physicalist currents, that a full explanation of mental acts by investigating neurological conditions is possible. However, this assumption implies the superfluity of regulatory orders, and that human actions could not be implemented differently than as provided by the neurological structures. Freedom in this case is a senseless concept. The possibility is suggested, towards the end of this chapter, of going to the *Treatise on the Soul*, by Aristotle, as a more appropriate source to transcend physicalist reductionism. The latter has two limitations: one, to reduce the explanation of mental acts and free decisions to its neurological conditions; another, that of not being able to open up to other explanatory possibilities that go beyond the determinism of neurosciences. Aristotle, we suggest, provides the elements to overcome these difficulties.

Keywords

Mind • Body • Soul • Free Will

The interest in the current mind–body problem across a great portion of contemporary philosophy needs no justification. It is raised by the discoveries made concerning the brain's anatomy and physiology and their relevance on the brain's functioning in mental acts. Not to simplify excessively, neuroscience is supposed to find the fundamental explanation of the mind in relation to cerebral organisation and even with regard to

J.M. Barrera, PhD
Facultad de Filosofía,
Pontificia Universidad Católica de Chile, Santiago, Chile
e-mail: jorge.martinezbarrera6@gmail.com

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_5

human acts. Neuroscience promotes a theory of causality whose main objective is to displace explanatory prototypes that cannot take into account those mental acts that respond to the demands of the current scientific conceptions of the world as an element of material things.

This void with regard to the explanation of the mind—which is supposed to be filled when thorough brain topography may be reached—has been a parallel concept to that which considers the soul as an obsolete concept. Renowned scientists such as Francis Crick maintain that the discovery of the DNA helix means that we can manage without a supernatural reality, such as the soul, as stated in a famous article [1]. "To understand ourselves, we need to know how nervous cells behave and interact. We are nothing but a package of neurons" ([2], p. 3). According to Crick, the soul would connect us with a religious realm that is not at all connected to our scientific view of the world. In fact, the term 'soul' has been replaced by 'consciousness', which has also been considered as an equivalent of the mind, as a product of neural activity. Many problems arise from this position from a philosophical point of view. For philosophy, at least, this has already been revealed well below cognitive neuroscience's questionings. One of the major difficulties is based on the existence of liberty. If every human act is featured as one of free will, there would then exist an impassable incompatibility between the liberty of such acts and their cause, which according to neuroscience's testimony is the product of complex electrochemical combinations.

In Michael Gazzaniga's works, there is room for a new discipline, neuroethics, which goes far beyond its original meaning [3]. The neuroethics concept was first introduced by W. Safire on 10 July 2003 in the *New York Times*. Safire coined it in referring to the reflection about the licit and illicit in investigations of the human brain, though Gazzaniga extends it to considerations of normality, sickness, mortality, way of life, and philosophy of life with regard to our knowledge about cerebral mechanisms, especially since all our acts invariably remit to a neurochemical base. There is an evident difficulty when it comes to reconciling this *physicalism* with the certainty that we are free that we feel at the time of decision. Jaegwon Kim tries to harmonise both sides while affirming that the intentional mental cognitive activity linked to behaviour is reducible to a neural factor, whereas *qualia* [4] is not. In this way, according to Kim, *physicalism* would be 'manqué, but not by much', mistaken but not by much.

Certain argumentative acrobatics cannot be denied in some of the works that try to explain free will directed to the cutback on mental acts on its neural conditions. Conclusions from Gazzaniga or Kim go like this: brains are automatic, but will is free. This reductionism leads to the mind being placed out in the physical world; it has been addressed as a friendly philosophy for neuroscience, which denies neither freedom nor the mind's physical character. This might seem like squaring the circle, but in fact that is what the effort of philosophers of the mind is about. John Searle has exhaustively taken care of this matter. The topic of the mind is approached in *Minds, Brain and Science* [5], and the subject of free will, in *Liberté et neurobiology* [6].

According to Searle, the mind consists of a sequence of thoughts, sensations, and conscious and unconscious experiences which sum up our mental life. It is necessary to get rid of the ghost of old philosophical theories which occupy our concept of what is mental. Our main concern consists of a personal representation of common sense as free and thoughtful beings, which does not match with the one in which science tells us that the world is made up of physical particles neither with mind nor with meaning. Above all, the solution might be somewhat easier than we think, since we are determined to find an exit provided by an obsolete philosophy and an outdated vocabulary such as the one used during the seventeenth century. Of course, it is Descartes who Searle is talking about. To Descartes, the issue was how we could deal with the link between two different realities or such different genres. Physical science's success has brought up the relocation of the essence of mind, especially the fact of subjective

mental states and that these are as real and irreducible as any other part of the universe.

Four different aspects of mental states are identified by Searle, which seem impossible to articulate from our scientific idea of the world as something made up by material things.

The most important feature is *consciousness.* Consciousness, he states, "is the central fact of human existence, due to the fact that without it all the other aspects of our existence, which are specifically human—language, love, humour—would be impossible".

The second feature is what philosophers call "intentionality". Through it, our mental states relate to objects and refer to things which are different from mental states. The area of intentionality is quite vast. It condenses belief, wish, hope, fear, love, hatred, lust, loathing, shame, pride, irritability, fun and all those mental states, whether conscious or not, related to a world beyond the mind itself.

The third feature of the mind seems hard to harmonise with the subjectivity of mental states. This undeniable subjectivity is marked by evident facts: No one can feel another's pain. That said, how is it possible to deal with the subjectivity of mental states and the scientific view of reality as something objective?

Finally, there is a fourth issue about mental causation. We all believe that our thoughts have a causal impact on the physical world. If someone decides to raise their arm, the arm is raised, reminds Searle. If our thoughts have a non-physical nature, how is it possible that they have a physical effect? How come a mental issue has a physical influence? With a slight irony, Searle states: "Are we supposed to think that thoughts may wind up the axons and shake dendrites or even strain through the cellular membrane and attack the cell's nucleus?"

A philosophy of the mind which is concerned with seeking harmony between body and mind cannot neglect these four issues. The good news is that the solution to this matter is simpler than one might think, says Searle. Actually, our only difficulty is we keep subscribing to insufficient philosophical theories. Therefore, Searle's proposals to solve the mind–body conflict are the following:

> Mental phenomena, each and every one of them, whether conscious or not, related to sight, hearing, pain, tickles, itchiness, thoughts, all our mental life are, indeed, caused by processes occurring in the brain.

The explanation of this issue consists of a detailed description of the physiology of pain, taking an example on mental phenomena. This physiology condenses those processes occurring in the brain. This complements Searle's previous thesis:

> Pain and other mental phenomena are just features of the brain (and perhaps also from the rest of the central nervous system) ([7], pp. 15–21).

At this point, Searle addresses the cause of mental acts from another perspective. He tries deconstructing quite a rooted idea, namely, the one of material physical events being the cause of immaterial events. This is, in fact, a mistake, he says. To overcome this apparent difficulty, Searle proposes a more adequate concept of causation while observing other kinds of causal relations in nature. A common distinction in physics is the one about micro- and macro-properties in small- and large-scale systems. For example, if we take a glass of water, we must deal with the water's micro-particles which are constituted by atoms and molecules. These features explain the liquidity of water. The nature of the interaction between water molecules has a microscopic expression, just as the interaction of solid molecules explains the features of that solid state at the touch of a hand. It could be said, then, that the superficial feature is caused by the conduct of the microelement, and at the same time it is formed by a microelement system. There is a cause-and-effect relationship, but at the same time these superficial traits are only features a level above the system itself whose micro-level behaviour causes such features. In the case of any objection against it, Searle argues that scientific progress precisely consists of the fact of an expression originally defining itself in terms of superficial traits which are accessible to the senses, and being

subsequently definable in terms of a microstructure which causes these superficial traits. This provides a fine explanatory model for the risky business between the mind and the brain. It could be said, then, that mental phenomena are caused by processes which take place at a neural level in the brain and, at the same time, they are presented in the system itself constituted by neurons, which seem a first-level equivalent to other physical phenomena such as micro-particles. In this respect, Searle cannot escape from reductionism's objection: why would anyone assume mental acts are not being reduced to physical conditions? As Thomas Nagel points out: "Reduction is the analysis of something identified at a level of description, in terms of another and more fundamental level of description which leads us to think that the first one is nothing more than the second one: water can be described as H_2O molecules, heat as molecular movement, or light as electromagnetic radiation" ([8], p. 128).

Searle's response to these objections, at least in quoted texts, consists of two arguments: First, the need to find an explanation of the kind of cause that produces mental acts, and how it is connected to neuroscience progress. The second one, which complements the first one, is the argument of behaviour or processes at a microlevel as the real explanation of behaviour in observable acts. In this way, quoting Searle: "There are no logical or philosophical obstacles, neither metaphysical, to account for the relation between the mind and the brain in terms of being completely familiar to us based on nature. There is nothing more common in nature than superficial traits of a phenomenon being caused and carried out in a microstructure, and those are exactly the kind of relations exhibited on the link between the mind and the brain" ([5], p. 27).

Then the question follows: how is it possible that mental acts may cause physical effects? How is it possible that something that lacks importance and is so ethereal may materialise in an action? Searle states then that thought neither lacks importance nor it is ethereal. When a thought is produced, there is an evident cerebral activity which causes physical movement through physiological processes. Could the mind be compared to a computer, then? Searle says no, due to the fact that a computer is just a *syntactic* device, while the mind is also a *semantic* one.

Just to summarise, Searle emphasises that mental states are biological phenomena. Consciousness, intentionality, and mental causation are all part of our biological history, alongside physical development, reproduction, bile secretion, and digestion.

This is the position of the physicalist mind philosopher. There are different positions for every specific topic, and Searle's ideas represent a vast part of what has been written about the links between brain and mind [9]. The first conclusion about this matter coincides with neurologist Arnaldo Benini, who without turning his back on a physicalist perspective, states that neuroscience's optimism—meaning that the impressive amount of data gathered about the brain leads one to believe that science might one day clear out the consciousness enigma—neglects the fact that the affected subject agrees with the organ that leads the study—i.e., the brain itself [10]. Benini quotes, in turn, Von Hayek who states that no explanatory agent may explain objects of its own nature or at a similar level of complexity, therefore the human brain will never be able to explain its own operations. However, one of the greatest difficulties for physicalism—neuroscience's reductionism—consists in explaining the permanence of an autobiographical consciousness: to recognise ourselves as subjects that remain in time, while the matter which builds us up renews itself every 3 months.

With regard to the matter of liberty, there are ways of considering it:

If decisions are made by the brain, which is a physical object which obeys physical laws, then will is not free. In what measure are we responsible for our acts if we are bound to do what our cerebral mechanisms impose on us? Benini states that the mind and brain's identities imply that we are not able to do what we want, as the illusion we imagine, but that we want what we do. The illusion of being the architect of our own destiny is part of the neurological mechanisms of choice.

In *Liberté et Neurobiologie,* Searle opens up a non-deterministic possibility in the explanation

of human acts, without giving up the material character of the origin of our mental acts. The possibility opens up through the twentieth -century progress in physics, when nature was discovered not to be entirely deterministic. In fact, the usual causality theories have made us think that everything that happens in our lives is the result of previous causal conditions. A cause, Searle states, is prior to its object, which seems obvious, though that anticipation might lead us to think it is also temporal, which is not obvious at all. This model of temporal anticipation is the one to be shaken by quantum physics, which introduced non-deterministic explanations to particle behaviour. The issue with literal extrapolation of a causal model of quantum physics to the brain–mind issue, and especially to the free will issue, is that in the world of particles, the only possibility to open up towards a non-deterministic causality consists of the inclusion of *chance*. It is certainly a problem to sustain the parallelism of the hypothesis according to which fate guides the dynamism of the basic structures of the universe, and the hypothesis that our properly human acts are free. We cannot equate fate with liberty. We cannot say that our free acts are given by chance. Searle states, anyhow, that "a certain number of attempts wish to explain consciousness and even free will in terms of quantum mechanics" ([11], p.21). In any case, the value of resource to quantum physics is in the fact that it is possible to introduce an explanatory model of human liberty without implying a resignation to the central hypothesis of mental acts, despite the fact of the deficiency that Searle himself confers the given hypothesis.

The problem seems to linger, however, due to the fact that Searle recognises a subjective nature in consciousness, which possesses an unyielding first-person ontology subjected to a third-person or objective ontology. If it is already hard enough to define consciousness as a brain feature, it is because of our dualist tradition and because if consciousness is assumed to be unyielding neural behaviour, then it should be added to that given neural behaviour. So then, the consciousness ontology in the first person is what prevents us accepting the hypothesis that consciousness might be added to the biological microstructures of the brain. In this way, Searle makes a case by stating that his explanation has nothing to do with an ontological reductionism; thus, he does not discuss ontology in the consciousness's first person. However, a causal reduction must be admitted: the causal power of consciousness cannot be extended beyond the power of neurobiological structures. It seems, then, we need to start from scratch, as it is impossible to escape from the notion of free will as a neurobiological matter, as Searle states:

> "(…) consciousness is a superior or systemic feature of the brain, caused by inferior elements such as neurons and synapses. I have already emphasised that the philosophical solution to the traditional matter about the link between body and mind consists in highlighting that all our conscious states are superior or systemic traits of the brain. At the same time, they are caused by inferior micro processes that are produced within it. At the level of the system, there is consciousness, intentionality, decision, and intention. At a micro level, there are neurons, synapses and neurotransmitters. The microelements' behaviour that build up the system produces the system's features" ([11], pp. 43–44).

The conclusion drawn up to Searle's argumentation might be put in the following terms: consciousness—or the mind, to be more exact—is not material itself. The mind is nothing but "the state in which the system of neurons is found, in the same way that solidity is nothing but the state in which the system of molecules is found" ([11], p. 56). To ask then if consciousness is or not material does not make any sense, much as asking whether the solidity of a metal bar is material. This ingenious explanation lets Searle escape from the schematic assumptions about the two combinations of causes, consciousness and neurons. What there is, in turn, is a unique combination described in different levels. The description of the mind as a state is important, since that would make of it an epiphenomenon of neurochemical states. Searle denies this through other arguments whose strength greatly resembles a word play.

Marcelo Boeri has already stated, at the beginning of his striking article previously quoted, that in favour of a practical philosophy Aristotle has

been rediscovered. Aristotle has also been mentioned by some of the contemporary philosophers who reflect upon this matter.

The reference to Aristotle would then be structured upon some major points, based on a possible answer for those difficulties laid out by physicalism or even by Searle himself. What Aristotle describes as *the soul (psyché)* is what will be dealt with. It is a crucial matter, as it is the exact matter which seems to have been excommunicated from neuroscience's language. Based upon this, the neuroscience's evidence about the nature of the mind will be superficially reviewed, as it is in itself a cause of physical acts. This might lead us to outline an explanatory possibility of the mind and mental facts based upon non-physicalist terms.

Let's examine an excerpt from *On the soul* whose importance cannot be denied (the text is on 407b 13 ss.)

> The view we have just been examining, in company with most theories about the soul, involves the following absurdity: they all join the soul to a body, or place it in a body, without adding any specification of the reason of their union, or of the bodily conditions required for it. Yet such explanation can scarcely be omitted (…). All, however, that these thinkers do is to describe the specific characteristics of the soul; they do not try to determine anything about the body which is to contain it, as if it were possible, as in the Pythagorean myths, that any soul could be clothed in any body—an absurd view, for each body seems to have a form and shape of its own. It is as absurd as to say that the art of carpentry could embody itself in flutes; each art must use its tools, each soul its body [12].

On his predecessors' theories, Aristotle makes a significant turn: it is not about explaining the soul, at least not in the way they have done it, as if the soul were a vital attachment of the body, or a substance added to another one. It is about explaining the reason why the body is what it is, that is to say, what makes a body be what it really is. It is about finding an answer to the question why the body is an organic element. It is not about finding how the carpenter's art gets into flutes, but to explain why flutes are what they are, not only what they are but why they are how they are. For that, the carpenter's art (who is also a bit of a musician) must be admitted to be a previous stage to the production of the actual flutes.

The problem arises not to give a reason as to what the soul is. The problem is not about the ontology of the mind or soul, or to go into the physicalist mode, in accepting its alleged immateriality starting from the physical data about the neurological structure. Aristotle inverts the question: how is it possible that there is a body with such characteristics? For a contemporary reference: how is it possible that such an extremely complex organisation as the human brain exists, and that it is widely developed during the process of embryonic development? What presides over biomolecular, cytological, histological, and physiological organic dynamics, which is oriented towards an extraordinary somatic result, namely, not just the body in its completeness, but towards the most complex organ of all, the brain? Gathering all evidence, if there is an ontogenesis principle and a *fortiori* about neurogenesis, this principle would become a cause whose anticipation cannot be contradicted. This principle might be the soul or the mind, as it is clear that it is prior to what causes it and that it cannot be the result of a somatic complexity; therefore what needs to be described is that complexity. It is inevitable that we address the existence of an anterior organisational scheme, with a causal power over what is potentially drawn to be a body or a determined organ. The cause of the mind or the soul cannot be explained without referring to a physical influence, from a neuroscientific point of view. To sum up, the problem is the mind, according to neuroscience. To Aristotle, the problem is the body.

The fact is that the nature of the mind is not Aristotle's problem; it is not an impediment for him to have an idea about it which is, indeed, not compatible with neuroscience. Physicalism, at least in Searle and Antonio Damasio's school of thought, does not guarantee an ontological rule with regard to the mind. Searle addresses the mind (consciousness) as a state. Damasio, on the other hand, states:

> (…) to solve the consciousness problem is to discover the biological support of the human ability to build up, not only the mental patterns of an object,

> but the mental patterns that transmit, automatically or naturally, the sensation of *self* in the act of discovering. Consciousness, as we usually see it, is a merged mental pattern where the object and *the self* mix up.

Further on:

> Consciousness is a private phenomenon, in the first person, which occurs as part of an intimate and own process that we call mind ([13], pp. 27–29).

Consciousness does not own a clear entity. It is a result and a cause of something. In turn, the mind or the soul owns a substantial entity according to Aristotle. He defines it as a substance in the sense of the idea of the body of a determined living organism:

> Now given that there are bodies of such and such a kind, viz. having life, the soul cannot be a body; for the body is the subject or matter, not what is attributed to it. Hence the soul must be a substance in the sense of the form of a natural body having life potentially within it [De anima 412a 17 - 21].

The soul is a substantial organisational scheme. It is not a result of anything else. It is on its own a cause of the body, just like the art of carving flutes cannot be a follow-up of the final flute. The explanation of human embryological neurology addresses just a tiny bit of the problem. The process of the brain's formation cannot be denied, yet it is essential to determine what guides this formation and why.

It is clear that this previous organisational scheme does not have a physical causation: it is not an electric current which produces visible results if the somatic potentiality permits it. However, this does not mean that physical causality has no room, hence it contacts the potentially organic body and organises it. This gives an idea of 'energy', or *en-ergon*, a metaphysical concept which also addresses a teleological dimension such as entelechy, highlighting its dynamic aspect.

Neuroscience, even with its great progress on physiological cerebral description, and its links with other sciences, has not been able to answer the big question, mainly because it has not been formulated. The big question, with Aristotle's help: what is the reason why the brain is what it is? Neurological description is a long way from exhausting the causal possibilities.

The question needs a level of causal explanation and cannot be drawn into self-reference. Physicalism starts off with a vast quantity of physical data, which do not amount too much with regard to epistemic consistency in an extra-physicalist explanatory dimension. The empirical argumentations need not to be dismissed: naturally, the mismatch between a purely physical conception about the mind and the existence of free human acts needs to be examined. The evidence provided by metaphysical explanation is not comparable in its character to the one provided by physics; however, this may not be a reason to omit information about it, given the implicit seriousness of the acceptance of the physicalist model. A coherent physicalism must restrict deontic expressions about what is human to ethology or perhaps to neuroethics, assessed by Benini:

- The moral sense is linked to the morphology and physiology of cerebral centres which produce and transmit it.
- The sense of good and evil springs up with the evolution of the brain, so that morality would become an ensemble of moral instincts. A kind of universal moral grammar, comparable to the innate consciousness of mathematics or the universal grammar of language described by Chomsky, natural to all human beings.
- The brain creates beliefs.
- Beliefs and religion would constitute a sociobiological aspect of human culture and their main purpose would be safeguarding life itself; etc. ([10], pp. 67–68)

To attribute a physical causal power to the mind is undeniable, something that Descartes had already dealt with and which he wanted to address by creating a link between the body and the pineal gland. The Cartesian solution was mistaken, since the problem remains unresolved, as we still need to ask ourselves how is it possible that the mind, being an immaterial substance, may cause an actual action on a material gland. However, the given difficulty remains only if we

stay on the etiological scheme, proper to physicalism. To Aristotle there is no doubt about the mind (or soul) having causal power on the body. This being such an intricate matter does not mean we need to give up the immateriality of it, as Aristotelian theory of causality does not necessarily imply physical contact either with effect or with a chronological anticipation.

The physicalist *cul-de-sac*, or its refined expressions such as Searle's, may be based upon the type of causal evidence expected from the nature of the mind. This theory favours a resignation to factual evidence, rather than admitting its serious limitations for giving account of the reluctance of its reduction to a physical fact, assuming the lack of another level of explanation. The topic of the link between the mind and the soul in Aristotle's work has not been mentioned. This is a well-known topic, especially after F. Nuyens' work which might be summarised as follows: how is it possible that the mind, being immortal and eternal, might be part of a somatic organisational plan of a body which is not immortal? If the mind were part of this plan, it should then transmit immortality to that organisational plan. For this reason, the mind seems to be another kind of substance for Aristotle. It seems to be detached from the soul, in the sense of not being part of an organisational plan of any kind of organ. This issue was resolved by Thomas Aquinas and it is not a matter of discussion for neuroscience, since the concept of the soul and all discussions about it have been completely removed [13].

In order to conclude this chapter, it must be stated that physicalist neurosciences lead us to a second level of reductionism. The first one portrays the mind as a product of neurological organisation. The second one, even more worrisome, could be addressed as a methodological reductionism, which implies the reduction of any kind of scientific knowledge to a very specific epistemological paradigm which seeks a specific kind of evidence. However, this leads us to serious conceptual fragilities. Physicalist neuroscience is based upon the hope of a self-made promise by assuming that by obtaining an exhaustive cerebral map, the inconsistencies will disappear. This methodological reductionism has extrapolated what we might call the functioning conditions or execution of human acts, to the causality realm. A condition is not necessarily a cause. In the theory of the causality realm, there is no reason not to assume unknown causes or even unknowable ones in the field of neuroscience and in a vast part of the contemporary philosophy of the mind, always overwhelmed by neurological discoveries. The physical account resource should make room for an explanation beyond itself, that is, a metaphysical one.

In this way, the revival of *On the soul*, by Aristotle, where neuroscientific data might be added to complement a review on the theory of causality, would certainly lead us to a provocative path in the realm of the philosophy of the mind.

References

1. http://edant.clarin.com/diario/2003/03/11/s-03401.htm.
2. Crick F. The astonishing hypothesis. The scientific search for the soul. London/New York: Touchstone; 1995.
3. Gazzaniga M. The ethical brain. New York/Washington, DC: Dana Press; 2005. Id.: Human: the science behind what makes us unique. New York: Harper Collins; 2008.
4. Kim J. Physicalism, or something near enough. Princeton/Oxford: Princeton University Press; 2005.
5. Searle J. Minds, brains and science. 1984 Reith Lectures. Harvard University Press; 1984 (Spanish Edition: Mentes, cerebros y ciencia. Madrid: Ediciones Cátedra; 2001. All quotes of this work are from this edition).
6. Searle J. Liberté et neurobiologie. Réflexions sur le libre arbitre, le langage et le pouvoir politique. Paris: Bernard Grasset; 2004.
7. Nagel, T. Other minds. Critical essays 1969–1994. New York/Oxford: Oxford University Press; 1995 (Spanish Edition: Otrasmentes. Ensayos críticos 1969–1994. Barcelona: Gedisa Editorial; 2000. Quotes are from this edition).
8. See: Boeri M. "A propósito del alcance de las explicaciones funcionalistas de la psicología aristotélica y del carácter causal del alma", *in* ELENCHOS. Rivista di studi sul pensiero antico. 2009;(1):53–97.
9. Benini A. Che cosa sono io. Il cervello alla recerca di sè stesso. Milano: Garzanti; 2009.
10. Liberté et Neurobiologie.
11. Aristotle. On the soul. In: The complete works of Aristotle. The revised Oxford translation. Edited by

Jonathan Barnes. Bollingen Series LXXI-2. Princeton University Press; 1984.
12. Damasio A. The feeling of what happens: body and emotion and the making of consciousness. USA: Harvest Ed.; 2000. Spanish Edition: Cuerpo y emoción en la fábrica de la consciencia. Spanish Translation by Pierre Jacomet. Santiago de Chile: Editorial Andrés Bello; 2000. All quotes of this work are from the Spanish edition).
13. Nuyens F. L'évolution de la psychologie d'Aristote. Louvain: Éd. de l'InstitutSupérieur de Philosophie; 1973.

Life Project

6

Ricardo Aranovich

Abstract

José Ortega y Gasset states that men *are* their lives, and that these lives consist in existing in a circumstance not chosen by themselves, but into which they were thrown, and to stay afloat and live they must do something, swim to shore like someone who has fallen into the sea. And whoever falls into the sea swims before *thinking* they need to swim. *That* is the life project. Once an individual is already swimming, he may think what needs to be done to escape this situation: that is just a life plan. But to fulfill that plan, he must already be swimming: if he weren't, he would drown. The life project is what someone is *already* doing in his life without forethought, and in which he stands. And when that *doing* responds to a defined wish in form and objective, it provides satisfaction no matter the results. *That* is vocation. One of the consequences of the current cultural crisis is the loss of contact with ourselves, living shallowly and without taking vocation into consideration. For the life project being the expression of vocation should be a fundamental objective of psychotherapy and of *living* in general. Paying attention to this matter would help solve the anomaly and lack of sense that burdens our lives, and which many times leads to addictions and violence.

Keywords

Life project • Vocation • Anomie • Cultural crisis • Sense • Psychotherapy

Jose Ortega y Gasset refers to the "life project" in different parts of his work. He considers it what characterizes the human being; since humans do not have a nature, they do not have a fixed way of being. For example, the tiger, even if it wanted, would not be able to "detigerize" itself.

R. Aranovich
ACTA Foundation (Fundación ACTA),
Academic Council (Consejo Académico),
Marcelo T. de Alvear 2202 piso 3°,
Ciudad de Buenos Aires C1122AAJ, Argentina
e-mail: raranovich@hotmail.com

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_6

> While the tiger can't stop being a tiger, and can't detigerize, the human being lives at a permanent risk of dehumanizing. [1]

In front of a cub of any species, even those closest to our stage of evolution, we can predict, with some certainty, what that cub will become once it matures. This is impossible when it comes to a human baby because:

> The life that has been given to me, I have to live it myself. It has been given to me, but it is not complete, unlike how stars and stones are given their fixed and untroubled existence. [2]

For Ortega, human life is not definable as a unit, but as a relationship between who lives, *me*, and the context that surrounds it. This relationship takes place in time, and its history, tale, or drama are what every life is going to be, there's nothing fixed. How is the body included in all this? Ortega places it as part of the *context*. What's meaningful is not the body itself, but all that it does by taking advantage of the conditions or accepting the limitations it is provided. Exactly like any other circumstantial condition it is in, such as the family, the country, or the time in which it was born.

> What's human is a human's life, not its body, not even its soul. The body is a thing, and so is the soul, but the human itself is not a thing, it is a drama: it is its life. Humans have to live with the body and soul they were given by luck. One and the other—body and soul—are the closest devices with which the human must live, meaning, those with which he has to exist in its context. [3]

Meaning that just as, for example, the financial means a family has, the city or the neighborhood, the education it has access to, the social or cultural opportunities that characterize the medium in which the human's life takes place, are going to exert an indubitable influence on what its life will become. The human's psychophysical conditions also exert an influence on the final result.

> Our life is like this: I have to live and develop myself in the world, among things and other men, with a body I got by chance and which suffers diseases, with a soul which may be lacking in will, memory or intelligence. [4]

But what should catch someone's eye, and we assume it's not because it is such a common and frequent fact, is the lack of a fixed relationship between context and the future development of existence. We are far from stating that context lacks importance, but it is still a factor that forms part of every individual's life. Even if:

> … Life consists of humans finding themselves, without knowing how, having to live in a determined and inexorable context. [5]

On the other hand:

> … Our life is our being. We are whatever our life is, and nothing more; but that being is not predetermined/solved from the beginning, we have to decide it ourselves, we are those that decide what we want to be. [6]

Where and when is this choice born? What is it that causes children in the same family, who were raised in similar conditions and were under similar influences, to turn into great people on one hand, while another ends up as the "black sheep"? Furthermore, what causes some teenagers from poor families to turn to illegality, whereas others overcome their situation and insert themselves in society? What should be taken into consideration are "psychological factors". Of course! That's it! But what are we referring to when we mention these "psychological factors"? Most people tend to relate psychological factors to environmental or family influences, which would later cause their lives to develop down particular paths, different and unique to each person. If we were to look at the wide variety of actions that humans take, we would be able to relate these paths to the different ways people have of facing their "struggle for survival". If human conduct were logical, it would be probable that *needs* would have already vanished from Earth a long time ago. Rationalism finds in the needs of subsistence the main engine of human action. But what if the human action is not related to subsistence, and even more so, endangers it? For example, the mountaineer that sets out to climb the Alps? What need would this action fulfill? For such a big goal, much effort, time, money, and training would be required. All of this suggests that this action implies the fulfillment of a need, but not one related to subsistence. What about the young man who decides to become an artist, even though he knows that if he were to join the family business, he would

have a much better subsistence? It is this that causes us to meet businessmen, politicians, artists, masters of different trades, scientists, sportsmen, women who choose to have children over following a career (or vice versa), etc. And even among each of these trades and occupations, not one is the same as another, they all differ in the way each person chooses to integrate it in their lives. Two lawyers can work as such for reasons and with objectives completely different from one another, and the same would apply to two politicians, two businessmen, or two doctors. Listing the different actions the human being performs is practically impossible, but their existence leads us to suppose that a factor different from "psychological influences" or practical needs exists. There is, in fact, a need, which is to fulfill a life project, which is the way in which life's force tries to manifest itself in each human being, and which in turn grants each person the energy required to push onwards as the protagonist and leading actor of their own personal drama, their life. Without it, living would not be living, but just existing with no reason to exist or an objective to fulfill. The cultural crisis in which we live does not favor life having a meaning beyond comfort and consumption. The unease that prevails is the most eloquent proof of humans having other needs that are sometimes ignored, which we refer to as "spiritual needs".

> ...Those diverse vital projects or life programs that our fantasy elaborates, and among which our will, another psychic mechanism, can freely choose from, do not present themselves to us as something familiar, but as something strange, coming from who knows which personal and dark secret coming from deep within us, calling us to choose one life project and discard the rest. All life projects present themselves as possible—we can choose *any* we want—but only *one* presents itself to us as what we *must* be. This is the weirdest and most mysterious ingredient of humans. [7]

Genesis of the Project

Maybe the expression "Life Project" is not the most fitting, because it alludes to a certain definition. For example, in architecture the project consists, precisely, in determining with the highest precision the details of what the house/building will later look like. However, in the topic at hand, the life project is more a state that a plan. And even more so, it should not be confused with a plan because plans are constructed through intellect, and the life project is prior and previous to any intellectual elaboration.

> "I find myself being the project that I am before I even think about it; in fact, no one has ever completely thought of what kind of project he or she is."[…] Basically, it is the course of life that shows us, little by little, what kind of *project* we are. [8]

The project is born from the energy which everyone displays in living, and *that* energy is not born from intellect, but from life itself. Intellect may *guide* this energy, but it does not *create* it. On the contrary, when convictions are born from intellect, they create doubts, because that same intellect is what gives people alternatives to all their choices. Intellect opens crossroads, while life is the force that pushes us down the road that, at one point or another, meets a crossroad.

> …All the things that happen to us, happen to us because we live. Since we accept this fact, it is evident that anything that happens to us, even the hardest and most maddening things, happen to us because we want them to—because we want to exist. Human beings want to live—want to exist, to subsist—and they want to exist in a way they will fulfill their own individualism, to be themselves. [9]

In our minor brothers in the biological scale, the life project matches their species. The "fulfillment" of the tiger consists in fulfilling, in all its characteristics, the project "tiger". Even if someone were to envy the size, strength, or invulnerability of an elephant, nobody would ever dream of becoming one. In the human being the life project is not limited to species, it is not enough to just fulfill the early biological stages of its life. In fact, that would be a (and is) a conviction. Those that go to jail have their biological needs more or less taken care of. However, their situation is completely unsatisfactory. It can be said that captive animals also suffer; the only difference is that we do not find out. But if they were to suffer, it would be because they cannot do by themselves actions particular to their species, not their individual actions. The human being, on the

other hand, can live his life *even* in those conditions. Such is the case of Miguel de Cervantes, who wrote Don Quixote while in prison. Each life is unique, and no matter how similar it is to the life of another person, never ceases to be our own. Lives are not exchangeable. They have absolute singularity, not biologically…

> …humans have no nature. They are not their body, which is a thing, nor their soul, conscience or spirit, which are also things. Humans are no thing, they are a drama—their life, a pure and universal event that takes place for each person, in which everyone is nothing but another event. [10]

…but symbolically, culturally. Human's habitat is not nature, which is to them an inadequate medium. Humans have built their own medium, culture, and from this situation have developed to become cultural beings, on top of already being natural beings. It is hard to determine which is *cause* and which is *effect* in this process. Meaning, human beings are individuals that develop in a cultural habitat and that instrumentally utilize a body inserted in a social situation.

But having a body is no minor fact. It is the source of the vitality one will require to develop one's project in the cultural world.

Vitality

> There is, in fact, a part of ourselves that is infused or rooted in our bodies, and acts as a sort of corporal soul. From this "soul" come the instincts of offense and defense, of power, organic sensations, pleasure and pain, the attraction between opposite sexes, sensitivity to music, dancing, etc. This corporal soul acts as a foundation to the rest of our person. [11]

Ortega, in an essay titled "Vitality, Soul and Spirit" decodes to describe these factors that intervene in human action. We will return to the soul and the spirit later on.

We can say, imaginatively, that this vitality or vital impulse meets, after birth, a world in which it must find a way to survive. We come to this world with the aforementioned energy or "will to live" that will have to find its way in the face of different aspects, some favorable and some unfavorable. It is in these situations that the most adequate attitude for survival is born. This is the seed of the life project. But it is not, as we can see, a plan that has been thought through. If we fall to water, if we know how to swim, we don't "think" we should start swimming. We find ourselves swimming before even thinking. Thoughts will come later: distance to the coast or a boat, temperature, ways to call for help, etc. This is the reason that makes the life project anything but a plan, since it is the action that takes place *before* any plan. Plans are born from the collision with reality, and therefore, cannot have been thought before the crash occurs.

> In its radical lines, life is always unpredictable. We have not been announced before entering it—life's stage, which is always a certain and determined one; we have not been prepared. [12]

The life project is, therefore, a reaction that cannot be called either rational nor irrational, the most adequate would be to call it pre-rational. Reason will come into play later, but the decision to fight, to beat difficulties and live on is not the result of a previously meditated choice, it is something decided by life itself. This distinction is important in view of the utility these concepts have to psychotherapy, since the therapist will find himself with patients that, in the face of minor predicaments, have lost the skill to react, or on the other hand, with patients that hold on to life and who fight even in the most unfavorable of situations. Rationalism, which puts everything on the same level (since reason is unique and universal), does not prepare us for these distinctions, but the therapist should possess the skills that will allow him to face what we have carried upon our shoulders, the singularity of the human being manifested in its life project. If vitality were to be missing, its empty space should be filled by psychological resources complemented with other resources that favor its recovery. In this characteristic we have called pre-rational lies the human's biggest strength, since it is a spontaneous manifestation of vital energy. It is known that humans are born completely defenseless. Without the care from its parents (not only food and warmth, but also motherly love) it would be impossible for the baby to keep on living. Such is

the need of care that the baby reacts whenever it is separated from its mother, since it does not know where she is or what may have happened to her. From the baby's interaction between his sensibility and the care received, a way of feeling in the world will rise, and this way the baby feels this will affect what kind of life project he'll eventually choose.

Once this stage of absolute dependence is left behind, the stages of social insertion will follow. First comes the insertion in family life, then school life, and finally, through adolescence, the child will become an adult. All these stages are opportunities that will affect and modify the child's life project: the place he fills in the family, in the succession of siblings, or if he's an only child. The role in educational institutions: better or worse student, interest in certain subjects or sports, social roles, being more or less "popular", the pretty girl, the ugly girl, the fat guy, the nerd, the transgressor, etc. All these experiences consolidate an idea of ourselves that, in turn, affirms the project. The problem is that the idea may be wrong or may have been born from an isolated experience (a bad grade in one subject, being rejected by your crush, etc.). This condition may be invoked by someone as "I'm not meant for that", "I'm not good at…" and they may turn away from what is most important: vocation.

Our subject finds himself, as he lives in the world spread before him, and he *knows* he needs to do something to live. Favorable and unfavorable experiences act as hints, and so do the adult figures whose activities may appear interesting or attractive; basically, all he sees through the course of his life. But none of these things are enough to explain the singularity and individuality we have explained earlier. At one point in life, an enlightening occurs, a certainty coming from an unknown place: "I will be…", "I'll work as…" which will take, without a doubt, materials from experience of life and world. However, the way in which these materials will organize at a certain moment and in a certain way to form the personal project cannot be deduced from the mere presence of its horizon, seen from afar. There is an act of choice, of will, a decision, which comes from the spirit.

In a house where a passion for chess exists, in which the different family members compete against each other, be it between siblings or fathers and children, there's a really high possibility for one of them to choose to devote himself to chess. But this is not the most important thing. That young man or woman, at some point had to *make* that choice, and that choice, even though it was facilitated by the environment, is as much of a decision as if he had chosen a career or a job, or even become a sailor. Because one should ask oneself; if it's a large family, why didn't all the siblings choose the same path, if they were all under the same influences? Some may argue that "not everyone lives things in the same way", something with which I wholly agree, but there's the issue: "not everyone *lives* it…" and in that particular leaving which is personal to everyone, the spirit is manifested.

The Spirit

We have already talked about vitality earlier. Ortega claims that spirit is no metaphysical entity, hidden or hypothetic reality, but a phenomenon that everyone can find within themselves with all the evidence, and then describes this phenomenon:

> …the act in which we understand with enough evidence a scientifically proposition can only be executed by the core of our being, which is our mind or spirit. Thought does not come only from the body or only from the soul. In every authentic "understanding", "reasoning", etc. an immediate contact between the spiritual "me" and the understood is produced… The understanding that 2+2=4 happens in an instant…Same happens with love, you fall in love or you don't in just a moment. Volition, even if it takes time to form, is a ray of intimate activity that completes the choice. [13]

While in the soul, home of emotions, phenomena extend through time (you "are" sad, you "are" happy), spiritual phenomena are instantaneous. Because of this, the volition, the choice that defines a life project, is spiritual. It is not necessarily unique; events and experiences in life

modulate it, but in each case it is a new act of volition, as "explosive" as the previous ones. In every "thought" the same occurs:

> While we "are thinking" we go through the successive series of many acts of thought, each of them being a mental lightning. [14]

The choice that stands as a starting point for a life project has all the characteristics that an action coming from the spirit would have. It is a volition born from the intimate, which has the kind of certainty that does not depend from any argumentative development. Arguments comes later, and if something changes it is because another spiritual intervention has occurred. Choices coming from the spirit are not permanent or unchangeable. They are modifiable, and context may render them unfit, so they are modified by other choices which also come from spiritual intervention. When it is not like this, and a choice is modified by secondary considerations, the subject feels uncomfortable and that he has failed himself. The same occurs when Ortega states that the life project cannot be changed:

> Our will is free to *do or not do* that vital project that we ultimately are, but it cannot correct it, change it, dispose of it or replace it. We are only a programmatic character that needs to fulfill itself. [15]

Every life is a project, and only one, which can slightly change depending on the context. These modifications must stay coherent with the characteristics of the initial project. We can compare the project with the individual itself: throughout its life it can experience changes as things around him and within him change, but this does not mean that he stops being himself. It can change, for example, the activity he does, but not the reason why he does it. Each individual can develop its life depending on the project, or let himself be carried away by conveniences and conventions and do what "people do". The spiritual I is the one in charge of perceiving or correcting the turns of this project, the loss of coherence, and its interventions appear as volitions, choices. It would be like a ship's captain's orders to stick to a course, making it a task to complete.

> Life is a drama, because it is the frenetic struggle against things, and despite our personality, we choose to become those we are in the project. [16]

This character, the spiritual I, is the manifestation of the spirit in every person, because it represents the purpose of every individual existence. Life is organization, growth, and purpose; and on the human level, this is manifested as spirit. The difficulty for a particular existence to develop according to its respective project is, precisely, the lack of contact between itself and its spiritual level. This is the consequence of the cultural crisis and life's subsequent superficialization. In all pathologies, a loss of organization, a chaos can be perceived. If the purpose of existence is consumption, fun, and pleasure, we move away from the possibility that what guide our actions are guidelines from our own uniqueness. Asking ourselves what do we really want to do with our lives implies a questioning of our own spirit, which is often unknown to us and cast aside, unheard and unseen, without being taken into account because time flies and we always have other more important things to do, which pay off, are fun or pleasurable. The spirit tends to be a killjoy when it's time to find out if what we are doing, the life we are leading, is or is not truly wanted. That life project which is the manifestation of the spirit puts things into order and gives them a purpose in each life, causing all calls taking us down that road to come from the spiritual plane. That is, the subject will feel like taking decisions, but those decisions are dictated by something previous to him, which is the spiritual route we call, yet again: "life project".

The importance of these concepts to psychotherapy is evident. Disorientation and lack of feeling our own existence are epidemic. Even more so, the epidemics of more objectified psychological diseases, such as depression and anxiety problems, may be thought of as manifestations of the preexistence of the previously mentioned disorientation and the lack of a way of giving some sense to existence. After some time of living letting yourself be carried along by what "people" do, then comes a moment of emptiness that evolves towards depression or anxiety. Panic disorder, in some cases, is accompanied by a highly suggestive depersonalization syndrome of this mechanism. Suddenly, the patient loses the synesthesia with himself, and, after this terrible experience, comes the panic. Or marital issues with their also epidemic sequel of divorces, originating from the conception of

marriage as a coexistence that must be at all times enjoyable and not as a realization of a project in common for which the gratification will be the satisfaction of developing the capability to take care of others and to help each other to be better human beings. But to improve we must perceive values that aren't just those of instant gratification, and that perception also depends on the place the spirit occupies in each person's life. Because of this, the project is unknown. It is not something one "wants" to do, it is what generates the action. No one says: "Since my life project is to be an architect I'm going to join the architecture faculty." He finds out his life project is to become an architect *after* he felt he wanted to study architecture. This means that the project and the spirit are two different aspects of the same phenomenon which surrounds them, which is life itself.

The life project is the manifestation on the human level of life's formidable tenacity, which extends itself even in the most precarious conditions as soon as it is given a minimum chance to do so. The understanding of the project's origin is confused with the fathomless mystery of life itself.

The Soul

Between vitality and spirit, Ortega places the soul. The soul is the sphere of emotions. While spiritual phenomena are instantaneous, emotions extend through time, lasting. You "are" sad, happy, angry, in love, excited. Spiritual phenomena are punctual; when a choice is made, that choice is produced, there's no other simultaneous choice happening at the same time. Meanwhile, one can experience discovered feelings which were not known to be there. Between vitality and soul there is no precise limit:

> …There is no way to determine where our body ends and where our soul begins… . [11]

It is because of this that emotions interact with vitality. A moment of enthusiasm carries a greater disposition to act, and sadness often has the opposite effect, inciting inaction. But the motivating action of emotion would be unorganized if the spirit's organizing intervention was inexistent. The spirit decides and organizes the emotional and motivating world, giving it a course to follow into action.

> The will does nothing but decide, choose between one inclination and the other: it prefers what's best, but it would not want anything by itself if it weren't for this metaphorical keyboard of emotions, where our force of will chooses which keys to press…. The spirit or ***I*** cannot, for example, create a feeling, or directly eliminate one. [17]

That life project takes shape from the choices that the spirit makes. Emotions tend to fill a privileged place in the therapeutic sphere and, depending on the school the therapist has been formed in a bigger or smaller importance to its expression or "catharsis" is given. But from the perspective that the spirit's presence suggests, it could be understood that when an emotion is expressed, you are "presenting" it to the spirit so it can perform its organizing function, causing that emotion to stop being disturbing. There are other resources, in and out of the therapeutic sphere, that may help achieve emotional balance, and whose way of action is understood by taking into account the spiritual body and the project. Such is the case of meditation in its different ways, the techniques of Directed Dreaming, the different religious experiences, a shocking experience forcing a deep pondering. All therapists with a certain degree of experience have had cases where the patient presented a particular difficulty in stepping away from a certain emotion. A particularly significant case of this is the so-called "PTSD" or Post Traumatic Stress Disorder, though its interpretation would not be very different. Other dramatic cases, such as not being able to forget a grudge with a relative, or holding a conflictive relationship with someone you live with, are examples of the difficulty of causing the spirit to intervene in its function of arranging emotions and achieving the emotional harmony that eases the continuity of existence.

Reflection

Our current way of life does not make the spirit's intervention in existence any easier. One is focused in the outer reality, with its obligations,

distractions, urgencies, problems, dangers, satisfactions, joy, and misery. In that, the human being answers to what has been its main occupation since hundreds of thousands of years ago, which paleontologists estimate to be the date of apparition of our species. During almost all of that time period, the attention focused on the outer reality made the difference between life and death. Being alert was essential, both to find food and to avoid *being* the food. Some of this still takes place, and an example of it is stress-related or -caused diseases. In fact, as long as we don't have an adequate mechanism to interpret the dangers of civilization, we will still react the same way we did back in the jungle. What is the use of generating an adrenaline rush which prepares the body for a fight-or-fly situation when you are facing a financial difficulty, a lawsuit or a risk of being fired? None of these situations will be solved with a physical confrontation, yet we still waste our time preparing the energy of our body as if it were to happen. Therefore, this is another terrain where the spirit's balancing intervention is required. And the way that we facilitate that intervention is to turn to a state of "reflection", a term which may refer to picturing a copy of ourselves in a mirror, though in this case it is actually an internal contemplation, or introspection. The term that Ortega uses to refer to this state is the one of "retraction", looking "into" ourselves. The opposite would be the state of "alteration":

> Almost the whole world is altered, and in that alteration man loses his most essential attribute, the possibility of meditating, of withdrawing into himself to agree with himself and determine what his beliefs are; what is it that he really cares about and what is it that he really hates. Alteration blinds him, forces him to act mechanically, in a frantic somnambulism. [18]

In the state of reflection or retraction the spirit has room to move, so it can organize the mind, to arrange the true objectives hierarchically and to organize the direction he wants to follow in life, distinguishing what he really cares about from his other shallow pseudo-interests. Again, psychotherapy comes into play. The therapeutic sphere is the most suitable for this process. It is a singular situation in which one of the participants, the therapist, decides to accompany the patient and make his state of reflection easier to achieve, so that he may find clarity about his true objectives. In our culture this is one of the few opportunities where one person hires another to receive help with their introspective capabilities, and receive another's support for that task, so that he can reconsider and modify the way he lives his life. When the life project is born from the spirit and a will to act, a spiritual state is required for it to be present in the subject's conscience. This is particularly necessary when the life project seems unable to develop, be it because of an accident, a change, or the context's evolution.

The Life Project's Evolution

Ortega defines life as the relation between the ***I*** and the context that surrounds it. This relationship is exposed to the alternatives of circumstances, and its alternatives make up what each life is.

> Life is what we do and what happens to us… living is what we do and what happens to us, from thinking or dreaming or being moved by something, all the way to playing games and fighting battles. [19]

As seen above, referring to life projects, Ortega states that many present themselves as possibilities "but one and only one presents itself as the one we *must* be". He also says that life is the relationship between the ***I*** and circumstances that take place in time, which is an "event". Meaning, to understand the human life we must resort to a tale; as therapists know it, a story. The human's life is… his story, "what he does and what happens to him".

> Man has no nature, he has history. History is the way of being of an entity that is radically variable and lacks an identity. And because of this, it is not pure naturalistic, eleatic reasoning which will be able to understand man. [20]

How do you make two characteristics as incompatible as the project being "only one" and that the being should "constantly change" compatible? It is obvious that the life project cannot remain unchanged throughout the course of life,

especially taking into account of the fact that it has been defined as the relationship between two objects, neither of which is fixed. So: the project is "only one" at all times. At all times, life places us in front of the need to make a choice, and in each case, the choice must answer to the life project. We have already seen that the life project is not a plan, a timetable known from the beginning. You get to know it as you live. Precisely, the successive choices you take are what shape the life project, *that's* why it is unknown at first, just as you don't know the circumstances that will shape it. However, if we follow its development we can see the course it will take. At every crossroads you meet, reflection is what reveals "what you will believe in, what you hold dear and what you hate". Therefore, at all moments, the answer to what is happening must be the expression of the true will and, as we have seen, that changes at all moments. This is what makes it the manifestation of the spirit. At any of life's alternatives, many options present themselves but only one will express the subject's true will. It is like this that in the face of any disagreement, some lives may follow the right way and some may turn off. The situation known as "the mid-life crisis" is paradigmatic. It is the moment when the life project stops acting as motivation because there is something that has dried it up: it has been fulfilled, and therefore has lost its capacity of generating new actions. It is not as if the project of life is lost. An argument that the life project used was lost, and therefore must now create a new one. It is not different from what at one time caused its creation. What happens is that at that time it was a succession of elections that were taken imprecise, which eventually led to that project to dry up. What happens now is that, because of the lack of exercise, the skill of finding what you "really want" to do with life has been lost. We have lived from "rents" of the project in course and it was supposed it would always be like this. But the capacity to project is intact. Actually, the unease that the situation provokes is the most eloquent proof of something missing, that life asks for a project, to dedicate to something. It is at this moment when one must recur to the spirit, reflection, or withdrawal. Searching in yourself is what matters now, not what mattered 20 or 30 years ago and which was active at that time. Because there are stages in life where the project's choice is facilitated. When couples marry, or a career is chosen, or you journey to a certain place. These are magical times because you take fundamental choices, though thanks to the time they occur, they are facilitated and require less effort. But at another stage of life, choices are harder, and in that case, you must be aware of the usefulness of recurring to a spiritual state, which is the one that'll give the conviction necessary to face change. It is no different from the process that took place back then, it's just harder. The same goes for when an unexpected turn of events affects the project. It may be the death of a loved one, a disease, an unexpected economic problem, or any possible "disgrace" which life throws your way. In each of these cases, suffering occurs because a situation which was supposed to last in time suffered a sudden interruption which affected the project in a negative way. How do you continue with life? It may not be easy, but it is possible (which from the therapeutic point of view is no small feat). Sometimes it becomes practically impossible to recover, but in other cases "spiritual" resources awaken, which develop into the possibility of reinstalling oneself in life with a certain degree of well-being. Life is not over; a way of living has ended, and it is urgent to find a new one, just as you found the first one, with the same elements it was made up of, through thought and imagination. Life must always "be lived", both in the easy times and in the hard times. What raises the difficulty is the confusion between the end of a stage and the loss of the capability to recover a life project. The project is not born from context, but from the human being's capability of getting excited with what the context offers him. With the passage of time, you start to believe that "things" are what generate enthusiasm, and this causes the inevitable losses of the said things to become terribly catastrophical.

> When noticing we cannot live without taking interest in some things, many believe that, in fact, what was interesting *were* this things, and not the fact of

> taking interest in ourselves…It is not, then, the transcendental values what give life a meaning, but the other way around, life's generosity, which needs to get excited about something unrelated to it, gives it its meaning. With this I don't mean that all those big things are fictionally valuable: I'm just interested in warning that the human's capability of enlightening itself for all the esteemed aspects of life is no less valuable than all those material things. [21]

And that capability that life possesses of being excited about something unrelated to it finds its manifestation in vocation. Vocation is the bridge that may keep a firm path in life over all the collapses which originate in life's circumstantial changes.

Vocation

Why does the loss of the project cause so much grief? In therapeutic activity, it is necessary to always be on guard to not pass over the obvious without taking it into consideration. Why is it that human life, to be more or less enjoyable, needs to embark on a project? Animals have no project other than survival, and they don't seem to suffer from the changes in circumstance, as long as they are not endangering ones. What happens is that in animals, the purpose of life is fulfilled merely by existing. In the human being, the purpose has separated itself from the corporal form and has developed on a symbolical level which may be even more sensitive than the body. When the project is damaged, it hurts; not in the same way a headache hurts, but if they were given the choice, many would choose a headache over a failure of any kind. What happens is that the human being lives in a particular habitat, culture. And in this habitat, the conditions for survival are others. For example, some recognition coming from others is needed. The conditions of safety have to do with values that aren't strictly materialistic, prestige may be felt as a need on the same level as food. Anthropologists face the enigma of the relationship between the symbolic capability and the cultural organization, both suppose each other mutually but it is not possible to determine which created the other. Life is organization, growth, and purpose. In the human being, the purpose, while in the cultural habitat, is the life project. The purpose is what supports organization and growth. Depression may be seen as the consequence of the loss of purpose, as if it were even necessary to maintain an appropriate state of organic functioning, of well-being. The loss of purpose generates a state of disintegration which is terribly pitiful. The counterpart is, for example, the state of being in love, which poets mention and where the purpose is so exaggerated that it may fill almost the whole mind, leaving no room for other thoughts. Enthusiasm is the manifestation of the individual possessing a purpose.

> I mean that our life is never just *being*, a state of just existing. Living is always living, for something or because of something; it is a transitive verb. From here rises the fact that a human life with no vital interest can't exist, because this interest is what holds, makes up and organizes that life. At the moment when all vital interests are let go of, life would merely cease to exist. [22]

It sometimes happens that the need and well-being coming from enthusiasm cause a hurtful enthusiasm to arise, but that happens when you haven't taken a moment to reflect and establish a relationship between enthusiasm and the life project. The same can be said about addictions, artificial ways of generating states of enthusiasm which depend on substances. Because of this dependence, once the effect is over the need to take the substance over and over again is established.

Now, it just so happens that not everyone gets excited about the same things. When something excites everyone, it is usually unrelated to the most particular aspects of the subject. For example money, which at the current moment is an object of universal interest. Obtaining it does not excite us, what excites us and generates enthusiasm are all the things you can buy with it. On the other hand, finishing a career is a source of enthusiasm by itself, along with all the possibilities that come with it. It is the case that every experienced professional has had cases of depression in wealthy excited people who are going through a rough economic patch.

Luckily, the human being has a condition that allows it to get excited. That condition is called vocation. Vocation is an impulse towards a determined

activity that is enjoyable by itself. Artistic activities are the most evident example, you sing because you like singing, you play an instrument because it makes you happy, and all of that is nothing more than the confirmation that enthusiasm is a factor of well-being. Art makes up a kind of purposeless purpose which fulfills that need in life. The same can be said about sports. Of course, one should set professional artists and athletes aside, since once they turn their activity into their profession, they often lose the vocational way, because they must develop their activity without considering their will (enthusiasm) which they may or may not possess at the time.

But vocation is not limited to art or sports; there are, for example, professional vocations, though vocation is not determined by a certain profession or job, but it actually has a wider sense, because it is a way of existing in the world which *includes* a certain profession or job.

But the current cultural crisis, with its emphasis in the economic aspects of existence, has displaced the interest towards vocation to interest towards the chances of getting a job. This is what causes many successful people to experiment a state of unease even when they are in a situation that appears favorable. It is necessary to revalue vocation, not only among parents and teachers, but among therapists themselves.

> …Every man, among its various possible existences, always finds one that is his authentic existence. And the voice that calls that authentic self is what is called "vocation". But the biggest part of men chooses to shut down that voice of vocation and ignore it. They try to distract themselves from paying attention to it, and trick themselves by replacing their authentic self with a fake one. Instead one should leave as himself, because you only truly live through vocation, the one that matches its true self. [23]

Life has the need of existing "for something", but this is not an imperative imposed by ethical or religious ideals which aren't a part of it. When it is "for something" it is more of a life, and that happens when life is given to an action that answers to vocation.

> ...If that life of mine, which only I care about, is not given by me to something else, it will walk lifeless and "shapeless". These years we assist the gigantic show of countless human lives that march lost in the labyrinth built by themselves, because they have no one to give themselves to. [24]

Corollary

Life is order, growth and purpose. Along the zoological scale, the purpose is fulfilled through survival. In the human being biological survival is not enough, because the problematic advantage consciousness gives us includes the notion of time, and with it comes the notion of future and the uncertainty ahead. To make its existence more predictable, culture was invented, becoming the human's true habitat. Now he must achieve survival in this habitat which, even if it frees him from certain needs, also generates others, he must *do something* with its life that inserts him in culture: a life project. But the need is not enough if there is no capability of answering it. Need without capability is solved through failure. But the human being does not fail because along with consciousness and culture he has the vocations to do something with its life, of fulfilling a purpose, of becoming the being he feels he is meant to be. It is not culture which generates vocation but, on the contrary, it is vocation what makes human culture possible, which is made up by the threads of life's various projects. Human life is problematic because:

> It is, then, for man, impossible to be without an orientation regarding the problem his life is. Precisely because life is always, at its root, disorientation, perplexity, not knowing what to do, it is also always the struggle to become orientated. [25]

Before the inevitable perplexity, the only certainty lies in the attitude with which one faces it. To this end, humans return to the capability of locking themselves up within their own minds, what Ortega called "retraction", and finding in the mind's interior the certainty that the situation denies to him.

> The world is the total exteriority, the absolute *outside*, which does not consent to anything further outside of it. The only outside other than that *outside* is, precisely, an *inside*, and *intus*, the intimacy of men, of their *own self*. (27)

And it is from this inside that this life project is born, where man will affirm himself to do what every man must *do*: his life.

> …Man is impossible without imagination, without the capability of inventing a life figure, of "imagining" the character that will exist. [26]

On the contrary of what many minds may suppose, creativity is what supports culture and makes it possible. The human being must return to its creativity to hold itself within culture. Vocation is the source of creativity with which he will affirm itself and acquire confidence in that uncertainty. But for that it is definitely necessary for the individual to keep listening to that voice in his head that we know as vocation.

> …The biggest part of men decides to ignore the voice of vocation and pretend they don't hear it, they make as much noise in their mind as they can to cover that voice, so they can replace their authentic self with a fake one. They should only live their true self, because you only truly live by living your vocation, the one that matches your "true self". [23]

This means that the resource to face the uncertainty which characterizes living is to affirm ourselves through a life project which is born from vocation. But this requires, along with the disposition to be heard, the decision of living it and, through giving, turning it into a mission.

> In our hands is…to be faithful or unfaithful to our vocation. But this, I mean, what we really have to do, is not in our hands, It is inevitably proposed to us. This is the reason why every human life has a mission. Mission is the following: the consciousness that every person has of their most authentic self which is being called to be fulfilled [27].
>
> Buenos Aires, January 23rd of 2013

References

1. Ortega y Gasset J. Obras Completas (OC) T. V. Madrid: Taurus; 2006. p. 540.
2. Ortega y Gasset J. OC T. VIII. Madrid: Taurus; 2008. p. 584.
3. Ortega y Gasset J. OC T. VI. Madrid: Taurus; 2006. p. 413.
4. Ortega y Gasset J. OC T. VIII. Madrid: Taurus; 2008. p. 593–594.
5. Ortega y Gasset J. OC T. VI. Madrid: Taurus; 2006. p. 469.
6. Ortega y Gasset J. OC T. VIII. Madrid: Taurus; 2008. p. 574.
7. Ortega y Gasset J. OC T.VI. Madrid: Taurus; 2006. p. 482.
8. Ortega y Gasset J. OC T. VIII. Madrid: Taurus; 2008. p. 436.
9. Ortega y Gasset J. OC T.VI. Madrid: Taurus; 2006. p. 387.
10. Ortega y Gasset J. OC T. VI. Madrid: Taurus; 2006. p. 64.
11. Ortega y Gasset J. OC T. II. Madrid: Taurus; 2004. p. 568.
12. Ortega y Gasset J. OC T.VIII. Madrid: Taurus; 2008. p. 356.
13. Ortega y Gasset J. OC T. II. Madrid: Taurus; 2004. p. 575.
14. Ortega y Gasset J. OC II. Madrid: Taurus; 2004. p. 575.
15. Ortega y Gasset J. OC T. V. Madrid: Taurus; 2006. p. 124.
16. Ortega y Gasset J. OC T. V. Madrid: Taurus; 2006. p. 125.
17. Ortega y Gasset J. OC T. II. Madrid: Taurus; 2004. p. 576–577.
18. Ortega y Gasset J. OC T. V. Madrid: Taurus; 2006. p. 534.
19. Ortega y Gasset J. OC T. VIII. Madrid: Taurus; 2008. p. 579.
20. Ortega y Gasset J. OC T. IX. Madrid: Taurus; 2009. p. 557.
21. Ortega y Gasset J. OC T. III. Madrid: Taurus; 2005. p. 601–602.
22. Ortega y Gasset J. OC T. II. Madrid: Taurus; 2004.p. 747.
23. Ortega y Gasset J. OC T. VI. Madrid: Taurus; 2006. p. 483.
24. Ortega y Gasset J. OC T. IV. Madrid: Taurus; 2005. p. 46.
25. Ortega y Gasset J. OC, T. VIII. Madrid: Taurus; 2008. p. 632.
26. Ortega y Gasset J. OC T. VI. Madrid: Taurus; 2006. p. 66.
27. Ortega y Gasset J. OC T. V. Madrid: Taurus; 2006. p. 350.

A Brief Bioethical Perspective on Work in the Field of Health

7

Gilberto A. Gamboa-Bernal

Abstract

Work in the field of health has been distorted over the years, with the emergence of new health systems that have made the delivery of services a real business. As a result, the field has lost not only the motivation with which it originated, but also the human quality of providing health care. It is not new to say that exercise of the medical profession is in crisis. The causes of this predicament can be found in policies and health systems that are poorly imitated, poorly administered, mismanaged, and poorly regulated. However, there is no denying the crisis is also due to the loss of the Hippocratic spirit that gave force and vitality to the medical profession from its beginning.

Several aspects of work in health and health care, namely, scientific and technical competence (knowledge and knowhow) and human skills (knowing "how to be"), are examined in this chapter, based on a brief look at the patients and the professionals who serve them. The author goes on to discuss three fields of professional activity where these competencies play out: the Hippocratic tradition, social responsibility, and constructive dialogue. In conclusion, and in light of the above, several initiatives and strategies to humanize health services are suggested. They involve example, communication, accompaniment, correction, purpose, and professionalism.

Keywords

Person • Health • Responsibility • Humanization • Professionalism • Dialogue

G.A. Gamboa-Bernal, MD, MSc, PhD
Universidad de La Sabana,
Cra. 43E 7D-14, Medellin 050022102, Colombia
e-mail: gilberto.gamboa@unisabana.edu.co

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_7

Introduction

The topic in question could be examined by talking about specific situations in medical practice; however, illustrating this field of activity, which is also an art, would require a far longer chapter than this one. Other thoughts on how to resolve the problems in clinical practice are discussed but, before tackling them, it is important to say a few words about patients and the professionals who serve them.

Although some find it difficult to accept, embryology and biology provided an answer, some years ago, that is fundamental to understanding what it means to work with human beings in the perinatal and neonatal stages; namely, that patients are human beings [1]. We have to start there.

In the 1970s, that assertion cost French geneticist Jerome Lejeune the Nobel Prize in Medicine. As you will remember, it was he who discovered trisomy 21 in patients with Down's syndrome [2]. Dr. Natalia López Moratalla, in an article entitled "The Zygote of Our Species Is the Human Body" [3], combines scientific data in embryology and biochemistry with anthropological applications to show how the life cycle of a body, with its own character and individuation, begins with the fertilization of two gametes. It also answers the question of what makes the human genome human, and clearly identifies the competencies that each field of science has to study this reality.

With these two examples, we are talking about the anthropological and biological statute on the human embryo. They provide all the rationale that is needed to say, with certainty, that every member of the human species, from the dawn of its existence, is a personal, relational, and acting being, one who also has a legal status that demands respect and protection [4].

Patients are persons, some healthy, others with illnesses or malformations. They passively await care and attention in proportion to their degree of defenselessness but, above all, commensurate with the dignity they hold. These two characteristics, dignity and defenselessness, make patients very special, and condition the care and attention they should receive. In the face of dignity and defenselessness, one must act with respect, care, prudence, and a great deal of science.

Expertise in Health Work

Scientific and technical expertise is the first requirement for work in the field of health. To find meaning in professional practice, but particularly to provide good service, one must start with specific training that includes knowledge and knowhow. However, these are not the only aptitudes medical professionals need to develop. They also are required to deploy a range of other skills that must be at the core of those mentioned already, namely, human capabilities, competencies of being [5].

More than being a good doctor, one must try to be a good person. Patients are the first ones to recognize these qualities, followed by family members, parents or companions. Colleagues and support staff also know what kind of person they have at their side. And, since work in the field of health is usually as a team effort, personal relationships are very important, especially if the outcome of that work is to benefit the patient.

Characteristics of Professional Activity

All these capabilities in terms of being, knowledge, and knowhow are reflected in three areas of professional activity: the Hippocratic tradition, social responsibility, and constructive dialogue.

It is not new to say that exercise of the medical profession is in crisis [6]. The causes of this predicament can be found in policies and health systems that are poorly imitated, poorly administered, mismanaged, and poorly regulated. However, there is no denying the crisis is also due to loss of the Hippocratic spirit that gave force and vitality to the medical profession from its beginning [7].

There are now new factors in medical practice that tend to denature its basis, which is the doctor–patient relationship. Part of this concern facilitated the origin of bioethics. Potter saw how

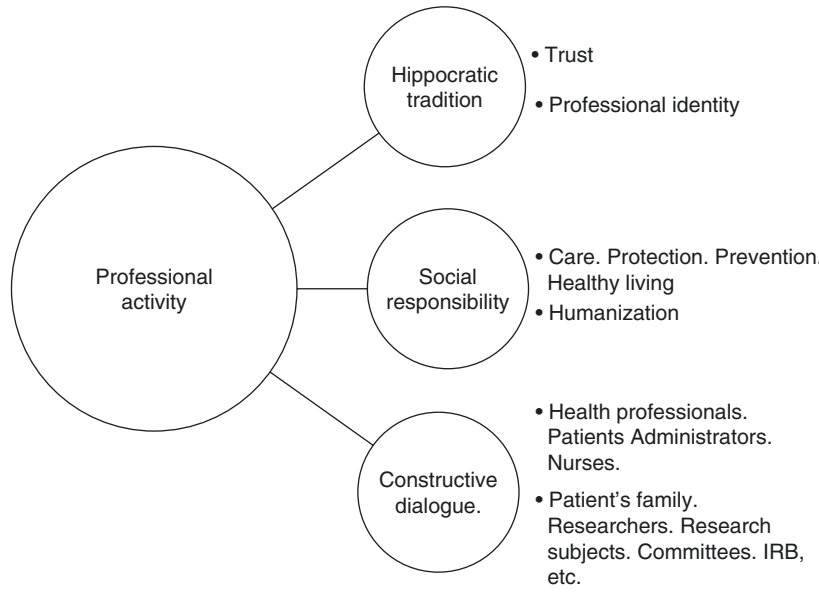


Fig. 7.1 Areas of professional activity

instrumentalization and techno-scientific advancement, when handled poorly, produced a gap that further removed physicians from their patients. It was not only a crisis of medical paternalism or old medical ethics, as claimed. It was the weakness of fundamental concepts inherent in modern globalization. For that reason, Potter proposed bioethics as a "bridge" between the humanities and the biosciences [8] (Fig. 7.1).

The Hippocratic tradition must not be lost. It is what safeguards professional identity and offers a core value such as trust, which is at the heart of medical practice. Thanks to that tradition, it is possible to give medical practice back its original force, which prompted practitioners to think first about the patient's welfare and then about administrative interests; in other words, to do patients no harm, as opposed to benefiting from them as possible subjects of research; to give them the best care, rather than abusing them through haste, paperwork, or procedures.

This is not to say that the Hippocratic tradition operates to the detriment of economic and administrative matters. This tradition will lead to more rational use of resources, getting the most out of them without waste, respecting times, meeting schedules, and being patient with administrative procedures.

The patient, however, will always be paramount. This is based on a comprehensive vision of patients and those who care for them, one that knows how to combine the patient's autonomy (or in the case of minors or the disabled, the autonomy of the parent or guardian) with that of health workers, preventing the imposition of patterns of action devoid of ethics and humanity. It is a holistic vision that knows how to exercise conscientious objection [9], when necessary, without allowing abuses by employers or contractors; one that knows how to apply a moderate form of paternalism that is a balm for the indifferent and aloof protection provided by health systems.

Only with professional practice supported by the Hippocratic tradition will it be possible to get past the frustration, discouragement and, frequently, the feeling of impotence in an environment that is hostile to humane and humanizing medical practice. Only with professional practice based on the Hippocratic tradition can the medical profession maintain its identity and repair or construct health systems on the basis of that identity, ones that genuinely contribute to the change found at the heart of the new notion of health [10], which goes beyond the concept formulated years ago by WHO.

The second characteristic of professional activity that merits comment is social responsibility. For the medical profession, social responsibility is derived from the people it serves, among other things. It is not at all poetic or lyrical to say that the present and the future are in the hands of health professionals, and how the present and future turn out depends on the care, skill, and competence with which patients are treated.

One of the practical applications of social responsibility is the ability professionals have to train the parents of their patients. Part of the education that new generations receive will depend on this. However, you cannot think that parents of patients only help them in medical topics. In addition to guidelines on care and protection, the physician also will give recommendations on prevention and healthy living to ensure a safe and positive course in life.

There is another front that warrants mention in relation to social responsibility. I am talking about the strategies that have been proposed in many health institutions to improve humanization [11]. Thanks to quality control offices or the concerns expressed by patients, their families, or the staff at these institutions, areas and situations have been detected where opportunities for improvement cannot wait.

It is often suggested that humanization can be achieved in one of two ways: through common sense and awareness. While common sense is extremely valuable and can explain many situations "*that aren't working out,*" it is not enough in itself to get to the cause, much less to propose applicable solutions to the problems it is able to detect.

The other way is to help staff members become "sensitive" to the need for humanization. This can be accomplished through "dynamics" and conferences designed to give people a "sense" of how they need to improve and why. Yet, this is not enough. Sensitivity is temporary, contingent, and variable; and what is built on that basis can change very easily, be forgotten, or cause fatigue that can chip away at efforts to humanize medical practice, making that goal fruitless.

There is a third option that allows for a better approach to the problem: raising consciousness. When reasons are taken into account, it is a different matter. If the objective is to humanize, you will want to enhance what is authentically human, both in personal and professional action. However, appreciation of what is authentically human depends on the conceptual framework being applied [12].

That conceptual basis can be found in humanism. However, the question is: What kind of humanism are we talking about? There are many versions in the history of human thought. Renaissance humanism, socialist humanism, existentialist and hermeneutic humanism, antihumanism and the new humanism or integral humanism are some examples.

It is not necessary to explain each of them, only to jot down the characteristics of humanism that seem to respond more to reality. The new humanism is based on the following propositions: man is a personal being, human uniqueness demands defense; it is essential to emerge from relativism and any sort of reductionism. In addition, it assumes that truth exists and can be known; the human person is free and capable of responsibility; freedom is connected with the individual good and the common good; the human person is worthy and deserving of respect; the human body is a human person (Fig. 7.2).

The points of reference used in person-centered bioethics are supported by those assumptions; namely, life is a fundamental value; the person is a relational and transcendent value; a holistic conception of the person is essential, a priority and complementary relationship must exist between the person, society and the environment; human love is a stable, exclusive, and enduring form of dedication (Fig. 7.3).

In short, social responsibility also translates into effective efforts that are made and maintained to bring about genuine and stable humanization of health services, based on adequate integral humanism.

Finally, the third characteristic is constructive dialogue. Ever since Plato's dialogue, this has been a tool of unique value to man. In the course of time, its use has proved to be crucial to human development and peace among people [13]. Dialogue also can deliver its best fruits in clinical practice, research, teaching, and social projection.

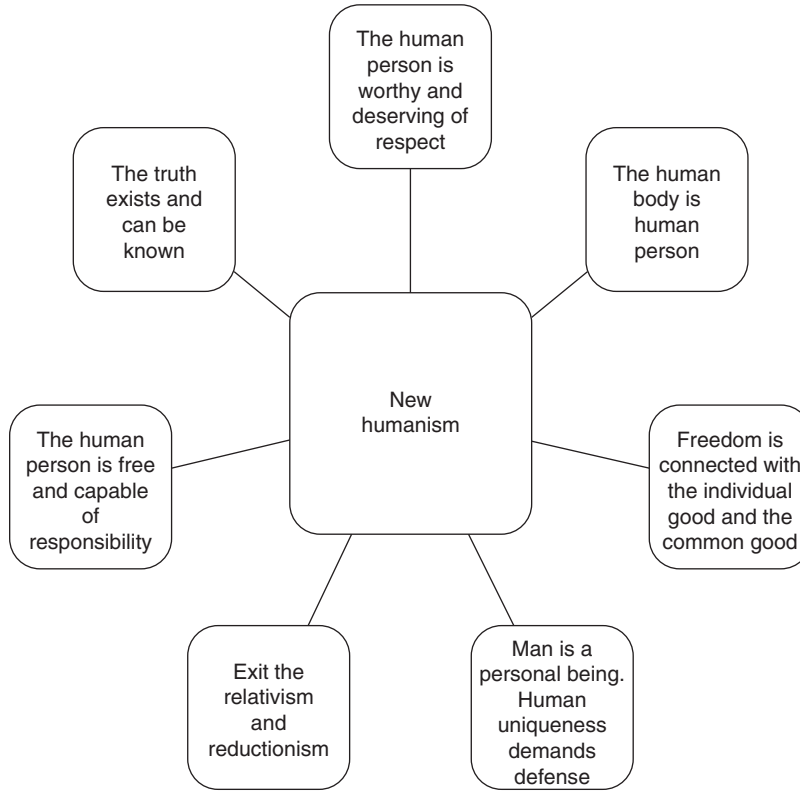


Fig. 7.2 The new humanism

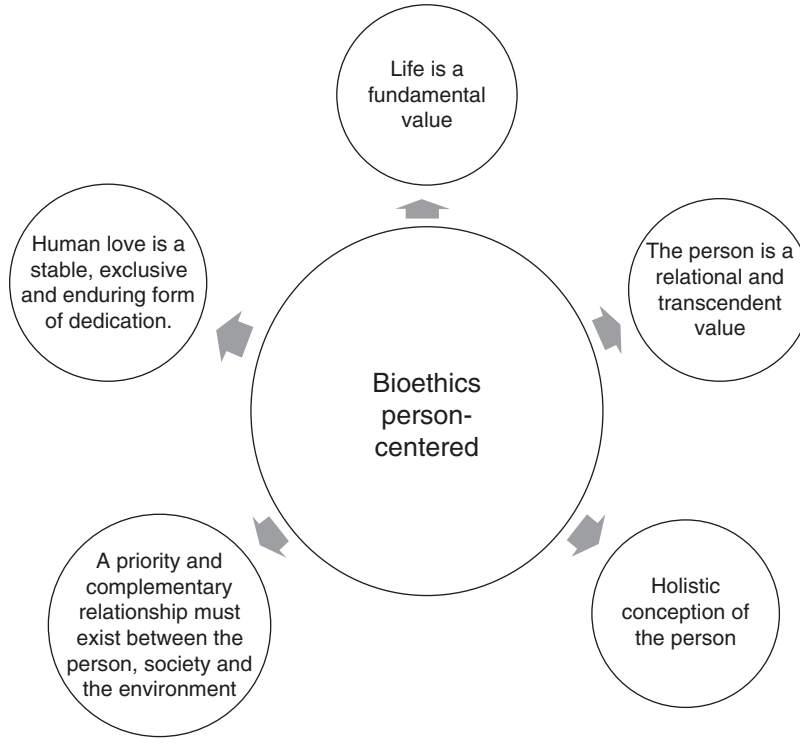


Fig. 7.3 Person-centered bioethics

In these areas, the exercise of dialogue is extremely important and it must be "constructive," since the point is to build, to add, and to propose the positive and good that can and should come from human action.

Dialogue has to play out at vastly different levels: dialogue between health professionals and their patients, among physicians themselves, to achieve real teamwork; between patients and doctors; between doctors and administrators, and doctors and nurses; between health service providers and the patient's family; between researchers and research subjects; within committees on bioethics; etc.

Humanizing Strategies

We can try to make these concepts more practical by combining the last two fields. In short, dialogue is needed to institute strategies that favor humanization (Fig. 7.4). The following are several possible humanizing strategies that require constructive dialogue.

- Example: while not acting to be seen by others, example is a major factor in change. Its influence has been known since ancient times. Julius Caesar's decision to separate from Pompei Sila, his second wife, when she became embroiled in a scandal perpetrated by Clodius during the festival of Bona Dea, is famous. He supported his decision by saying: "Caesar's wife must not only be virtuous, she also must seem to be so." We must set a good example in our personal lives and professional practice. Example is the result of coherence between what we think and what we do.
- Communication: Marshall McLuhan is one of the pioneers and leading thinkers of the information society. Concepts such as "the global village", "the medium is the message," "we are what we see," or "we shape our tools, thereinafter our tools shape us" have made him a visionary in our globalized world [14]. He also argued that communication is an extension of the person. This is what makes it so important; it is crucial to know how to establish, maintain, and direct communication. The causes of many of the problems addressed in clinical bioethics committees at health care institutions stem from difficulties with communication. Therefore, strategies to improve communication will always be important if we want to advance in the process of humanization.
- Accompaniment: medical students and interns are not the only ones who need accompaniment to perform well. Prudence dictates it is always good to seek accompaniment, especially when problems are more complex, even if one has a great deal of experience. It helps a lot to have an outside opinion, frequently free of the passion and bias that can come with proximity to the problem. Time and again we need someone to help us when we can't see the forest for the trees. Loneliness is almost always a bad counselor. It is an inseparable companion of professionals in health and education, who move in a hostile and disorganized system, one that often is imbued with ideologies that are harmful to the human person, the family, and society, and where corruption has also spread its tentacles. This environment is not the most conducive to making decisions, but when professionals know they are accompanied and supported by their colleagues, the institution, and their immediate family, they are far more likely to work well, sometimes with genuine heroism.

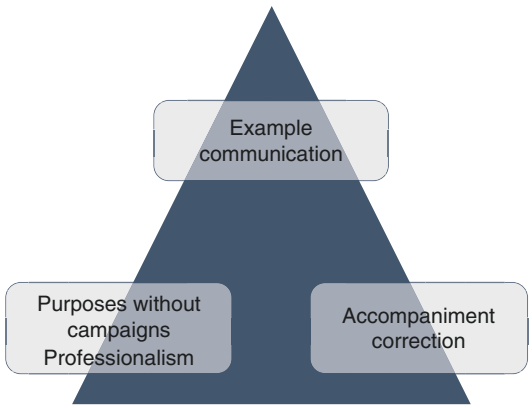


Fig. 7.4 Humanizing Strategies

- Correction: one of the finest expressions of charity is correcting someone who has made a mistake. Nevertheless, this practice is rare, not least of all because we work in an environment where selfishness and a culture of indifference prevail. Before proposing to help someone who has blundered, we often reject, ignore, or condemn them outright. It is crucial to promote a culture of joining in solidarity to help those who have blundered. This also would cure another disease of the medical profession: complicit silence. We must not perpetuate this culture of silence, which hushes up mistakes. If the goal is to humanize medical practice, we must take it upon ourselves to help those who have made mistakes by correcting them in a kindly, clear, and sincere way.
- Purposes without campaigns: as we have seen, an integral form of humanism is supported by human virtues [15]. Without them, professional practice does not go beyond the scope of what is technical or procedural: it seemingly is enough to "follow protocol". Campaigns not are the right vehicle to help build human virtues. These generic initiatives usually propose achieving a particular institutional value in a certain amount of time. However, it should be absolutely clear that situations or conditions are humanized not with values, but with virtues. And, how do you build virtues? You do so by helping to formulate specific purposes, setting achievable goals, and providing accompaniment (companionship, once again) to evaluate performance on the specific points that are slated for improvement. This task falls first and foremost to those who lead the work groups, and is based on two important assumptions: knowing one's subordinates and being close to what they do.
- Professionalism: health professionals in private practice and teachers in the academic community labor under the growing imperative that procedures be performed quickly and dictated, in many cases, by the economic interests of the agencies that have come to mediate in the health agent–patient–family relationship. We can longer assume neither that the circumstances of medical practice will stimulate an expression of professional virtues, nor that teachers can assume that students will see these qualities in action [16].

On the contrary, students might witness acts or omissions that damage this relationship and might adopt, for themselves, a standard that is not consistently professional. These constant encounters with unprofessional attitudes and behavior have jeopardized the standard of excellence that has characterized professionals in medicine, education, and research.

Professionalism is based on a service mentality, trust, altruism, accountability, excellence, duty, honor, integrity and respect for others. However, in the current environment of work in the health sciences, this notion is being challenged constantly and requires active and repeated reaffirmation from professionals to sustain it.

The key to practicing any medical specialty appropriately, from a bioethical perspective, is the ability to recognize and be conscious of the magnitude of the *dignity of each person*, so as to act accordingly. Above all, that action must be coherent: you must proceed according to what you think, which will always stem from what you are.

References

1. Pastor-García LM. Scientific evidence and bioethical discourse. Cuad. Bioét. 2009;XX(3):453–69.
2. Muñoz-Pérez B, Sarricolea-Errausquin ML. Dr. Lejeune: the educational challenge of bioethics 50 years later. Cuad. Bioét. 2009;XX(3):531–2.
3. López-Moratalla N. The zygote of our species is the human body. pers.bioét. 2010;14(2):120–40.
4. Steinbock B. Life before birth. The moral and legal status of embryos and fetuses. New York: Oxford University Press; 2011.
5. Barnett R. Learning for an unknown future. High Educ Res Dev. 2012;31(1):65–77.
6. Patiño-Restrepo JF. The health care in a model of commercial insurance. Rev Colomb Cir. 2011;26(1):9–10.
7. Gómez-Fajardo CA. Notes on some ideas of the Constitutional Court. Iatreia. 2006;19(2):155–63.
8. Hottois G. Definir la bioéthique: retour aux sources. Rev Colomb Bio t. 2011;6(2):86–109.
9. Tomás y Garrido GM. (Coord.). Understanding conscientious objection. Murcia: San Antonio University Foundation; 2011.

10. Huber M, Knottnerus JA, Green L, Van der Horst H, Jadad A, et al. Should health how we define it? BMJ. 2011;343:D4163.
11. Sánchez-Naranjo JC. Humanization of health care, art and humor therapy. Rev Med Risaralda. 2013;19(2):154–7.
12. Gamboa-Bernal G. Philosophical anthropology as key of Bioethics. Rev Roman Bioethics. 2013;11(3):130–7.
13. Alfaro RM. A communication for another development. Lima: Calandria; 1993.
14. McLuhan E. McLuhan, then and now. Infoamérica. 2012;7-8:23–9.
15. González-Blasco P, Pinheiro TR, Rodríguez-Ulloa MF, Angulo-Calderón N. Medical ethics: an educational resource that facilitates learning. pers.bioét. 2009;13(2):114–27.
16. Wynia MK, Latham Jr SR, Kao AC. Medical professionalism in society. N Engl J Med. 1999;18: 1611–6.

8 Cosmetic Psychopharmacology, Inauthentic Experiences, and the Instrumentalization of Human Faculties: Beyond Post-emotional Society

Luis E. Echarte

Abstract

This chapter explores the phenomenon of inauthentic experiences as a negative consequence of the new trends in cosmetic psychopharmacology, concluding that they are also the psychological manifestation of the tension, in late modernity, between two rival versions for a moral paradigm—Classic and Modern—and in which transition, rationality, affectivity, and will are being progressively and consecutively instrumentalized. Moreover, it is argued that this post-emotional scenario poses a threat to mental stability as well as social cohesion. The second general objective of this chapter is analyzing three types of psychological complaints by patients about inauthenticity—those related to the artificial origin of emotions, to the physical nature of its content, and to its episodic coherence; on the other hand, I present and compare three rival contemporary solutions to the problem of inauthenticity: the psychological, the organic, and the narrative.

Keywords

Medicalization • Authenticity • Transhumanism • Personal identity • Cultural paradigms • Cosmetic psychopharmacology • Transcultural psychiatry

No man is an island entire of itself. John Donne

Introduction

Not only surgical procedures such as deep brain stimulation (DBS) but also psychiatric drugs, as well as psychotherapy, can unintentionally change personality. Some patients describe these consequences as harmful unpleasant experiences

L.E. Echarte, MD, PhD
Mind-Brain Group, School of Medicine/Institute for Culture and Society, University of Navarra, Irunlarrea s/n, Pamplona, Navarra 31008, Spain

Unit of Medical Education and Bioethics, School of Medicine, University of Navarra, Pamplona, Navarra, Spain
e-mail: lecharte@unav.es

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_8

around identity, in which it seems to be corrupted or damaged [1]. The fear of inauthentic life is, in this context, fear of an artificial, disjointed, non-self-determined way of being, among other things.

Antonio Damasio's model of the self provides a neuroscientific basis to help psychiatrists distinguish and classify different types of inauthentic experiences (IEs) and treat each one appropriately [2, 3]. Basically, Damasio identifies three levels of identity: (1) *Protoself*, at the lowest level, would comprise a set of multiple practical identities, (2) *Core self* would emerge from Protoself as a first type of unified moral self, and, in turn, (3) *Autobiographical*—coming from Core self—would be a temporal representation of the Core self/environment relationship. In this context, IEs may be understood as contradictory representations that can co-exist at the same level (horizontal dislocations or experiences of alienation) or at different levels (vertical dislocations or, strictly speaking, experiences of inauthenticity). If so, a significant part of suffering IEs would be related to the innate human tendency to keep and protect coherence in the webs of beliefs within and between levels [4].

Damasio also points out the importance of social factors in the development of human identity at each of these three levels, in particular the last one. On that basis, some authors even speak of a fourth one, where shared cultural ideals constitute the highest corporeal meta-representation of the human being—a sort of cultural embodiment [4]. This chapter explores the particular Western cultural etiopathogenesis of inauthentic experiences—what is called in the field of clinical ontology the *problem kappa* [5].

Losing Me in Society

As a starting point, I use Charles Taylor's book, *The Ethics of Authenticity*, which is one of the most widely cited works on this subject. According to Taylor, we are currently witnessing a psychological and social battle between two cultural worldviews: Classical and Modern. This scenario would explain many of the malaises of modernity, concretely the experience of "loss or decline, even as our civilization 'develops' " [6] (p. 1). Despite this war being fought on many fronts, Taylor identifies and links three central worries: individualism, disenchantment with the world, and, as a consequence of the other two, the emergence of industrial–technological societies. In section one, I discuss certain aspects of the notion of authenticity in the classic worldview, which, as we shall see, has certain areas of convergence with the reason–passion binomial, as interpreted through Damasio's perspective. I then go on to identify the most important factors related to the change of worldview and, finally, the connection between the technification of society and IE.

The Great Chain of Being

The classic worldview, in place for 2 millennia and in decline for the past 200 years, holds that a hierarchical order in the universe gives meaning to our world and our activities in society. Those who still consciously embrace this paradigm believe that "[t]he things that surround us were not just potential raw materials or instruments for our projects, but they had the significance given them by their place in the chain of being. By the same token, the rituals and norms of society had more than merely instrumental significance" [6] (p. 3). From this perspective, the hierarchies of human society both reflect a natural harmony and also participate in it because, among other things, human beings are not exceptions to the structure of the cosmos. So, if we are part of this cosmic order and have our proper place in the universe, no individual should completely arrogate her own existence. On the other hand, the author concludes, a *belief in natural law* also implies that some realities and relations are good simply in themselves, beyond their spatial and temporal context. These immutable ideals, written in the stars or in God's mind, are knowable by human rationality, and are applicable to all individuals.

Nowadays, many historians hold that this worldview comprises one of the deepest roots of Western culture. For example, Werner Jaeger

locates its origin in the ancient Classical educational model, which is built around the belief in harmony, objectivity, and intelligibility. "By discovering man, the Greeks did not discover the subjective self, but realized the universal laws of human nature. The intellectual principle of the Greeks is not individualism but 'humanism' […] It meant the process of educating man into his true form, the real and genuine human nature […] the man revealed in the work of the great Greeks is a political man. Greek education is not the sum of a number of private parts and skills intended to create a perfect independent personality" [7] (pp. 16–17). In fact, that type of education—the *Paideia*—was adopted in the Roman Empire and was used all through the Middle Ages and the early modern period [8, 9, 10].

Aristotle is one of the most well-known and influential exponents of theoretical systematization in the classic worldview. Only in this light it is possible to fully understand why, in his theory of emotions, rationality has an important educational role. Thanks to rational emotions, human beings learn to "find enjoyment or pain in the right things" [11] (II, 3, 1104b 13–14). However, rational emotions depend on the acquisition of virtues, namely, on the individual's efforts to interpret and moderate basic emotions, memory, perceptions, and so on [12] (p.105). When rationality is introduced into our desires, they then become as good as any other universal value. Put in normative form, the optimal human moral condition is to desire cosmic harmony. In Aristotle's words, "Anyone can get angry—that is easy—or give or spend money; but to do this to the right person, to the right extent, at the right time, with the right motive, and in the right way, that is not for everyone, nor is it easy; wherefore goodness is both rare and laudable and noble" [11] (II, 9, 1109 a 27). In this situation, the whole body, with its different parts, with its actions and passions, reaches a dynamic unity that is a reflection of the highest order of things. I should like here to clarify that, in this chapter, I use the term *passion* to refer indistinctly to emotion and feeling.

In the Classic interpretation, affectivity has a double dimension: pragmatic and referential. With regard to the first dimension, emotions help us to move toward the realization of the human ideal—in which, as I said, emotions have more than an instrumental role; that is to say, they do not disappear when one's goals are achieved. With regard to the second dimension, when treated appropriately emotions are valuable sources of information about the world and about oneself. Returning to the context of authenticity, the human heart is disclosed here as being an important mirror of identity—looking into it, I can know who I really am, and by showing it to others I can sincerely express myself. Furthermore, passions reveal the tension between the *factual me* and the *ideal me*, between what I am and what I should be. From the Aristotelian perspective, both are part of *my* identity because *I* am also my emotions (the mirror in which I look at myself is in fact part of myself) and, further, because they are present in the ideal of the human being.

The distance between the ideal and the real human being, in the classic worldview, is not completely bridgeable. Entities of the sublunar world will always be an imperfect image of those beings that make up the incorruptible, supralunar reality. In other words, some inauthentic experiences are connatural to individuals—hence the tragic human condition. However, the feelings that follow are not as disturbing as they might seem and, in fact, are part of human growing-up. It is thanks to the matter–spirit duality (not to be confused with the matter–soul binomial) that human beings are able to reach a degree of unity that is qualitatively superior to those beings without it. As Robert Spaemann writes, "Aristotle has made a radical ontological break between 'soul' and 'spirit'. God, for him, is pure spirit, and the being of the spirit is life; soul, on the other hand, is the principle of a lower form of life, the life of material bodies. There is a class of ensouled beings that also possesses spirit; that is the class of human beings" [13] (p. 151).

However, this Aristotelian identity, typical of free men, is only attained by having knowledge of the *good of the universe* of which the human ideal is also part. "And all things are ordered together somehow, but not all alike—both fishes and fowls and plants; and the world is not such

that one thing has nothing to do with another, but they are all connected. For all are ordered together to one end, but it is as in a house, where the freemen are least at liberty to act at random, but all things or most things are already ordained for them, while the slaves and animals do little for the common good, and for the most part live at random" [14] (XII, 10, 1075a l2–23). The experiences of both human gaps—between the ideal and the factual, and between the material and the spiritual—do not disappear; instead, they are eclipsed by the experience of *eudemonia*, which emerges from understanding and following this supreme order.

Applying Aristotelian perspective to the IE problem, it could be argued that inauthenticity should be perceived as part of human life—a physiological symptom—at least for young people, that is, people with immature selves. However, this natural suffering is also an opportunity, a means, to arrive and express higher levels of perfection.

The Greek *polis* is the principal sublunar expression of this supreme order, and individuals should sacrifice even their own lives for its sake—here, we have the heroic human condition. In this way human identity reveals its true shape—alterity—and also where the pursuit of well-being acquires meaning. Of course, it must also be assumed that at least some alienated experiences will appear over the course of the social process of achieving a life of excellence. However, they should soon be neutralized by new and noble sentiments.

Being a good citizen means aspiring to fulfill human ideals in the above-mentioned context, that is to say, with the right intention and with a willingness to engage in combat—both features conform to what Classical writers called *Areté*. Thus, the battlefield is the best place to know myself—to prove who I am. There, the pragmatic and referential dimension of passions reveal their intimate and ultimate relation: emotions appear as the best mirror—for they offer an authentic reflection—when they are being directly used not to describe myself or anything else, but to change—improve—the polis. In short, the heart must be viewed from the outside.

The Aristotelian connection between reasons and passions fits, in many respects, with Damasio's approach. Firstly, neither of them thinks that passions are stupid, purely passive and mechanical reactions. In fact, what Aristotle calls "*deliberative desire*" bears some resemblance to what Damasio defines as feeling [11] (VI, 2, 1139 a 21). Secondly, because of this kind of connection, for both authors affective education must not rely on a despotic imposition—a productive action—but, as philosopher Leonardo Polo has pointed out, rather primarily on a dialogic learning—a governing action [15] (p. 198). This means that the acquisition of virtues is not just a question of *osmosis*, that is, of mere exposure to values, but of understanding. Thirdly, by the same token, Aristotle and Damasio share beliefs about how the abstraction of ends (the decoupling of behavior and means) generates intensely pleasant, creative, and productive subjective experiences [3] (pp. 294–297). Undoubtedly Aristotle goes a step further, recognizing that teleology is not a creation of mind but a real natural cause. The difference is great, because believing that human ideals have more than an evolutionary meaning and survival value increases the enjoyment of the contemplation of such ideals.

Humanism is one of the most totalizing narratives that has ever existed. With it, human beings have been furnished with many effective strategies to control and use passions and, ultimately, to promote cooperative behavior. No matter whether they are true or false, these beliefs have offered the human brain, for a long time, an optimal breeding ground for the consolidation of a unique and strong self… at least to some extent.

However, there is a problem with this paradigm from Damasio's point of view: it ontologically justifies the final steps of the evolutionary escalation of the representational process. Agents with an Autobiographical self tend naturally (have a biological predisposition) to think of themselves as timeless and spaceless (as substances and responsible agents), which are beliefs that may give rise to dualistic lifestyles and finally to the worst type of vertical IE—one of those that lead to pathological conditions.

Understanding our own nature implies accepting, according to Damasio, that the time has come for us to escape the chains of physical events: in order to survive we must divert ourselves from our current biological direction. The philosopher of mind Daniel Dennett supports a similar thesis in chapter 8 of his work *Freedom Evolves*. However, Dennett's alternative is not a Spinozan one. For him, the solution involves giving up attitudes associated with realism and choosing beliefs in accordance with the human need for survival. The difference is enormous. For Damasio, knowing the true implies rejecting false beliefs and, thanks to that, surviving. For Dennett, surviving implies rejecting the belief in truth itself [16]. But ultimately, both Damasio and Dennett defend the notion that human substantial identity is only a fiction generated by the autobiographical self.

Of course, this kind of criticism makes sense only if we reject the idea of the good of the universe. In contrast, if there is such a good, Aristotle is right to point out the useful and transcendental connection between the Autobiographical self and *cultural identity*—between subjects and the polis, and between subjects and their spirits. If there are substances, human beings should follow the path that provides their biology.

Whoever is right, it is a matter of fact that the classic worldview is still present in our contemporary culture and hence continues to percolate down to at least our Proto- and Autobiographical selves.

Lovers: A Fragile Balance

The later introduction of Judeo–Christian ideas into Western culture boosted even more, on one hand, the pursuit of deliberative desire and socialization and, on the other hand, a sense of coherence and security. After all, the Universe has been created by an omnipotent and benevolent God, who has revealed Himself to the world—to the human mind—a God who deserves to be loved and who commands us to love.

However, Christianity gives greater weight to the *subject* (from the Latin *subiectus*—lying beneath) in the sense that each individual is not understood as identical to her nature. The new perspective implies, among other things, that individuals are responsible for many of their decisions: they are able to own their nature. *Moral conscience* and *personal identity* are other expressions linked to this self-understanding. The defense of responsibility, a belief the Greeks considered to be a natural delusion—we could say that a delusion associated to vertical IE, is first of all based on theological arguments. In addition to disease and ignorance, there is another reason for loving the darkness: a free personal choice. In fact, as Spaemann claims, only on this condition can isolated individuals be deserving of punishment by a righteous divinity [13] (p. 21).

A third gap is introduced into the puzzle of identity: *what I am*, *what I should be* and, the new element *what I want to be*. With the last, new meaning is added to the Greek *Areté*, namely, the role of intention and struggle in the definition of individuals. There are two main consequences. First, the outlines of the *human ideal* become blurred, because not everything seems to be written in heaven. *God listens to those who pray, and grants things to those who ask with faith*. The new framework leaves room for creativity and a certain amount of self-government [17, 18]. Secondly, the gap affects the sense of community. The call of God is vocational: it is not just open to novelty, but is personal as well. Along these lines, the early Christians believed that everyone, after death, must go through a *particular judgment* before God, in which the repayment for the acts committed by individuals will be determined independently of the sins that communities have committed. In fact, these followers of Christ believed that individuals are prior to the polis. It was the discovery, Spaemann concludes, that the man who dies for his fatherland is more than his fatherland [13] (p. 19). But, if this is so, the lines of humanistic ideals are blurred even more.

The Christian solution to the three gaps bears some resemblance to that of the Greeks' because, for the former, alterity is also a central element. The doctrine of the Trinity shows that there is one

God in three Persons. On the one hand, God's nature is one. On the other hand, the divine Persons maintain a reciprocal relation of infinite love for/with each other. Both features explain, from different perspectives, God's oneness. In the same way, for Christians, each individual, as *an image of God*, shares her nature with other human beings—a nature that we need to know and implement in order to attain happiness. Besides, as persons, we have to find ourselves in extending our love to others [19]. It is only in this way that what is initially a fracture becomes the most valued gift. "No one can have greater love than to lay down his life for his friends" (John 15:13). This is not a (Greek) heroic end; instead, it's the way for an individual to gain resurrection—the final triumph of the subject. Apart from this, in practical terms, Christians for a long time followed the theory of deliberative feelings. Faith and reason support each other and, together, nourish the heart. It is the heart, in turn, that defines the individual. Only the heart in action—a loving heart—can reflect the agent's identity, the agent's unity.

In the promise of resurrection, we discover the last important difference from the Greek point of view. Christian salvation comes from God's intervention, both during our earthly life as well as in the afterlife. Of course, this *grace* is mediated, according to early Christians, by the community, which reinforces even more the recognition of human alterity. A similar result develops from two other Christian beliefs: first, the proclamation of the Kingdom not only to Israel but to all the Gentiles; and second, the universal judgment at the end of the time. The civilizing purpose of Humanism is extended to the entire human race. In addition, as mentioned, this *extended love* is not just a matter of pure altruism. Each individual's happiness is put at stake.

On one hand, the Christian revelations lent even more credibility to the congenital human belief in substances and against fatality but, on the other, they introduced certain important tensions into the humanist worldview: subject–citizen, particular judgment–universal judgment, moral conscience–grace, life–sacrifice, ideals–creativity… If the central nodes of such network were able to remain united—incorporated in our mind—it is because its structure is held by a powerful religious belief: divine wisdom—*Deus ex Machina* solution. However, it is true that, from very early, believers begin to use Greek philosophical tools to give a rational robustness to Christian faith—theology.

Nevertheless, this mighty effort appears to have been insufficient: the course of history would still tilt the balance toward the subject. The process, which would take more than one and a half thousand years, reaches its peak with modernity. I shall present, in the next section, three catalysts for this change (C1, C2, and C3 from now on), particularly those directly related to the emergent ideal of authenticity.

The Fall of Humanism

The first milestone (C1) in civilization's downfall has to do with the introduction of a Cartesian approach. Catholic philosopher René Descartes breaks with the hegemony of the Schoolmen—and their Aristotelian influences—when he proposes a trialistic ontology: *res extensa* (extended being), *res cogitans* (mental substance) and *God* are the three substances that make up reality. This means that each one can exist without the existence of the other two. The conclusion also has epistemological implications: the natural sciences, humanities, and theology enjoy significant autonomy with respect to each other. The only link among them is God, who assures that there is a unity among the sciences.

Concerning human beings, Descartes holds that the mind and the body are separate substances. In the words of Damasio, Descartes suggested that "reasoning, and moral judgment, and the suffering that comes from physical pain or emotional upheaval might exist separately from the body" [20] (p. 249). Just as important, in Descartes' anthropological dualism, passions are placed on the side of the physical world, and the self is on the spiritual side. In other words, the first are generated by purely mechanical laws, and therefore they have to be governed—have their excesses corrected—just as humans control

machines, namely, by changing their physical causes. In contrast to the theory of deliberative feelings, for Descartes, the passions are not susceptible directly to convincing arguments, but only deserve—respond to—despotic impositions [21]. Passions, like perceptions, are moved by physical forces. However, according to Descartes, this does not mean that they are only linked to physical realities. In fact, some of them speak of the soul and some of the body–soul connection (Principles of Philosophy, AT VIII 23, CSM I 209). But how, then, do passions reflect the soul? This is no merit of theirs, but rather of the soul which, by humbling itself to the level of the material world, can receive and control this kind of physical images, namely, neural encoding."Nature likewise teaches me by these sensations of pain, hunger, thirst, etc., that I am not only lodged in my body as a pilot in a vessel, but that I am in addition so intimately conjoined, and as it were intermixed with it, that my mind and body compose a certain unity. For if this were not the case, I should not feel pain when my body is hurt, seeing I am merely a thinking thing, but should perceive the wound by the understanding alone, just as a pilot perceives by sight when any part of his vessel is damaged" (Descartes, Sixth Meditation, Part 13). How is this possible? Here lies the main difficulty of the Cartesian proposal. Descartes does not explain the nature of this particular *movement of the soul.* This difficulty is usually formulated as the *mind–brain problem*.

Other consequences of this new ontology (in this case, in relation to paths of self-knowledge) are, first, that passions seem to offer very poor images of the self. They are only mirrors of what is going on in the body. Intellectual introspection is now the optimal method for understanding the essence of an individual's identity—including her ideals. In fact, Descartes denies that teleology is an inherent feature of physical entities. If we can infer goals from them it is because they—like machines—have received these goals from God or from another intelligent being. In other words, to understand the meaning of physical phenomena (including our own behavior) we need to look away, toward their external sources. Notice how the Cartesian approach is opposed to the idea of the *primacy of action*, described above. In order to explain behavior, the first factor is the monolithic self; it is only afterward that the material execution of its plans comes into play.

Within the Cartesian framework, it makes no sense to talk about a dislocated self. The gap is only in the relation between the self and the body. The pursuit of unity between the two worlds has to do mainly with an intellectual self-knowing achieved through introspection. There, the ideals of the human being are perceivable: for example, loving God and one's neighbor. However, human beings must tame the body: it is only in this way that they can put these ideals into practice. Phrasing it in biblical words, *the spirit is willing, but the flesh is weak*.

The undermining of human behavior is the second catalyst (C2) along the slippery slope. Here, the intellectual and social phenomenon in question is due to theological arguments, concretely those defended in the Protestant Reformation. Theologians like Martin Luther and John Calvin expounded the belief that salvation is not earned by good deeds but through faith in Jesus Christ. From this perspective, God keeps an even greater distance from the earthly world than in Cartesianism [22] (pp. 157–159). The physical world is corrupted by sin: no ideals can be inferred from it, even from the human body. Contrary to what Catholics believe, God does not intervene in this mess in any way—nor does He intervene in our behavior, which tends inevitably to evil and ruin. God's grace affects only the soul— a self that, because of original sin, cannot direct the body toward good. Moreover, saving grace comes not from the community but solely from the individual's faith. In fact, what leads to God is the subject's recognition, first, of this tragic divorce between body and soul, and second, of her incapacity to remedy this problem without divine aid [23].

The reformist view gives priority to introspection over objective knowledge much more than Cartesianism. *Your heart, not your behavior, tells you who you really are*. Here *Areté* ends up being radically transformed: the fight for identity and salvation takes place in the mind — in the task of attaining good thoughts. Of course, the reformers are referring to the intellectual heart — not the emotional, which is part of the perverse world [24] (pp. 63–64).

From very early on, numerous philosophers began to rationally justify Puritan theological attitudes. One of the best-known examples is Immanuel Kant. According to him, duty must be the motor of good choices, not the passions. There are two reasons for adopting this strict posture. First, the passions tell us almost nothing about reality or even about our own body [25]. Second, morality is not about the pursuit of identity and happiness; as Judy Hughes writes, it is "about becoming worthy of happiness by heeding the call of duty" [26] (p. 72). In this sense, Kant goes further than Descartes: the passions are not even worthy of being governed tyrannically, they merely deserve to be ignored. This means that the individual's happiness — the overcoming of identitarian gaps — is a matter of God's choice. In sum, if Descartes takes God out of the physical world, Kant takes him out of the moral world, for it isn't necessary to believe in God in order to recognize what is good or bad.

Like Descartes, Kant infers human ideals through rational introspection but, unlike Descartes, he makes them dependent on the subject and not on the chain of being. This becomes clear, for example, in Kant's *Principle of Respect:* never treat a person merely as a means, but always as an end. This is also the case of Kant's *Categorical Imperative*: you are to "act only in accordance with that maxim through which you can at the same time will that it become a universal law"(*Groundwork for the Metaphysics of Morals*, 4:421). In both, any ideas about higher orders have disappeared, and with them the classic solution to IE.

Kant's ideals are oriented toward enabling the encounter between different subjects, each one with particular interests. The Kantian solution is to think of one's neighbor as a potential enemy, and then regulating the fight so that it can be as beneficial as possible for both. In this context, it is clear why the golden rule ends up being formulated in its negative form: don't do to others what you would not like them to do to you.

Likewise, Kant asserts a "negative" ideal of freedom. "It is only in a Society which possesses the greatest Liberty, and which consequently involves a thorough Antagonism of its members — with, however, the most exact determination and guarantee of the limits of this Liberty in order that it may coexist with the liberty of others — that is the highest purpose of Nature, which is the development of all her capacities, can be attained in the case of mankind" (*Principles of Politics*, Fifth Proposition). In short, *my freedom ends where that of the other begins*. It was not only Kant: many other modern philosophers, like John Locke, Adam Smith, and John Stuart Mill, have helped to popularize the idea of negative freedom and the necessity to separate public space from private space. Thanks to all these thinkers, the gap between the physical and the intellectual worlds finds its correlate in a new way of understanding community.

The third milestone (C3) of the fall of Humanism is the process of secularization of Christian faith. This has had a variety of consequences. First, because in the Cartesian view, the unity of sciences depended too much on theological arguments, secularization fuels the phenomena of hyper-specialization and disintegration of academic discourses. It is no accident that many contemporary authors blame Descartes for what Charles Percy Snow named The Two Cultures: the contemporary divorce between the natural sciences and the humanities (the sciences of the spirit). If nowadays there are many difficulties that hamper establishing a dialogue this is because, first, for a long time, each of these spheres of knowledge saw themselves as self-sufficient and, second, because neither of them knew, thanks to Descartes, how to carry on such a dialogue. Undoubtedly, this scenario does not help one to believe (without divine help) in the unity of being, and hence, in the unity of the sciences [27].

This kind of scenario is, in turn, linked to the emergence of the various monistic approaches. Many idealist and positivist movements may be considered consequences of the fruitless attempt to solve the mind–brain problem. What results is a renunciation of understanding or a refusal to accept the existence of the physical or spiritual world, respectively. Finally, a goodly number of

authors, beginning from these reductive approaches, end up rejecting realism. How could the solipsistic mind, by itself, produce objective statements? Or how could the physical body do it? David Hume's theory of causation is one of the first and most radical modern examples of epistemological skepticism. It may be the case that objects are connected by causes but, for Hume, we do not know this with any certainty. If the chain of being exists, it is beyond our reach [28] (p.10). The fact that a large number of non-believers simultaneously maintain relativistic positions seems to have more to do with the history of ideas in the West than with timeless strong arguments.

The loss of faith and a widespread disbelief in the natural law is another frequent (but not necessary) connection in modern societies. From this perspective, there is no individual or community responsibility to a supreme order — no more externally imposed blame. We human beings thus appear, in theory and practice, to be the only source of goals and rules. For the same reason, the pursuit of identity and happiness will depend, basically, on the subject's decisions and social agreements. So, it is the end of external ideals. The true *me* is what I choose because my will is the sole criterion (the ideal) of authenticity. Friedrich Nietzsche is one of the first modern exponents of this idea about the will as cohesive criterion of identity and moral behavior. For example, in *Beyond Good and Evil*, he writes, "The individual has always had to work hard to avoid being overwhelmed by the tribe. If you try it, you will be lonely and sometimes frightened. But no price is too high for the privilege of owning yourself" [29].

Human beings seem to be released, in the final stages of the secularization process, from physical and rational bonds. The only limits — the only logic — are those which the means impose on us. In this scenario, human intelligence is no longer the faculty with we attain human ideals, but rather the tool with we overcome obstacles to our will. Taylor calls this new understanding of the role of intelligence "the *primacy* of *instrumental rationality*." "By 'instrumental reason' I mean the kind of rationality we draw on when we calculate the most economical applications of means to a given end. Maximum efficiency, the best cost–output ration, is its measure of success" [6] (p. 5). In contrast to reason, the passions begin to be understood as the new and indisputable source of morality: they offer the legitimate goals of self-fulfillment. For instance, it is because of his theory of causation that Hume defends positions that are close to instrumentalism. "Reason is, and ought only to be the slave of the passions, and can never pretend to any other office than to serve and obey them" (A Treatise of Human Nature, 2.3.3). Reason cannot evaluate ends or passions. Only passions can select and evaluate ends. In a similar but most radical way, Nietzsche contrasts, in *The Gay Science*, rationality with a "veritable delight in madness" — namely, the passions. Clearly, this revulsive rejection of Puritan principles does not return sentiments to their original place — to Areté. Instead, they acquire a self-referential sense: a positive emotion is justified by its own presence, independently of any external logic, of triggers, or even of the agent's will.

The hegemony of the passions has, among other important consequences, that of the strengthening of the introspective attitude toward happiness. This ultimately leads, according to Taylor, to hedonistic and narcissistic lifestyles, which in turn leads individualism to unprecedented levels in Western societies, levels at which the classic worldview comes closest to realizing its point of maximum instability [6] (pp. 16–17).

From Instrumental Rationality to Instrumental Emotions

The above-mentioned catalysts, each in a different way, have all contributed to the arising of the modern paradigm, whose gravitational center, according to Taylor, is the ideal of a *self-determining free will*. I have an authentic life when I am radically free, namely, "when I decide for myself what concerns me, rather than being shaped by external influences" [6] (p. 27). Undoubtedly, in the historical process of the development of the subject there have been many

positive achievements, especially in human rights. However, there are also defects, problems, concerns, and risks associated with the new network of beliefs. In this section, I will touch on those directly associated with the experience of dislocation of the self.

As I have explained, we should not think about the implementation (embodiment) of the Classic and Modern paradigms through the structure of an *all-or-nothing* logic. There are several intra- and extra-epidermal identitarian planes, with connecting links but also with a certain separation that makes it possible for contradictory ideals to coexist. On one hand, some Classical ideals would survive, due to their capacity to remain, primarily, outside our awareness (those placed in the social structures — a sort of Protoculture — and even in the Protoself), and secondarily, in certain roles within the individual's private space (in the Core-culture and the lowest levels of Core self). This would explain, for example, why the Protestant reformation has had a significant impact on Catholic communities, and why the same applies to the process of secularization amongst all Christians, and similarly, why many agnostics continue to think in dualistic terms and to value the general good over particular interests, and so on.

On the other hand, Late Modernity is a troubled era where numerous important things have happened in a short space of time. As a result, many conscious ideals (that are somewhere between the Core and Historical Culture and the Core and Autobiographical Self) are perceived simultaneously as conflicting and as tempting. It is precisely in this context that the greatest number of potentially pathological IE occur [4] (section 1.3.C, pp. 150–163).

Social changes are inevitable and are not necessarily harmful. Or, at least, they are inexorably harmful, even though they come about in a very short time. However, there are other more intrinsic problems with the Modern paradigm: those related to the effects of the ideal of free self-determination in the development and living out of human identity. In the next sections, I will argue that these effects are creating a new kind of vulnerable group that are characterized by the inability both to manage suffering and to resist the influence of biotechnological marketing.

Individualistic Sufferings

The first problem relating to the ideal of a self-determining free will concerns the surrender of coherence to autonomy. Continuous instrumental use of rationality would undermine Core and Autobiographical representations because the pursuit of emotions, always volatile and capricious, does not provide as strong a cohesive criterion as does the pursuit of objectivity. On the contrary, any unpleasant emotions (included physiological ones) would be *pathologized*. Indeed, when the *logic of passions* is in play, the better option to avoid *growing pains* is not to abstract any behavior out of the context in which it originates. At the same time, it is unavoidable that, due to such voluntary slowdown to the Protoself level, individuals will experience the oddness of the fragmentation: specifically, alienated feelings. Finally, in the Modern carpe diem lifestyle, it is expected that stress and anguish would appear as a result of the refusal of the Core and Autobiographical Selves to disappear — unconscious defensive mechanisms would be triggered in defense of the cohesive web of beliefs.

Another serious and paradoxical consequence has to do with the intensity of emotions. First, in regards to the above mentioned deconstructive process of the personality, the normative power of the hedonic attitude — the sole common factor among all distancing personal roles — loses steam because the individual's will is settled in the Core and Autobiographical selves. Second, the instrumental use of rationality implies, in practical terms, a reduction in the formation of deliberative desires. If existential ends cease to be contemplated (observed by reason) then the *sublime feelings* will also tend to go away. In other words, individualism places the human being in a *disenchanted world* where, as Taylor writes, '[p]eople no longer have a sense of a higher purpose, of something worth dying for. Alexis de Tocqueville sometimes talked like this in the last century, referring to the "petits et vulgaires plaisirs" that people tend to seek in the

democratic age. In another way of putting it, we suffer from a lack of passion. Kierkegaard saw "the present age" in these terms. And Nietzsche's "last men" are at the final nadir of this decline; they have no aspiration left in life but to a "pitiable comfort" ' [6] (p. 4].

That is the reverse of individualistic suffering: positive emotions are less pleasurable when they are not accompanied by strong motivations and meaning. Here, the terminal stage would be a total emotional disengagement: the moment in which the subject does not really care about passions. Then, experiences of an existential void — depersonalization or disembodiment — may become really destructive.

In the long run, practices associated with instrumental reason would induce not only apathy but also the wearying of the locus of control. First because the organism's self-control is supported mainly by conscious rational processes in which different ends are decoupled from means and behavior in order to compare them and to choose the one with most value. Leaving such a task in the hands of low-order automatic processes implies the elimination of profound experiences of autonomy. The second cause has to do with how the hegemony of the Protoself process in decision making (at the conscious level) is associated with the experience of the hypertrophy of the public self. The loss of autonomy is revealed as an obvious fact when the individual's passion reflects clear patterns of stimulus–response. Indeed, few things are less libertarian than a predictable heart. Of course, IE that originated in the Core self would be less intense than those whose origin is in the Autobiographical self, because the latter would be most susceptible to the logic of the timeless present.

The last sorrows come from the human social dimension. Individualistic ways of dealing with choices lead, in many people, to undervaluing those ideals that are maintained by the majority or, at least, those that are not generated within the subject's conscious system. In other words, it is believed that, first, authentic and autonomous behavior only arises out of the heart and, second, being original (even a *freak*) is a sufficient proof of the purity of that inner voice. By the way, this kind of beliefs has been especially justified and encouraged in certain neo-romantic and existentialist trends of thought in the second half of twentieth century. For example, Jean Paul Sartre, in his first philosophical stage, writes: "For I believe that a man always makes something out of what is made of him. This is the limit I would today accord to freedom, the small movement which makes of a totally conditioned social being someone who does not render back completely what his conditioning has given him" [30].

The problem with the existentialist attitude is that it implies wanting to set aside a kind of social input that, on one hand, continues to be processed by non-conscious systems and, on the other hand, is very necessary to the development and sustaining of the conscious self. Like it or not, if Damasio is right, every human being is partly *"others,"* right from the lowest level — the Protoself — to the highest — as a cultural being. Put in its simplest form, without social ideals, originality seems neither possible, nor meaningful, nor of any value. On the other hand, if the Modern ideal shrinks the limits of identity, then it is reasonable to believe that IE may emerge as the effect of a still stubbornly present cultural identity. Specifically, this type of IE would be similar to that described in the case of associative prosopagnosia or of body integrity identity disorder (see previous chapter, section 3.3.A). Finally, also here unconscious defensive mechanisms may play a role to protect social ideals, that is to say, culture identity.

To conclude this section, I want to highlight that the disturbing experiences described above do not just affect those who embrace individualism; rather, they affect everyone, at least as far as the question of recognition is concerned. Radical individualists do not want to feel properly recognized by others — only equals may do this, and individualists try to be different. And conversely, they do not care about giving recognition (compassion) to others because it would be boasting about a weakness: the subject's likeness. Moreover, this problem occurs even among those who share the pursuit of difference, since they would see each other as mere instruments or competitors. Expressed in poetic terms, individualists

would feel like strangers even in their own land. However, according to Sartre, that would not be a problem. "If you seek authenticity for authenticity's sake you are no longer authentic." This is because only when the pursuit of authenticity is a real movement of the heart, and nothing else, can subjects be alone and happy at last. Obviously, this is, for Sartre, a fanciful possibility [31] (p. 4).

Actually the problem is even worse than this, because complicity is not a necessary condition for individualistic sufferings. A community partly contaminated with such radical segregation may be harmful to both followers and non-followers of the Modern ideal. Here, IE would emerge at all levels, even in the representations elaborated on the basis of the Protoself.

Virtual Re-embodiment

Sooner rather than later, social miseries promote changes in lifestyles. *Technification* is Taylor's name for the shift in Western cultural dynamics that has taken place in the second half of the twentieth century. It is characterized by the seeking of "technological solutions even when something very different is called for" [6] (p. 6). This perspective also includes the pursuit of happiness. In this section I will blow up some of the main ideas about the causes of change.

Today more and more people tend to think, first, in terms of an identification between well-being and positive passions (see C3 in section 1.3); second, that well-being is achieved more by internal struggle than by actions in the world — *happiness comes from within* — (C2); and third, that emotions and feelings are part of the physical world (C1). In accordance with these three beliefs, one of the most important tasks of technology would consist in changing — conquering — the human body. It is in this context that technification takes the form of medicalization and, in special way, the form of *psychiatrization* of the human condition. For example, Peter Conrad defines this social phenomenon as "a process by which non-medical problems become defined and treated as medical problems, usually in terms of illnesses or disorders." [32] (p. 209).

Western countries are facing an exponential rise in social demand for psychiatric drugs [33]. Many causes are involved. First, the sciences of the neural system have extended their reach to the point that there are seemingly no issues today that remain untouched by them. To a lesser, yet still important, extent, the development of neurotechnology is making us face up to problems that have never been dealt with from a neuropsychological perspective. However, these advances themselves do not seem to provide sufficient justification for the current changes in psychopharmacological habits [34]. It is very revealing that neurologists and psychiatrists are no longer the sole providers of behavior- and emotion-modifying medications [35–38]. On the contrary, many recent studies show that, in recent years, most mental health problems are being treated by physicians in primary care [39–41]. And to boot, there is a world-wide black market for drugs such as Prozac, Diazepam, Modafinil, and other substances that legally must be prescribed by physicians. In addition, illicit purchases are not just for purposes of therapy or enhancement, but now for dangerous games as well [42]. *Cosmetic psychopharmacology* is the term used by some authors to describe the application of biomedical knowledge and technology for subjective circumstantial ends. It is the step from *what I should think, feel, or do* (questions orbiting around rational criteria) to what I want to think, feel, or do (questions orbiting around the solipsistic self) [34].

If we accept Taylor's view, then psychiatrization would be an expression of the new cultural source of morality. Psychotropic medications would be used to bring back the lost paradises of *deliberative feelings* or, at least, to escape from the hell of individualistic disengagement. It is not a matter of mere feelings, but of recovering the body and the world that surrounds the person. It might even be said that the final goal is to bring back the meaning of life through chemistry. Indeed, Elizabeth Wurtzel hits the nail on the head with her observation that what consumers of Prozac want is to *shut the brain off and turn the heart on* [43] (p. 7). Of course, drugs cannot provide reasons but can make people feel as if they exist. Enough is enough. I will call this way of being-in-the-world *virtual engagement*.

If I understand it correctly, IE precedes medicine intakes. Worse still, it may, at least partially, be one of the main causes of the psychiatrization process. Undoubtedly, there are more (post-intake) causes involved in the aggravation of suffering. Let us take a look.

First at all, among patients, there is a certain suspicion about the authenticity of drug-induced emotions [1]. I am going to classify their complaints in three groups: those related (i) to the artificial origin of emotions — *I do not like feeling in a way caused by a drug*; (ii) to the physical nature of its content — *I do not like feeling happy for no reason, just because*; and (iii) to its episodic relevance — *I do not like to have incongruent feelings*. These grievances could be also applied to thoughts and behavior; however, they do not generate such uncomfortable experiences as feelings do. This is yet more proof that we live in an emotional culture.

These three types of complaints reflect, in a different way, that some classic ideals are still present, not only at non-conscious levels but also in the conscious experience of Western people — ideals that are taken into account when we voluntarily choose or reject anything. There are reasons that suggest, therefore, that these three laments mainly originate at the Core and Autobiographical levels, or even at social levels.

Complaints of type (i) seem to have to do with a belief in the intangibility of emotions, as if they shouldn't be submitted to human will. In fact, today many people think that the more spontaneous the sentiment is, the better it reflects the person (it is more authentic). From an historical perspective, this belief would be the effect of a vestigial attitude that derives from the classic way of creating and managing deliberative feelings. As we have seen in sections 1.1 and 1.2, when all passions are despotically controlled, the subject loses three things: a rich source of information about the world, the most precise mirror of herself, and a valuable tool of communication to express what is *truly me*.

Related to this idea but closer to our time, it is very likely that the high value currently given to spontaneity may be also influenced by the Romantic ideas of the eighteenth century, which represent a transitional stage in the inflationary process of the subject mentioned above — in the material colonization of the spirit. According to Taylor, "[h]uman beings are endowed with a moral sense, an intuitive feeling for what is right and wrong […] The notion was that [our] understanding of right and wrong was not a matter of dry calculation, but was anchored in our feelings. Morality has, in a sense, a voice within" [6] (p. 26).

In Romanticism, the heart takes the place of the Cartesian self, like, using Taylor's own expression, a disembodied ghost "inhabiting an objectified machine" [6] (p. 106). Ironically, there are no limits to the modification of the body, but heart is not part of the body — humans do not have a heart, they are their heart. Heart is, in this framework, sacred. It is no coincidence that this view of the heart has an interesting connection with Sartre's notion of authenticity. For different reasons, yes, but both sides hold that feelings should come before reflection.

The subject's inflation helps to explain both the current hegemony of *emotional labor* over *emotion work* and why this hegemony evokes similar or even stronger feelings of desecration and inauthenticity. Both terms were defined by Arlie Hochschild to explain the links between feelings, roles, and rules in the general context of human *emotional management*. *Emotional labor* has to do with, in certain circumstances, the human necessity of suppressing private feelings in order to attain a benefit — tangible or intangible. On the contrary, *emotion work* has to do with some social scenarios in which the person is able to impose her own emotions in the transaction of goods [44] (p. 56). Only these emotions are useful for expressing the self, but, unfortunately, they are not the most popular in our days. Whatsoever, we still conserve some memories of the referential dimension of our sentimental life, echoes that resonate with force in the loneliness of the subject's private space, where roles are interpreted mainly to oneself — the toughest audience. We can find, in these echoes, the root of some intense IE. Attitudes are more dangerous than drugs.

Stjepan Meštrović notes a second kind of sorrow in the *time of intimacy*. The positive emotions

conquered by drugs or by trade on the social battlefield, which are most often enjoyed in home or during one's free time, are disappointing and destructive. Why? The existence of a whole authenticity industry places in evidence, according to him, our postmodern obsession of manufacturing 'real feelings' — deliberative feelings. The result is dreadful: the *McDonaldization of society*, as he calls it: taken-for-granted ideas are saturated with emotions in order to launch them to the mass market [45] (pp. 73–74). For example, in our *culture industry*, freedom, equality, and dignity seem to be presented more as feelings than as ideas, and therefore they are malleable stuff which everyone can interpret as they see fit, provided that the basic aesthetic codes are respected. It should be noted that Meštrović is talking here about how the Western culture industry — like the pharmacological one — is producing and promoting strategies of virtual engagement too. However, according to him, the spell of this simulation doesn't continue very long. The consequences of this failed attempt at re-embodiment are feelings of emptiness, frustration, and anger about fraudulence, and, worst of all, the globalization of vulgarity, which implies that alternatives to more efficacious sources of — sublime — pleasures are eliminated. Will the rapid impoverishment of cultural life that we are experiencing allow us to keep this masquerade going more than two or three generations?

It is understood that Meštrović is referring to our current age when he uses the expression *post-emotional society*. If passions are less and less satisfactory, present enjoyment ceases to be the focus of attention, to be replaced by future enjoyment — expectations of pleasure [46]. As a Brazilian saying asserts, *hope is the last thing to die*. This implies, among other things, that one's own emotions are increasingly used for obtaining physical goods, social status, greater autonomy, etc., all of which are believed to lead, in the long term, to the awaited emotional fulfillment. With this attitude, the pragmatic dimension of emotions is further boosted and the referential is severely decreased, in particular its self-referential value (as we saw in section 1.3). As can be guessed, this shift toward utilitarianism, where emotions are pure means, is a step further in the process of virtualization of engagement — *I am not my body and this is not my world… yet*. The Modern subject is trapped, in this way, in a vicious cycle: individualism and utilitarianism impoverish emotional life, and this, in turn, fuels the former.

I will end this section by presenting a final important cause for the emergence of a post-emotional society: an (uncontrolled) *mimetic desire*. According to René Girard, "mimetic desire aims at the absolute slenderness of the radiant being that some other person always is in our eyes but we ourselves never are, at least in our own eyes. To understand desire is to understand that its self-centeredness is undistinguishable from its other-centeredness" [47]. A part of our passions (both emotion and feelings) are related to the natural human tendency to desire what other people desire. The more people are seeking an object, the stronger my desire is — *I expect more*. Under normal conditions, this emotional affinity can be very useful for building intersubjective spaces and for establishing social cohesion. The problem is that, in a culture like ours, where emotions are not ruled by reason, mimetic desire drives us to become part of *mass society*.

Five features characterize mass dynamics [48] (pp. 135–142). The first is a conditioning homogenization: the bigger shared desires are, the more impossible it is to resist them. The second is the presence of strong feelings of rivalry: the fewer people have the object of desire, the higher its value. Therefore, it is as important to prevent all others from obtaining it as it is to achieve it myself. The third is the separation and violence amongst the elites (those who are envied) and the support of the masses (those who envy). The fourth is a general feeling of frustration. This is because, among the elites, the spell of a high social expectation is broken — *a now I have it, but I do not feel like all of us imagined* — and the same thing occurred amongst the masses, due to the excessively long delay when awaiting positive passions. Finally, the last feature is *cathartic violence*, which is the consequence of and remedy for frustration: it is the way to purge social

tension through the annihilation of some shared victim. For Girard, this second kind of contagious violence — as old as humanity itself — takes the form of a ritual act, where sacred spaces are destroyed and other ones appear, as signs and symbols of a new order and peace. As Girard writes, in cathartic violence, the victim — the scapegoat — is "chosen only because it is vulnerable and close at hand" [49] (p. 2). This seems the right way to approach taboo territory, where human logic and will are prohibited from entering.

In short, uncontrolled mimetic desire is another key to understanding why the seeds of individualism and utilitarianism create a society marked by a superficial, sterile, and monolithic way of feeling, where *the others* are the enemies and, at the same time, the last powerful tool for engaging with the world.

Mass society would act, on one hand, as a shelter against the sorrows of intimacy mentioned earlier and, on the other, as a substitute for communities. It would offer a kind of *virtual recognition*: there, individuals would feel in some manner that *they are not alone* — even though time inexorably shows that it's not true. However, if Girard is right, it is not clear that, in such an individualistic culture, violence can have a sufficiently cathartic effect against anguish and frustration, namely, IE. The issue here is that the Modern ideal of self-determining freedom does not appear to mesh successfully with the acceptance of new sacred spaces, forbidden places, from whence one can ground new webs of meanings and values.

Would the end of violence only come with the end of society? The only (almost) sure thing is that, in the meantime, the using of drugs in higher and higher doses and the recrudescence of violence would be the only relief, if a change of mentality does not occur. In addition, it is, regrettably, to be expected that unscrupulous money-grubbers will capitalize on the desperation of this emergent, vulnerable group. According to Conrad, many private companies and even governments may already be doing this [50]. Social control is not a consequence like any other: with that, there is no turning back because even the lives of a sales manager and of a populist leader would be affected by this kind of destructive process. Even the presence of elder brothers would not be a necessary condition for the development and fatal end of the mimetic cycle.

Further Reasons to Sentimental Desacralización

In complaints of type (ii), patients express their discontent with the virtual nature of their feelings. Following Elizabeth Anscombe's distinction between *motives* and *intentions*, when I say that *I am happy because of a pill*, I am not giving any reason, namely, a meaning for my feelings [51] (p. 9). The drug is merely the motive for my non-rational (and hence unintentional) sentiments. On the contrary, when I say that *I am happy because I found my keys,* because of *your glowing smile* or because of *the relaxation of the law on immigration,* I am giving meaning to my feelings, which are the basis for my choice and not the other way around. Underlying this complaint is, in this light, the belief in a natural order through which I can judge what kind of emotional response is appropriate at a given time.

Anscombe's perspective would help also to understand, for example, why many people prefer psychotherapy to drug treatment, even when there is increasing evidence that the first may alter the functioning of the brain as much as the second [52]. The key is to understand that they are not just thinking about issues that one should be cautious about, but also on how to face reality. In the depths of their consciousness, these people may hold, mysteriously, that the frontiers between physical law and natural law are found there — again, not to be confused with the Cartesian idea about the limits between body and soul. These grounds forge the argument about why the brain or, at least, the limbic system (the emotional brain) should be considered to be sacred territory: certain changes may cause us to feel not just differently, but even in the wrong manner. A similar argument seems to underlie the preference toward natural remedies over scientific–technological medicine. It is as if imitating the paths of nature were the only permitted means to jump from one

side to the other of such a bridge, that is to say, the only secure way to enter sacred territories.

Finally, complaint (iii) would be the sentimental version of our biological and cultural tendency to think of ourselves as substantial identities. In this context, Heart seems beyond time and space; hence we should find common elements in its temporal projection. This perspective gives way to critical opinions about why congruity should not be invented and enslaved by the will — for example, with the decision to take pills to maintain an emotional balance. In so doing, subjects would be unable to distinguish between the timeless heart and a fake, pretend heart.

Empiricism, and then positivism, were the main modern philosophical approaches that would justify and boost the idea of personal identity as continuity of consciousness, also known as *Psychological Continuity Theory*. In this secularized version of the Cartesian self, the human quintessence depends on the capacity to create a stream of consciousness, that is to say, to project past and future in every present instant. For example, in 1689, John Locke, in his famous work An Essay Concerning Human Understanding, claimed that persons depend upon the persistence of consciousness. Personal identity (the self) "depends on consciousness, not on substance" (Book II, Chap. 27, paragraph 17). In summary, possessing time is the naturalized form of being timeless. To do this, memory, abstraction, and other cohesive functions play a decisive role, where the final result is the human being. It is particularly significant that, in such a definition, the bodily organs of these functions are not part of who we are, but are only instruments. The idea that humans are not their body reappears. The practical implication would be that serious changes in the subject's consciousness would make the subject disappear. Expressed in medical terms, some drugs would not cure patients but would instead replace them.

Coming to our current *emotional culture*, 'continuity of consciousness' theories are being transformed into a belief in the "continuity of heart." In this new second version, it is accepted that human feelings, including their internal logic, may have been created by circumstances and individual and collective behavior. However, this does not mean that such a web of feelings is entirely available to the subject's control — among other reasons, because every individual's will depend on this kind of web, and wanting to change the web sounds like a contradiction in terms. *Should I modify what makes me want what I (deeply) want?* Sentimental consistency also looks, in this approach, to be sacred.

Rivals Logotherapies

We are at last prepared to face the issue of how the different logics of self-understanding (now adequately contextualized) result in alternative dialectics for managing IE — in clinical oOntology, the *problem lambda* [5].

Much has been written about the possibility of finding meaning in these disturbing experiences and, with that, making them more bearable or even making them disappear. However, in the second part of this chapter, I would like to focus on three rival solutions, which I describe as *psychological*, *organic*, and *narrative*. In particular, I will analyze the last-named as being the most likely to take root in our society.

Cultural Persistence?

Nowadays, the *psychological solution* is mainly supported by advocates of the Psychological Continuity Theory, such as Tristram Engelhardt, David W. Shoemaker, David Lewis and Derek Parfit, among others.

For instance, in Tristram Engelhardt's view — intensely debated in bioethics forums — the term person is identified (in a strong sense) with self-conscious being. That is, a being which utilizes symbols in order to communicate, which makes decisions, and which does all of this in a particular manner that reflects his or her imprint (personality) as an individual reality. That is what he calls *human personal life*: the type of life lived by an organism which has such capacities *in act*. In contrast, *human biological life* would be the life lived by an organism which doesn't have not

them or has them only *in potency*. Therefore, when the stream of consciousness ceases or changes, personal life also does so, which means that the subject is destroyed. At that point, there are no reasons to keep the rights and obligations biographically ascribed to her, because only human biological life remains [53].

If Engelhardt's argumentation is right, there is no other option but to acknowledge that patients' suffering and fears about psychiatric drugs and treatments as DBS stimulation are well grounded. IE are not illusions, but are the body's signals about a real danger. But, how does this interpretation help them? Firstly, it would give patients crucial information before they accept or continue treatment. Is the disease painful enough or disabling enough to warrant sacrificing oneself? It is therefore not surprising that, for Engelhardt, decisions about changes of personality should be contextualized and evaluated within the context of the euthanasia debate [54] (pp. 107–108). In a similar way, but more recently, Henry T. Greely draws an equivalence between the death penalty and certain controversial techniques in the rehabilitation of prisoners, such as chemical castration [55]. Secondly, Engelhardt's perspective would help patients reduce the intensity of IE. Usually, it is a relief for patients to know that they are not losing their grip on reality [56].

The difficulties that accompany Engelhardt's position are diverse. For example, from Damasio's perspective, it does not seem possible to establish clear separations between conscious, unconscious, and non-conscious processes. Although Damasio distinguishes hierarchical levels, all of them form a harmonic unity with regard to not only physical dynamism but also to the mind's intentional contents. Speaking in practical terms, who is going to bell the cat? Who will define what level, or what element of the level, is not part of human personal life?

A second criticism has been formulated in the paradox of the sleeper. There are phases of sleep in which certain psychic faculties are intact but not in act, which appears to imply the interruption of the flow of consciousness. But if this is the case, during this period it would be unjustified to qualify the sleeper as a person. Engelhardt responds to this paradox in two different ways: one of an ontological character, and another of a practical character. In the first place, he holds that it cannot be demonstrated that during sleep the flow of consciousness is completely interrupted. On the other hand, even if we lose the relevant ontological status during sleep, it would be senseless to seek to draw practical conclusions from that kind of an affirmation (for example, conclusions relating to respect for life), since such an attitude would generate a society-wide lack of confidence concerning sleep which society simply cannot support. Of course, in his view, patients in a persistent vegetative state or with advanced Alzheimer's, as well as embryos, would be a different matter.

Does Engelhardt attach too much importance to biological function in order to explain personal identity? In my opinion, Derek Parfit, a most sophisticated defender of the Psychological Continuity Theory, understands the importance of cultural identity better. Both authors claim that what is important and particular about each human being does not have anything to do with the existence of spatial or temporal limits, but rather with the fact that we can describe ourselves as individuals. However, for Parfit, this is a conventional capacity, just as the penalty area of a soccer field is: both kinds of limits cannot be understood apart from the rules established for the game [57]. (pp. 245–280). So true is this that it is not even necessary that each player be always and entirely conscious of such rules. In other words, neighbors have an important role in the makeup of the limits of any individual: if they keep thinking about someone as a person, there are strong reasons to recognize her identity. It goes without saying that, in such contexts, much of the problem of IE would derive from the individualistic attitudes of Western societies, where persons with a weakness are not assisted, but instead pay a heavy social price — the stigmatizing gaze.

Nevertheless, Parfit's view has a very weak point: the availability of intersubjective meaning and values, which he attaches to dialogue and consensus. For authors as Alasdair MacIntyre, Jürgen Habermas and Harry Frankfurt, the com-

munitarian *ethos* is only susceptible to being known and protected as (or at least, like) a sacred space. Therefore, the question about who is a person (those whom we recognize as persons) should not be submitted for democratic arbitration [58–60].

Change Is Good

In the *organic solution*, supported by authors as far apart as Gordon Allport and Eric T. Olson, human identity refers to an organic whole, with respect to which the conscious self is only a part, dimension, or type of activity. More specifically, Allport describes personality traits in functional terms as optimal tools for the adaptation of the individual to her environment. On the other hand, the self would be the fruit of the experiences of physical and psychic interactions, which also improve the ability of the organism to act as a harmonious whole [61] (pp. 46 and 377). So, any change of personality or self-understanding should be well received, provided that it helps to maintain organic homeostasis.

The organic solution takes personality to be both a cause and a part of the unity. It is a cause because it collaborates in the preservation and unity of the subject, and it is a part because its products (the different selves) are going to interact as yet another element that must be harmonized. In addition, personality turns the living being into an extremely dynamic system, because its mode of protecting the being against external variations presupposes, paradoxically, its own modification — growth; that is, a certain variation in that which it seeks to preserve.

Based on these statements, Eric T. Olson proposes *animalism*: probably the most radical version of the organic hypothesis. In his view, a proper noun provides information about what a particular individual can do and has done, but it does not talk about what an individual is. On the contrary, the essential aspect of human beings — what defines a rational individual with a particular personality and experience of herself — is not the fact of being a person, but rather the reverse: being a living (changing) human organism. Coherently, for Olson, human identity persists even though the absence of any sign of present or future consciousness [62] (Chaps. 2 and 3). Therefore, the fear of identity change is nothing but a consequence of the error of *entifying* that which is only operations and consequences. And escaping from this kind of confusion implies accepting the possibility that the introduction of new gaps between representational levels may help to improve the organism's adaption. Indeed, on certain serious occasions, it is reasonable to give priority to the body's homeostasis — what we are — over the mind's coherence — what we use.

Damasio's approach is also aligned with the organic solution. Evidence for this is, for example, his argument about how Autobiographical processes naturally induce us to accept belief in substantial identities, which are far from having adaptive value. However, his solution to IE — escaping from chains of physical events (see section 1.1) — should not be understood as a defense of the hegemony of the mental world over the physical, but as one among many formulations of the ideal of instrumental reason. The proper management of thoughts leads to survival, i.e., to positive passions.

To conclude, Spaemann's solution can also be classified within the organic category, although with nuances. The main differences between Olson and Damasio are that, for the former, on one hand, human unity implies something beyond physical causes and, on the other hand, survival is not the only criterion for understanding human capacities, and is not the only value for using them either. Spaemann resized human acts by introducing a transcendental dimension—making them worthy of *Areté* once again,

In spite of this, Spaemann also defends, like Olson, the position that the failure or destruction of a single organ, even if it is the brain, does not imply or, at least, does not warrant, the loss of personal identity — the individual's death. "Loss of somatic integrative unity is not a physiologically tenable rationale for equating BD [brain death] with death of the organism as a whole" [63]. This is because, according to him, humans are not defined by their highest deeds. Coherently,

if drug-induced personality changes improve the subject's ability to survive and, more importantly, to freely achieve natural and cultural ideals, then they should not be feared but intensely sought. This statement also serves to address the problem of personal ideals. Technology cannot change the human spirit (the basis of what Spaemann calls *numerical identity* — about 'someone' — in opposition to *qualitative identity* — about 'something') nor can it change who each individual is called to be.

Storytellers

Inspired initially by philosophers of the stature of Maurice Merleau-Ponty and Paul Ricoeur, the *narrative solution* proposes that human identity is based on the exercise of will more than on the continuity of consciousness or on organic unity. When choosing, we express our true nature: beings which perceive themselves as temporally extended, and which are continuously building a past and a future from a personal present. This is why, for example, Françoise Baylis claims that personal identity depends on every instant and place [64].

Another interesting defender of this approach is Marya Schechtman. In particular, she uses narrative premises to formulate what she calls *the person-life view*: a) teleological experiences constitute each particular human story (goals, emotions, beliefs, values...), and b) personal identity resides in selfhood, i.e., the capacity of the human being to understand him or herself in narrative terms. Nevertheless, for Schechtman, it is important to understand that intentions cannot be thought of as realities which are completely distinct from the medium to which a person is adapted. On the contrary, they manifest a specific type of interaction with the environment which links the person to other human beings and situates her in what Schechtman calls *person-space* [65, 66]. However, the rational and voluntary manner of occupying this intersubjective place is what determines personal identity. If there is a narrator, there is a personal identity. Therefore, it doesn't matter how many radical changes have taken place in the life of the individual. In fact, as Schechtman declares regarding the concrete context of change of identity after DBS, the lives of any human being are more similar to non-narrative histories than to fictional narratives [67]. A pill also may change a person's life radically, as can a wife's death, but this does not mean that the story has concluded or that it has become another story; rather, human narrative forms are more complex than those of the simple novels we are accustomed to reading. The only condition — the connecting thread — is the subject's desire to keep telling the story. Contrary to Engelhardt and Parfit's views, for Schechtman, biological functions, consciousness, and the common medium of our communicative practices are necessary but not sufficient for assigning personal identity.

The main difficulty in Schechtman's solution is that, as Antonio Damasio and John A. Teske defend, the intention to continue to tell a story seems founded on the search for consistency, and not the other way around. Humans seem biologically conditioned to appreciate the pleasures of good stories [68]. Put in its negative form, the anguish over an unstructured life does not disappear with an act of will but with meaningful answers or, at least, with intoxicating emotions. Thus, there are only two possibilities for which Schechtman's solution would be profitable. The first one involves assuming a moderate position: personal identity is not completely dependent on will. This is, for example, Taylor's position. Freedom depends on recognizing that our common cultural heritage (Classic and Modern) is not only a tool but a part of what gives us meaning. If this is so, we should also be able to understand that there are intrinsic limits (sacred spaces) in our capacity of self-determination. In turn, respecting such boundaries will enable us to manage the tensions arising between the eternal, the old and the new, between the limit and the unlimited, and between the individual and the collective. All of them make up what Taylor calls *moral identity*. In contrast to MacIntyre's version of communitarianism, Taylor claims that ideals must be discovered not only in particular forms of life and traditions but also in the free decisions

taken by communities and individuals throughout history, even if we disagree with them. It could be said, paraphrasing a biblical expression, that each human being takes upon herself, in part, the sins of the rest of her ancestors. However, both MacIntyre and Taylor reject the neo-Kantian, liberal view of Engelhardt and Parfit concerning the possibility of attaining to universal ideals without taking account of what really matters to human beings — what they in fact share in order to build communities.

The second circumstance would be that in which the storyteller accepts the use of lies. Certainly, coherence would emerge here from pure acts of will. However, this post-rationalist view pushes us out of the framework of what we call therapy, even in the context of Clinical Ontology. But there is a bigger problem, as we saw in section 1.4.B. This attitude is closely linked to the Modern ideal of self-determining free will, where passions have replaced reasons and, as a result, the greater pleasures are progressively eliminated. Consequently, the strategy of creating a fictional life could provide some (*petits et vulgaires*) pleasures in the short term, but in the long run would result in nothing but the most intense frustration and violence.

Breaking with the Genealogy of Morals

Nietzsche's counter-response to the problem of pleasure in a disenchanted world is in tune with post-rationalist and post-emotional sensibilities — which would explain why his thought remains a viable option and why it should be taken seriously in order to anticipate and evaluate his none-too-distant prophecies. Supporting the post-rationalist theory of fictions, he claims: "Why is the belief in such judgments necessary? — to understand namely that, for the survival of beings like ourselves, belief in the truth of such judgments is necessary: for which reason they may, of course, even be false judgments! (…) They are indeed all false judgments. But belief in their truth is necessary as a superficial optical illusion characteristic of the perspective optics of life" (F. Nietzsche, Beyond Good and Evil, part I, aphorism 11).

Apathy and other modern sufferings, according to Nietzsche — defined by Gilles Deleuze as the *philosopher of difference* — are the consequence of the relocation of emotions to a sacred place. "All passions have a phase when they are merely disastrous, when they drag down their victim with the weight of stupidity — and a later, very much later phase when they wed the spirit, when they 'spiritualize' themselves" (Nietzsche, *Twilight of the Idols*, Morality, 1). The cure would then consist in *re-enslaving* passions with the same boldness and energy that Modernity would direct toward reasons. In exchange, the individual will has to occupy these divine and inviolable territories. Nietzsche promises that putting this ideal into practice — *the will to power*, where reason and emotion are slaves of the will — will bring, as indirect consequences, extraordinary positive sentiments — higher than the lost deliberative feelings.

One could say that Nietzsche holds the same position as did the early Moderns about the need for a despotic control of passions. "What is needed first? A man who refuses to become master over his wrath, his choler and revengefulness, and his lusts, and attempts to become a master in anything else, is as stupid as the farmer who stakes out his field beside a torrential stream without protecting himself against it" (Nietzsche, Human, All Too Human, Part II, aphorism 65). However, there is a critical difference. Nietzsche gives a theoretical justification to a phenomenon — the instrumentalization of emotion — that has come about only in our time and, as we have seen, results from multiple factors. Even more, nihilistic attitudes represent the superlative expressions of virtual engagement (section 1.4.B): in them, the experience of meaning is not provided by the heart but by the will. *That is what would really make me feel like a body, as though I inhabited an enchanted world — enchanted by my own decisions*.

To get thinking, feeling, and acting to integrate in the way the subject wants is, for Nietzsche, the only goal that the human being needs to pursue in order to be authentic — in order to be unitary, one. This would be, therefore, the way to resolve fears about technology mentioned in sections 1.4.B and 1.4.C: only artificial emotions are truly mine (complaint i); there is no

reality beyond will, so there is no right or wrong way to feel (complaint ii); nor is there any necessity to be emotionally congruent (complaint iii).

Nietzsche is aware that the nihilistic plan — the reduction of the distance between *what the human is and* what *the human wants to be* — takes a long time, to the point of becoming a generational project. This is mainly because the first task (which I will call *alpha*) consists in interrupting the *genealogy of morals*, that is to say, breaking with the origins of our moral prejudices, many of them deeply rooted in feelings — Damasio would certainly confirm that last statement. And such origins, which affect both biological and social structures, are themselves beyond individual initiative. For this reason, the education of offspring is, for him, the most efficient tool for little by little replacing the *Old Man* with the *Superman*. As future generations progress on this task, it will be easier for them to advance with the second task (*omega*): master fictions.

In this second sense, Nietzsche is one of the first authors to push modern ideals beyond negative freedom [6] (pp. 27–29). However, Nietzsche's idea of education still drags along the problem of the *ghost in the machine*. The only difference is that will is now the *new homunculus* — truly safe from any decisions about future bodies and worlds. In the next section of this chapter, I will argue that this interpretation of the self is precisely the Achilles' heel of all contemporary nihilistic approaches. They focus their argumentation on the coherence of his theories and ideals about the human being, namely, on problem lambda, but hardly notice the biological and cultural circumstances that underlie such beliefs — the problem kappa.

Beyond Nihilism

Strong cultural trends from the last century have taken on the nihilistic challenge of building a post-human, better world. Concretely, the first tendencies (making their appearance mainly in the 1940s and 1950s) are characterized by taking on *task*α, namely, the pursuit of the identification and deconstruction of all suspicious mental categories and the social structures which keep them alive — philosophical, political, aesthetic, linguistic, etc. Heidegger, Foucault, Derrida, and Lévinas are probably the most famous critical theorists of the post-humanist group. *Posthumanism* is the general term with which many of these thinkers have been labeled, with or without consent.

Two decades later, an alternative version of posthumanism, called *transhumanism*, arose. In this philosophical current, emphasis is placed on *task* Ω: the search for ways to improve human power. It is *freedom of choice*, namely, the most general goal that can be formulated to assist the individual's indefinite desires — her boundless reality. John Haldane, Marvin Minsky, John Desmond Bernal, and Irving John Good are considered to be the fathers of transhumanism.

It is controversial to what extent transhumanism emerges from the atmosphere created by the heterogeneous posthumanistic schools [69]. In addition, a second important difference, not just from the posthumanist perspective but also from that of Nietzsche's nihilism, is that, in transhumanism, science and technology provide the main source of increasing human power. This is the key to understand the transhumanist interest in biotechnology and virtual reality interfaces. According to such authors as Max More and Stefan LorenzSorgner, it is thanks to technology that we will shorten the time limit for the coming of Superman. It might even be possible for currently-living human beings to materialize Superman in their own flesh — thanks to which, no further sacrifices would be necessary to save the unknown and nonexistent people of the future [70, 71].

This possibility of enjoying already and oneself transhumanist promises is very attractive to a more and more individualistic society. Indeed, somehow, psychopharmacology is already being used to eliminate vestigial passions (especially, feelings of guilt) and to induce what people want to feel at any given moment. On the other hand, the current proliferation of *online identities* is the clearest and most powerful expression of virtual engagement. According to Peter Lambor, defender of transhumanism, it is because physical presences are being substituted for concepts

that are situated within a new virtual space, with no material and cultural limitations, that human beings can now begin to truly enjoy hegemony of will [72] pp. 255–264.

However, within the logic of post-humanism, when the technification process ends up reaching nihilism's sacred core (the will), the second difference becomes unacceptable. Four factors give rise to this last desecration. In the first place, the belief in *human responsibility* is one of the main targets of attacks by transhumanism's defenders. It is the coherent consequence of studying freedom only through empirical methodologies. For example, Martah Farah, one of the founders of the *International Neuroethics Society* — an institution of a clearly transhumanist inspiration, writes: "the problem with neuroscience accounts of behavior is that everything we do is like a knee-jerk in the following important way: it results from a chain of purely physical events that are as impossible to resist as the laws of physics […] The idea that there is somehow more to a person than their physical instantiation runs deep in the human psyche and is a central element in virtually all the world's religions. Neuroscience has begun to challenge this view, by showing that not only perception and motor control, but also character, consciousness, and sense of spirituality may all be features of the machine. If they are, then why think there's a ghost in there at all?" [73]. Science is erasing, then, the ghost of will and, with that, reducing human being to mere mechanisms.

From transhumanist perspective, how do human machines deserve to be treated? The first thing that could be said about this issue has to do with what is called the compatibilist approach. According to Marta Farah and Steven Pinker, subjects are not responsible but autonomous: if their brains work adequately, then they enjoy the capacity of self-control. When they do not, subjects lose autonomy — a problem that is not anybody's fault, nobody is responsible for it [74, 75]. Notice that, for Nietzsche, the feeling of responsibility (*I am the author of my own life*) is one of the main pleasures, and is also a remedy against Modern apathy. Transhumanism would lose, then, the main advantage of any plausible nihilistic approach.

Secondly, transhumanism advertises, together with the ideal of autonomy, many other ideas about what happiness means, even about moral enhancement. On this point, Julian Savulescu writes: "Technology might even be used to improve our moral character. We certainly seek through good instruction and example, discipline, and other methods to make better children. It may be possible to alter biology to make people predisposed to be more moral by promoting empathy, imagination, sympathy, fairness, honesty, etc." [76]. Savulescu goes even further and defends the position that such technological improvements should be considered to be a moral obligation [77]. Again, transhumanism seems to revive, in a bizarre way, old ghosts about the possibility of finding objective moral rules, which are radically opposite from the ideal of the self-determining free will and, of course, from nihilism.

This is not entirely a step back. Transhumanist thinkers suggest we imagine the human being as a machine for survival or for pleasure, or both at the same time: we are realities without sacred spaces — completely malleable by the subject or by others. Here we find the third factor that pits post-humanism against transhumanism: for the latter, technology may also be used to direct the course of evolution of the human species. In this sense, Savulescu argues, for example, that parents have the duty to make better children, even if that means restricting the latter's *freedom of choice*, that is to say, the possibility of wanting to be dishonest. Not accidentally, Conrad identifies "the exclusion of evil" as one of the problems associated with the medicalization process [50]. Long gone are the days of the Superman. The horizons of alternative realities are narrowing in a future where the Old Man, under the supervision of science, wants to cast his long shadow over more and more conditioned and homogeneous descendants. This intergenerational ideal is a perfect display of what I call *the instrumentalization of will*, which is, in turn, the final stage, using Spaemann's terms, of the total *objectivation* of man [78].

The fourth and final divergent factor emerges from the idea of thinking as a form of computational

function. Let us see it in the Andy Clark and David Chalmers's *Extended Mind Theory* — frequently mentioned in transhumanist works. This can be summarized by two statements: a) cognitive processes can be constituted in part by elements that are not confined to the limits of the skin–skull barrier; and b) an agent's non-occurrent mental states (phenomenally conscious states) can be partially constituted by realities that are not bound by the body [79]. So, assuming that mind is a constitutive element of identity — as many transhumanist theorists do — any tool (such as computers) that I use to think should be understood and felt as part of me.

The issue is that a good number of defenders of transhumanism are beginning to use Clark and Chalmers's view to diminish the importance of the individual's limits. For example, with regard to the consequences of this conclusion, Neil Levy writes: "the extended mind thesis dramatically expands the scope of neuroethics: because interventions into the environment of agents can count as interventions into their minds, and decisions about such interventions become questions for neuroethics" [80]. Precisely in order to manage such questions, he proposes the *Ethical Parity Principle*, in which the external and internal elements of our cognitive processes are ethically equated. "Since the mind extends into the external environment, alterations of external props used for thinking are (*ceteris paribus*) ethically on a par with alterations of the brain" [81].

Levy's principle brings other major controversies together around the concepts of subject and subjectivity. First, this less locationist view of mental states jeopardizes and weakens human experiences of identity because, on one hand, tools are elements that fluctuate greatly in human life and, on the other hand, some of them are shared by many subjects. The Internet is the paradigmatic example. In fact, Clark claims that, in this sense, it would be more appropriate to call his position "Web-Extended Mind Theory" [82].

We come to a crucial point. There are signs that this path leads us to the collapse of the subject bubble. For example, according to the transhumanist Katherine Hayles, when we use the Internet and other technologies of our global age, we are not only sharing the same mind, namely, the same identity, but also, we begin to be part of a "higher order identity — a better reality" [83]. By the same token, many transhumanist thinkers are publicly expressing a great personal and intellectual interest in Buddhism [84]. It seems they are arriving at an answer that is similar to what had been formulated thousands of years ago: the solution to the problem of suffering is to stop trying to eliminate the identitarian gaps. The promise also includes many conscious IE: giving up any concern for the subject and personal interests would make IE disappear because there is no reason to fear or lament the loss of what we do not love. This clearly does not mean that biotechnological and fictional strategies lose their function in the struggle against non- and unconscious IE.

I would just like to make one small point. For most of the Hindu and Buddhist traditional schools, fictions and drugs are not permitted. The reasons reveal profound differences in the understanding of the universe compared with that of transhumanism: for the former, men are not machines and reality is not the construction of a machine. It is believed that the self can be dissolved by the encounter with a pre-existing highest and benevolent cosmological order, to which the released/enlightened subject is joined. Drugs and fictions would prevent us from reaching enlightenment.

Returning to the main point, the Web-Extended Mind Theory affects the compatibilist view of freedom too. If the locus that controls human behavior is not to be fully restricted to the biological parts of the organism, but to the network of a massive mind, then the biological concept of autonomy is also empty of meaning. Only digital masses could be identified as truly autonomous. But then what remains of the subject — without responsibility and without autonomy? A deterministic individual's self-understanding is the unavoidable and final result of Levy's principle.

In conclusion, the fourth factor definitely buries Nietzsche's nihilistic project: in more than one transhumanist utopia, the will of the subject seems destined to become part of an anonymous, *unblaming* magma of collective identities in which any preference for the individual's wishes would be stigmatized [85].

The End of Subjectivity

How is it is possible that nihilism, which promotes an inflation of the subject, resulted in the Man–God's death? And most importantly, why is transhumanism receiving a more enthusiastic welcome than post-humanism? As I anticipated, I think that Nietzsche, as with the rest of the posthumanist thinkers, overlooks the fact that the self and will are sustained by biological, psychological, and environmental elements. Therefore, changing the latter implies changing the subject's understanding of and choices about the pursuit of happiness. In this last brief section, I will present, in a very summarized form, some of the concrete causes that have catalyzed the transition between wanted and non-wanted beliefs.

The first cause is related to the difficulty of creating meaning. In the Modern ideal, as Taylor claims, "the artist becomes in some way the paradigm case of the human being, as the agent of an original self-definition" [6] (p. 62). The problem is that art means, in this context, something original, something not based on the pre-existing, the creation of the truly new. In other words, modernity is transforming us into *solitary artists*, who feel obligated to undertake a "heroic effort to break out of ordinary existence" [6] (p. 35). However, despite Nietzsche's promise, this self-made loneliness is closer to being experienced, Taylor concludes, as a condemnation than as a sublime and pleasant privilege [6] (p. 68). *Let the others decide for me*. It is easier. The existentialist literature of the 1960s confirms Taylor's criticism. Frustration drives conformism.

It is not good that the man should be alone. We need to be recognized by others. In this chapter, I have tried to show that the causes of this necessity (and of Modern suffering) are rooted in biology. Unfortunately, individualistic social habits direct us toward facing the problem with drugs and online identities, not with friends. Transhumanism is coming.

A second cause has to do with the instrumentalization of rationality and emotions. As was explained in section 1.4.A, the first seems to be at the origin of the predominance of the public self in the human expression of identity, which induces experiences of a stereotyped life –a phenomenon that is closely related to a Protoself hypertrophy. The second develops psychopharmacological habits, which reinforce the idea that the meaning of life and of vital choices is dependent on physical causes. If we add the weakening of the locus of control instigated by digital technologies, then it is only natural that many people listen to transhumanist ideas about freedom with pleasure. Such ideas are just justifying how they live as a fact, that is to say, like machines which are already being handled as machines.

The mimetic emotional storm would be the third important cause of the success of transhumanism. On one hand, without the necessary control of the reason, Girard's cycle would be pushing us into a mass society where *solitary artists* would be systematically exterminated. On the other side, and as a rebound effect, Modern ideals about individuality, associated with apathy, violence, and frustration, are being radically devaluated. There appears to be no middle ground.

The last cause has already been introduced: transhumanism offers, in a selfish society, a better destination for us — Old Men — than that proposed by Nietzsche. Better to be the merciless master of our offspring than to be slave of their unpredictable desires. Good or bad, it is clear that this picture of *Saturn Devouring His Children* distances itself greatly from what humanism has defended for millennia. Will dreams such as these be fulfilled or, as occurred with earlier versions of nihilism, will they give rise to the worst of our nightmares? There are grounds for pessimism so long as belief in a Cartesian self continues to frame our thinking, feelings, and choices. Time will tell.

Final Remarks

Advances in neuroscience are bringing about a better understanding of human neural function in relation to cognitive and behavioral performance. Furthermore, neuro-technology is developing

powerful ways to treat serious diseases, to improve lifestyles and even, potentially, to enhance the human body. However, this progress is also associated with new self-understandings, existential challenges, and problems never seen before.

In this chapter, I have explored the phenomenon of inauthentic emotions as an adverse effect of the neuroscience revolution. My main thesis is that IE are not only consequences but also precede medicine intakes. Indeed, IE are central causes of the psychiatrization social phenomenon, and ultimately of the process of instrumentalization of the human being.

Should we reverse such a process that generates a very vulnerable man? I believe that there are sufficient signs of how it is harming our minds, human rights, and Western social cohesion to provide a positive answer, at least so long as we continue to appreciate the dignity of every single person.

But is it possible to stop a threefold process of this kind? In my opinion, the key is to avoid idolizing individuals. Precisely, in this chapter I have tried to decode this paradox: to save the subject we need to go beyond the subject. That is to say, first, that authentic life implies internal and external causes, and second, that some of these causes ought not to be manipulated. Human nature is an appropriate term in describing the whole set of these causes. However, it does not exhaust itself in the delimitation of a particular and fixed sacred space, because, among other things, it can be resituated — it has to. Nevertheless, the important thing here is to understand that, from the human perspective, such changes are not comparable or susceptible to moral judgment. The pursuit of the improvement of human nature is neither good nor bad, but rather nonsense.

There is no magic recipe but, in order to finalize this chapter, I can suggest some safe paths that may be integrated in the heart of identity logotherapies:

- First, it is important to combat Cartesian dualism: philosophy and neuroscience show us that we are not experts on consciousness, which is the same as saying that our existential beliefs and feelings also depend on how our mind works. For the same reason, we must become aware of how our self is vulnerable to our own decisions and, of course, to those of others. Patients need to realize that others are necessary not only to grow properly and survive but also as constitutive elements of our selves. Finally, this conclusion also applies to the future: some ideals take us further from what was promised.
- Second, it may be desirable to learn how to reconnect reality and emotions in the right way: with goals and reasons rather than with drugs and fictions — sources of virtual realities. This would also help in the acquisition of intellectual, emotional, and behavioral habits [48]. The formation of these habits is the legitimate (natural) pathway that consciousness has for expanding itself over non- and unconscious processes: namely, to spiritualize the material body.
- Conversely, and at the same time, this kind of reconnection would be useful for materializing, in the right sense, the spiritual body: bringing the referential dimension of emotions back. Only in a true world can there be an authentic me — can my heart be listened to again. A reliable heart is the clearest proof of human re-embodiment, which in turn, is an expression of engagement with the real world.

Disclosures/Conflicts None.

References

1. Kraemer F. Authenticity anyone? The enhancement of emotions via neuro-psychopharmacology. Neuroethics. 2011;4(1):51–64.
2. Damasio A. The feeling of what happens: body and emotion in the making of consciousness. New York: Harcourt Brace; 1999.
3. Damasio A. Self comes to mind. Constructing the conscious brain. London: Vintage Books; 2010.
4. Echarte L. Paradoxes of authenticity: a neuroscientific approach to personal identity. In: Masferrer A, García E, editors. Human dignity of the vulnerable in the age of rights. Interdisciplinary perspectives. New York: Springer; 2016. p. 141–66.
5. Echarte L. Biotechnologies inside the self: new challenges in clinical ontology. In: Masferrer A, García E, editors. Human dignity of the vulnerable in the age of

rights. Interdisciplinary perspectives. New York: Springer; 2016. p. 123–40.
6. Taylor C. The ethics of authenticity. Cambridge, MA: Harvard University Press; 1991.
7. Jaeger W. Paideia: the ideals of Greek culture. New York: Oxford University Press; 1945.
8. Renaut A. The era of the individual. A contribution to a history of subjectivity. Princeton University Press: Princeton; 1997.
9. Cremin LA. The transformation of the school: progressivism in American education, 1876–1957. New York: Vintage Press; 1961.
10. Strauss L. Natural right and history. Chicago: University of Chicago Press; 1953.
11. Aristotle. Nicomachean ethics. Chicago: University Of Chicago Press; 2012.
12. González AM. Aristotle and Kant on practical reason. An annotation to Korsgaard. Acta Philosophica. 2009;1(18):99–112.
13. Persons SR. The difference between 'someone' and 'something'. Oxford: Oxford University Press; 2006.
14. Aristotle. Metaphysics. Cambridge, MA: Harvard University Press; 1989.
15. Ética PL. Hacia una versión moderna de temas clásicos. Madrid: AEDOS-Unión Editorial; 1997.
16. Dennett D. Freedom evolves. London: Penguin Classics; 2003.
17. Ratzinger J. Principles of christian morality. San Francisco: Ignatius Press; 1986.
18. Ratzinger J. Faith and future. Chicago: Franciscan Herald Press; 1971.
19. Cain J. The doctrine of the trinity and the logic of relative identity. Relig Stud. 1989;25:141–52.
20. Damasio A. Descartes' error. Emotion, reason and the human brain. New York: Avon Books; 1995.
21. Hoffman P. Cartesian passions and Cartesian dualism. Pac Philos Q. 1990;71:310–33.
22. Lazareth W. Christians in society: Luther, the Bible, and social ethics. Minneapolis: Fortress Press; 2001.
23. Holl K. The cultural significance of the Reformation. Cleveland: The World Publishing Company; 1959.
24. Pearson TD. Luther's pragmatic appropriation of the natural law tradition. In: Baker RC, editor. Natural law: a Lutheran reappraisal. St. Louis: Concordia Pub. House; 2011.
25. Korsgaard CM. From duty and for the sake of the noble: Kant and Aristotle on morally good action. In: Engstrom SP, Whiting J, editors. Aristotle, Kant, and the stoics: rethinking happiness and duty. New York: Cambridge University Press; 1996. p. 203–36.
26. Hughes JL. The role of happiness in Kant's Ethics. Aporía. 2014;14(1):61–72.
27. Alexander L. Liberalism, religion and the unity of epistemology. San Diego Law Rev. 1993;30:763–97.
28. The SG, Connexion S. Causation, realism, and David Hume. New York: Clarendon Press; 1989.
29. Nietzsche F. Beyond good & evil: prelude to a philosophy of the future. New York: Vintage; 1989.
30. Sartre JP. Sartre, itinerary of a thought: interview with Jean-Paul Sartre. New Left Rev. 1969;58:43–66.
31. Sartre JP. Notebooks for an ethics. Chicago: University of Chicago Press; 1992.
32. Conrad P. The medicalization of society. Baltimore: The John Hopkins University; 2007.
33. Chodoff P. The medicalization of the human condition. Psychiatr Serv. 2002;53:627–8.
34. Echarte LE. After medicine: the cosmetic pull of neuroscience. In: Davis J, Gonzalez AM, editors. To fix or to heal. New York: New York University Press; 2016. (in press)
35. Lazaratou H, Anagnostopoulos DC, Alevizos EV, Haviara F, Ploumpidis DN. Parental attitudes and opinions on the use of psychotropic medication in mental disorders of childhood. Ann Gen Psychiatry. 2007;6:32.
36. Slingsby SB. The Prozac boom and its placebogenic counterpart — a culturally fashioned phenomenon. Med Sci Monit. 2002;8:389–93.
37. Benkert O, et al. Public opinion on psychotropic drugs: an analysis of the factors influencing acceptance or rejection. J Nerv Ment Dis. 1997;185:151–8.
38. Clinthorne JK, Cisin IH, Balter MB, Mellinger GD, Uhlenhuth EH. Changes in popular attitudes and beliefs about tranquilizers. Arch Gen Psychiatry. 1986;43:527–32.
39. Van Rijswijk E, Borghuis M, Van de Lisdonk E, Zitman F, Van Weel C. Treatment of mental health problems in general practice: a survey of psychotropics prescribed and other treatments provided. Int J Clin Pharmacol Ther. 2007;45:23–9.
40. Hedenrud TM, Svensson SA, Wallerstedt SM. Psychiatry is not a science like others — a focus group study on psychotropic prescribing in primary care. BMC Fam Pract. 2013;14:115.
41. Sirdifield C, Anthierens S, Creupelandt H, Chipchase SY, Christiaens T, Siriwardena AN. General practitioners' experiences and perceptions of benzodiazepine prescribing: systematic review and meta-synthesis. BMC Fam Pract. 2013;13:191.
42. Teter CJ, McCabe SE, LaGrange K, Cranford JA, Boyd CJ. Illicit use of specific prescription stimulants among college students: prevalence, motives, and routes of administration. Pharmacotherapy. 2006;26:1501–10.
43. Wurtzel E. Prozac nation: young and depressed in America. New York: Riverhead Books; 1995.
44. Hochschild AR. The managed heart: Commercialization of human feeling. Berkeley: University of California Press; 1983.
45. Meštrović SG. Postemotional society. London: Sage; 1997.
46. Tamir M. What do people want to feel and why? Curr Dir Psychol Sci. 2009;18:101–5.
47. Girard R. Eating disorders and mimetic desire. Contagion J Violence Mimesis Cult. 1996;3:1–20.
48. Echarte L. Hábitos emocionales en torno a la salud y la belleza. Astrolabio: Pamplona; 2014.
49. Girard R. Violence and the sacred. Baltimore: Johns Hopkins University Press; 1977.
50. Conrad P. Medicalization and social control. Ann Rev Sociol. 1992;18:209–32.

51. Anscombe GEM. Intentions. London: Harvard University Press; 2000.
52. Barsaglini A, Sartori G, Benetti S, Pettersson-Yeo W, Mechelli A. The effects of psychotherapy on brain function: a systematic and critical review. Prog Neurobiol. 2014;114:1–14.
53. Engelhardt HT. Infanticide in a post-Christian age. In: MacMillan RC, Engelhardt HT, Spicker SR, editors. Euthanasia and the newborn. Dordrecht: Reidel; 1987. p. 81–6.
54. Engelhardt HT. The foundations of bioethics. New York: Oxford University Press; 1996.
55. Greely HT. Neuroscience and criminal justice: not responsibility but treatment. Kansas Law Rev. 2008;56:1103–38.
56. Tuckett AG. Truth-telling in clinical practice and the arguments for and against: a review of the literature. Nurs Ethics. 2004;11:500–13.
57. Parfit D. Reasons and persons. Oxford: Clarendon Press; 1984.
58. MacIntyre A. After virtue: a study in moral theory. London: Duckworth; 1981.
59. Habermas J. The future of human nature. Cambridge: Polity Press; 2003.
60. Frankfurt H. Concerning the freedom and limits of the will. Philos Top. 1989;17:71–81.
61. Allport GW. The person in psychology: selected essays. Boston: Beacon Press; 1968.
62. Olson ET. What are we? New York: Oxford University Press; 2007.
63. Spaemann R. Is brain death the death of a human person? In: Spaemann R, Schindler DL, editors. Love and the dignity of human life: on nature and natural law. Grand Rapids: Wm. B. Eerdmans; 2012. p. 45–69.
64. Baylis F. I am who I am: on the perceived threats to personal identity from deep brain stimulation. Neuroethics. 2013;6:513–26.
65. Schechtman M. Personhood and the practical. Theor Med Bioeth. 2010;31:271–83.
66. Schechtman M. The narrative self. In: Gallagher S, editor. The Oxford handbook of the self. New York: Oxford University Press; 2011. p. 394–416.
67. Schechtman M. Philosophical reflections on narrative and deep brain stimulation. J Clin Ethics. 2010;21:133–9.
68. Ramachandran VS. The tell-tale brain: a neuroscientist's quest for what makes us human. New York: Norton & Company; 2011.
69. Murillo JM. Does post-humanism still need ethics? The normativity of an open nature. Cuad Bioet. 2014;25:469–79.
70. Moore M. The diachronic self: identity, continuity, transformation. Ann Arbor: UMI Bell & Howell Company; 1995.
71. Sorgner SF. Nietzsche, the overhuman, and transhumanism. J Evol Technol. 2009;20:29–42.
72. Lamborn P. Violaciones de fronteras. In: Aronowitz S, Martinsons B, Menser M, editors. Tecnociencia y cibercultura. Barcelona: Paidós; 1998.
73. Farah M. Neuroethics: the practical and the philosophical. Trends Cogn Sci. 2005;9:34–40.
74. Farah M, Heberlein A-S. Personhood and neuroscience: naturalizing or nihilating? Am J Bioeth. 2007;7:37–48.
75. Pinker S. The blank slate: the modern denial of human nature. New York: Viking; 2002.
76. Savulescu J. Genetic interventions and the ethics of enhancement of human beings. In: Steinbock B, editor. The Oxford handbook on bioethics. Oxford: Oxford University Press; 2007. p. 516–35.
77. Savulescu J. The moral obligation to create children with the best chance of the best life. Bioethics. 2009;2:3274–90.
78. Sabuy P. Entretien avec le professeur Robert Spaemann. Acta Philosophica. 1999;8:321–32.
79. Clark A, Chalmers JD. The extended mind. Analysis. 1998;58:7–19.
80. Levy N. Rethinking neuroethics in the light of the extended mind thesis. Am J Bioeth. 2007;7:3–11.
81. Levy N. Neuroethics. Cambridge: Cambridge University Press; 2007.
82. Clark A. Supersizing the mind: embodiment, action and cognitive extension. New York: Oxford University Press; 2008.
83. Hayles K. How we became posthuman: virtual bodies in cybernetics, literature and informatics. Chicago: University of Chicago Press; 1999.
84. DeMoss D. Empty and extended craving: an application of the extended mind thesis to the four noble truths. Contemp Buddh. 2011;12:309–25.
85. Echarte LE, García-Valdecasas M. Identity and conflict in the ethics of neural implants. Cuad Bioet. 2014;25:415–25.

9 Dialogues Between Philosophy and Psychiatry: The Case of Dissociative Identity Disorder

Francisco Güell, Gonzalo Arrondo, Pilar de Castro-Manglano, Javier Bernacer, and Jose Ignacio Murillo

Abstract

In psychiatry, as in any other medical specialty, the clinician collects information from the patient's anamnesis, clinical observation, and diagnostic tests; evaluates these data; and makes a diagnosis. The most common manuals used to assess a patient's mental disease according to his or her symptoms are the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) and the International Classification of Diseases (ICD). This chapter focuses on the dialogue that philosophy and psychiatry have held for decades to achieve a better understanding of dissociative identity disorder (DID). The outcome of this dialogue is the expression of the diagnostic criteria for DID, as well as other dissociative disorders, in the medical manuals. Thus, we first analyze the evolution of DID across the different versions of ICD and DSM. We then show that the characterization of DID and other dissociative disorders is a lively debate that is far from being settled. We demonstrate that the core of this debate is the understanding of *person* after John Locke's philosophy: a person is defined by the apparent expression of consciousness and memories. This leads to what we have termed a primary conceptual dissociation: the mental qualities of the person are dissociated from the body. We propose an alternative account based on the dynamic nature of identity and the understanding of person

F. Güell, BS, BPh, PhD
G. Arrondo, PhD • J. Bernacer, PhD, MPhil
Mind–Brain Group, Institute for Culture and Society (ICS), University of Navarra, Pamplona, Navarra, Spain

P. de Castro-Manglano, MD, PhD
Psychiatry and Clinical Psychology Department, Clínica Universidad de Navarra, Navarra, Spain

J.I. Murillo, PhD (✉)
Mind–Brain Group, Institute for Culture and Society (ICS), University of Navarra, Navarra, Spain

Department of Philosophy, University of Navarra, 31009/Pamplona, Navarra, Spain
e-mail: jimurillo@unav.es

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_9

as a mind–body unity. We hope that our proposal, which results from the interdisciplinary dialogue between psychiatry and philosophy, contributes to a better understanding of this disorder and its underlying concepts.

Keywords
Consciousness • Dissociation • DSM-5 • Epistemology • ICD-10 • Personality

Introduction

> […] her answers implied coexistence and parallelism of thought for she explained certain lapses of knowledge by asserting that ordinarily, as she herself was not fond of books, she did not pay attention while Miss Beauchamp was reading; but when she did so, which was only when interested, she could understand and remember the text; that she liked different books from Miss Beauchamp liked, and that she understood some things that Miss Beauchamp did not and vice versa. A claim of this kind, to be able to pay attention or not as she pleased, when the waking consciousness was reading, required the coexistence and simultaneous action of two distinct and unlike streams of thought in one individual (Morton Prince, 1908, p 48).

This is a paragraph extracted from a work by Morton Prince, written in 1908 [1]. It was one of the first systematic descriptions of a patient with dissociative identity disorder (DID), termed "dissociation of personality" by Prince. The extraordinary complexity and attractiveness of this psychiatric disorder is just outlined in this piece of text, where the author explains how Sally, one of the alter personalities—or simply *alters*—of the patient, expresses a different set of memories, stream of consciousness, likes, and dislikes with respect to another, Miss Beauchamp.

In this chapter, we show the importance of a fruitful dialogue between psychiatry and philosophy, taking as a major example the evolution of the characterization of DID (and dissociative disorders in general) in the medical reference guides to assess a patient's diagnosis. Because of the complexity of DID, its description has undergone important changes in the revised versions of these manuals. Our main goal is to demonstrate that the dialogue between disciplines—psychiatry and philosophy—has been important to find a more accurate description of the disease, and that a richer theoretical reflection would have shortened the period of time to find that accurate description.

The prevalence of dissociative disorders is remarkable: 10% among the general population, and 46% among psychiatric inpatients [2]. From a clinical standpoint, dissociation is a mental state that appears in several pathologies or disorders, such as posttraumatic stress disorder. In this case, the patient may experience repeatedly the traumatic event through recurrent memories or nightmares. In addition, they can suffer dissociative states of a variable length, during which some features of the traumatic event are rekindled. Thus, patients with this condition may behave as if they were suffering the painful circumstance again. This mental state is usually experienced together with severe anguish and an intense psychological discomfort, together with maladaptive physiological responses. Other affective psychiatric pathologies such as anxiety or adaptive disorders may also entail dissociative symptoms. However, independent of these conditions, dissociative disorder is also described in the main medical manuals as such. It is defined as a disturbance of identity with a discontinuity in the sense of self, together with mood, behavior, consciousness, memory, perception, cognition, and sensorimotor alterations [3]. With regard to DID, its prevalence is difficult to assess, although it is estimated to occur in between 1% and 3% of the general population [4, 5]. As we will see in the next section, its description in the medical manuals has evolved in the subsequently revised versions. However, it is generally understood as a mental disorder where two or more different "identities", "personality states" or "alter personalities" alternatively take control of the

patient's behavior. This is usually accompanied by severe memory impairments.

Several interesting attempts have been made to go in depth about DID. Some of them have started from the clinical practice to achieve a deep philosophical analysis [6], whereas others have made a clinical proposal starting from a philosophical position [7]. Our attempt starts from an intermediate position, and contributes to the understanding of this disease both in philosophy and psychiatry. To achieve this goal, we first describe the evolution of the description and diagnostic criteria for DID in the medical manuals, where we highlight the conceptual changes that this disorder has experienced in recent decades. Then, we show how the characterization of DID and dissociation in general currently remains a debated topic, in spite of this conceptual evolution. The last two sections of our chapter have a stronger philosophical inspiration. First, we explain the critical influence of John Locke's philosophy in the past and current understanding of the dissociation of an individual's identity, which leads to what we call a *primary dissociation*: the extreme conceptual separation between a person and his or her body. Then, we outline a different way to understand the terms "person" and "identity", emphasizing the dynamic and relational aspects of these notions.

Evolution of Dissociative Identity Disorder in Diagnostic Manuals

In the last 50 years, the use of diagnostic manuals has transformed the practice of psychiatry. The most common manuals to assess a patient's state according to their symptoms are the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Classification of Diseases (ICD). Whereas the former is restricted to diseases of the mind and is more frequently used in the USA, the latter pertains to all types of disorders and is mainly used in Europe. In this section, we will analyze how DID has been characterized across the latest versions of DSM (from DSM-III to DSM-5), and we will compare it with the current version of ICD (ICD-10). How has dissociation been conceptualized in these diagnostic manuals? What has been the evolution in the diagnostic criteria? Are there disagreements between the main manuals?

Dissociative disorders were not included as such in the DSM until its third version (1980). For a schematic outline of the evolution of dissociative disorders in these manuals, see Table 9.1. The DSM-I (1952) included *dissociative reactions* (code 000-x02) under the category of *psychoneurotic disorders* (disorders in which anxiety is either expressed or controlled through defense mechanisms), whereas *hysterical neurosis, dissociative type* (code 300.14, which was specific for multiple personality disorder in DSM-III and dissociation identity disorder in DSM-IV and onwards) where categorized under *neuroses* in DSM-II (1968). Different symptomatic expressions such as depersonalization and dissociated personality (DSM-I) or multiple personality (DSM-II) were also present in these initial versions of the manual. The DSM-III included, for the first time, a specific section on *dissociative disorders*, with the diagnosis of

Table 9.1 Publication year of the main medical reference guides and categorization of dissociative identity disorder

	Year	Section	Name
DSM-I	1952	*Psychoneurotic disorders*	*Dissociative reactions*
DSM-II	1968	Neuroses	*Hysterical neurosis, dissociative type*
DSM-III	1980	*Dissociative disorders*	*Multiple personality*
DSM-III-R	1987	*Dissociative disorders*	*Multiple personality*
DSM-IV	1994	*Dissociative disorders*	Dissociative identity disorder
DSM-IV-TR	2000	*Dissociative disorders*	Dissociative identity disorder
DSM-5	2013	*Dissociative disorders*	*Dissociative identity disorder*
ICD-10	1992	Dissociative [conversion] disorders|other	Multiple personality disorder

multiple personality as one of four members in the category. According to the manual, individuals should have "two or more distinct personalities, each of which was dominant at a particular time and was a fully integrated and complex unit". The category of dissociative disorders received a substantial revision in the updated version of this third edition (DSM-III-R, 1987). In particular, two major changes were included: (1) the criteria for the diagnosis of multiple personality were altered to avoid the term "personality", which was henceforth transformed to "personalities or personality states"; and (2) criteria were somewhat loosened to allow various interpretations of the sense of self. This trend continued in the DSM-IV (1994) and DSM-5(2013), where the criterion of control was also loosened: from a strict "personalities take full control" in the DSM-III, to an eased "take control" in DSM-IV. In fact, the latest version of the manual makes no reference to "control" in this context. In any case, the most prominent modification in the DSM-IV was that the disorder received its current name of dissociative identity disorder. Following the trend of bypassing the term "personality", this version had a confusing equation of the terms *identities* and *personality states*, while reincorporating the symptom of amnesia which had been dropped from the criteria in the DSM-III-R. Finally, nomenclature was changed once again in the DSM-5 to refer to an alteration of identity which manifests itself through multiple personality states. The DSM-5 also highlights the existence of non-pathological cases of DID.

Overall, DID is considered the prototypical dissociative disorder in the latest versions of DSM; however, this view is opposed to that of the ICD. In the tenth version of this classification published by the World Health Organization (WHO), multiple personality disorder is one of four *other dissociative* [*conversion*] *disorders*. Moreover, the very existence of the syndrome as something different from a cultural or iatrogenic (therapy-induced) manifestation is put into question. In turn, this manual states that many personality changes only occur during suggestion-related therapies such as hypnosis. In any case, diagnostic criteria are similar to those employed in the DSM-III, indicating thus that personalities are complete and that communication between personalities is minimal.

In summary, this section shows that the conceptualization and categorization of DID in the main diagnostic manuals have been a debated topic in the last decades. In fact, this relatively recent debate is a continuation of the discussions that this topic has instigated in the last 100 years (for a review on the historical conceptualization of DID see [8]). We have reviewed how terms such as personality, identity, and personality states have been indistinctly used in the description of dissociative disorders. In the next section, we expose the trending debates that have arisen in clinical and philosophical grounds with respect to this disorder. Then, we will shed some light on the theoretical inspiration that underlies the current interpretation of DID, and will introduce another philosophical comprehension centered on the concept of person.

Current Debates in Dissociative Disorders

As we have summarized in the previous section, the characterization of DID is a lively topic that seems far from being consolidated in the near future. In this section, we discuss some of the issues around the theoretical conceptualization of dissociation and dissociative disorders in the scientific literature, in order to tackle these debates from a philosophical standpoint in the following sections.

The differences between the current versions of the American and WHO manuals expose the fact that the mere existence of DID is a major topic of discussion. If the existence of this disorder was accepted, another key debate would be what can be considered as a truly dissociative process and the implications of such a consideration. It has been widely argued whether dissociation refers to a division in the personality or to any alterations of consciousness. Finally, a pervasive and somewhat related issue within the theoretical works on dissociation is whether dissociation can be considered both a pathological and non-pathological human response. We will explain these three discussions in the following lines.

Is DID a 'Real' Disorder?

We have already reviewed how the DSM and ICD have different perspectives with regard to DID, and specifically its etiological origins. The DSM points towards trauma as a factor which is typically involved (posttraumatic model), whereas ICD indicates that it could be mainly iatrogenic (that is, induced by the therapist) or culturally related (both being part of the sociocognitive model). While the posttraumatic model has been dominant for over 100 years, the alternative view is supported by solid evidence and should not be dismissed out of hand. Some examples that support these alternative views include: (1) the fact that the number of alter personalities increased by the end of the twentieth century, which was in line with media depictions at the time; (2) an increase in the number of diagnoses during this same period; (3) a greater prevalence of the disorder among patients of therapists that use suggestion-prone techniques such as hypnosis; and (4) the appearance of the alters being typical during therapy [9–11]. In response to these critiques, other authors indicate that empirical studies support the existence of DID as a non-iatrogenic, "real", and valid diagnosis, since it has content, criterion, and construct validity [5]. Moreover, experts have also defended the usefulness of trauma-focused psychotherapy aiming to integrate identity fragmentation and to decrease dissociative amnesia [12]. The fact that the diagnosis of DID is highly controversial could be related to a broader problem concerning the lack of a specific definition of dissociation.

What Is Dissociation?

In a broad sense, dissociation means that mental processes that are normally integrated within an individual are abnormally detached. Hence, dissociation includes the domains of conscious awareness, memory, or personality [13]. If we accept the least restrictive definition of dissociation, it would include even the perception of a stimulus under the threshold of full consciousness [13]. Prototypical examples would be the attentional neglect of over-learnt sequences such as driving, or the cocktail party effect (the ability to focus on a particular conversation neglecting the noisy background) [14]. Nevertheless, this conceptualization does not take into account whether individuals can actually change their awareness status and shift attention towards the momentary "dissociated" perception [13]. An alternative definition subordinates the diagnosis of dissociation to the unexpectedness of the disrupted integration. Therefore, "dissociation applies to mental processes, such as sensations, thoughts, emotions, volition, memories, and identities, that we would ordinarily expect to be integrated within the individual's stream of consciousness and the historically extended self, but which are not" [13]. Typical examples of this definition of integration are post-traumatic amnesia or memory lapses in DID patients. It is important to note that any mental process—such as memory, volition, or emotion—could be dissociated. The current diagnostic criteria use this latter conceptualization [13]. In the particular case of DID, discontinuity of memory (e.g., stream of consciousness) leads to a deficit of self-integration and hence to the existence of multiple identities within the same individual, which are at least partially independent [13]. If this definition of dissociation is accepted, there is another issue to deal with: the degree of compartmentalization between the dissociated processes. In the case of DID, both the ICD and DSM-III proposed that it was almost complete, although very early historical accounts indicated that this was not the case [8]. As previously discussed, latest versions of the DSM are less strict with respect to the degree of independence of the disrupted mental processes.

However, we would like to note that neither of the two previous approaches takes into account the subjective experience of the dissociated individual. To the best of our understanding, both assume that subjective experience is mostly unchanged, in as much as patients are not aware

of the incoherence of their inner processes. Moreover, this assumption was explicit in earlier versions of the DSM and still remains in the ICD, but it does not fit well with clinical data. Taking into account the subjective state of the patient, a different interpretation of the concept of dissociation arises: it is understood as a subjective sensation of disconnection between oneself and one's environment, which is a byproduct of a lack of integration between mental processes [13]. The latest versions of DSM, together with recent scientific reports, have proposed a distinction between two types of dissociative processes or symptoms in the case of the clinical domain, namely detachment and compartmentalization [15]. Compartmentalization refers to the concept of dissociation that we presented first, i.e., the lack of conscious integration. Volitional processes are a key issue when speaking of compartmentalization, since it has been defined as an inability to control processes and take actions that non-pathological individuals would be able to do. On the other hand, detachment refers to the sensation of alienation, and thus to the subjective experience of an altered state of consciousness [15].

But this debate goes further and deeper. Recently, other authors have proposed that dissociation only refers to a lack of structural integration of the personality [6]. A critical point of these authors is the proposal that all dissociated personality elements involve a minimum level of sense of self or, in other words, a rudimentary first-person perspective. It is important to note that these authors acknowledge the fact that these "personality fragments" are not completely independent. In fact, they speak of "division" vs. "separation", and use the "corporation metaphor", according to which the different departments of the same corporation can share functions or aims, but are somewhat independent. With regard to this first-person perspective, they propose that the dissociated parts fulfill at the very least the minimal requisites for consciousness: "situatedness", phenomenal now, and transparency [6]. This means that every dissociated part of the individual live in a *here*, in a *now*, and experience their own version of the world as real. These are, as we mentioned, the minimum requisites of the dissociated parts or alter for being considered as such. However, alters will usually go beyond this minimum threshold. It has been argued that this phenomenological proposal avoids major philosophical concerns by comparing discontinuities of the self with dissociations of consciousness, especially in uncertain cases of dissociation [16]. Furthermore, this proposal has been criticized for being overly narrow at a clinical level, as it would consider DID as the only dissociative disorder, leaving thus other mental problems such as depersonalization, amnesia, or derealization outside this domain [17]. As a final note, and in relation with the following debate, this proposal has been found problematic as well by those who defend dissociation as a human disposition (i.e., not necessarily pathological [18]). We will explain this debate in some detail in the following subsection.

Could Dissociation Be Considered a Spectrum Spanning from Normal to Pathological Conditions?

The answer to this question has provoked a prevailing debate that started with the first descriptions of dissociation [8]. Those who advocate for dissociation as a human disposition propose that it occurs within a continuum ranging from the pathological, non-adaptive clinical syndromes, to inconsequential daily-life dissociations such as day-dreaming [15, 19]. There is statistical evidence indicating that there are some qualitative differences between pathological and non-pathological dissociation: for example, amnesia or identity disturbances are rarely present in the latter. Furthermore, patients diagnosed with a dissociative disorder are more prone to experience "normal" dissociative symptoms, such as absorption in one's own thoughts. These differences have been termed the dissociation taxon [19, 20].

From a more philosophical perspective, Braude has proposed that dissociation is a human disposition, that is, an ability that can bring both positive and negative consequences: this is termed the "capability assumption". This interpretation of dissociation involves not only

everyday phenomena, but also extreme pathological dissociative states, since in both cases—and the whole spectrum between them—the own mental states of the person are dissociated: this is the "ownership assumption" [7]. If the thesis of dissociation as a human disposition is accepted, there is a deeper debate to be held: should dissociation be treated? If so, at what point within the spectrum should dissociation be considered pathological? These are extremely relevant questions for the clinical practice: if dissociation is considered in an excessively loose way, it could deny the existence of a maladaptive state of the person that entails an extreme psychological suffering. This denial would prevent the person receiving an adequate assistant to recover from this suffering mental state.

Interestingly, dissociation has been used as a therapeutic tool to overcome severe mental conditions. For example, hypnosis benefits from the suggestibility of the person to achieve certain therapeutic goals, although it is not successful for all patients. From this point of view, dissociation could be understood as a defensive mechanism to overcome the original conflict that caused the pathological mental state. However, it should be considered that this "transitional" (dissociative) state is maladaptive itself, since it prevents the person from properly adapting to the environment. An important fact to consider is that DID patients are highly suggestible to hypnosis [21]. Another problematic issue of considering dissociation as non-pathological is allowing the subject to freely going back and forth from his or her dissociative state. Once again, this would entail psychological suffering and a lack of integrity of the self. For those who are able to self-hypnosis, the dissociative state may become a mechanism of self-protection against traumatic memories [21]. However, this protection should not substitute the final goal of the therapist with respect to the patient, who should achieve: (1) a proper adaptation to the environment; (2) an acceptance of their condition; and (3) the integrity of the self.

Overall, we have outlined some of the prevalent and recent debates in psychiatry with respect to dissociative disorders and, in particular, DID. In our opinion, these debates are better understood if the philosophical framework that underlies this psychiatric condition is further clarified. Therefore, in the following section we will summarize the Lockean inspiration of the concept of dissociation, and then we will propose a novel alternative that may help overcome the above mentioned debates.

Philosophical Framework of Dissociative Identity Disorder: The Primary Dissociation

The main goal of this section is to show how the understanding of DID across the history of manuals, most scientific research about it, and philosophical reflections on the topic have a common theoretical background based on the philosophy of John Locke. The matters included in the analysis of dissociative disorders are so radical and important that the interest that these disorders have awakened among philosophers is not strange. In fact, the mere description of the most recent contributions of philosophers to this topic would be such a complex quest that it is beyond the scope of this chapter. At the same time, some psychiatrists find these philosophical approaches interesting to solve the great questions at stake. Undoubtedly, the deep study of dissociative disorders, and in particular DID, is an ideal ground to promote the dialogue between psychiatry and philosophy.

The main philosophical problem in the study of this issue is the clarification of the notions of person, identity, and personality (or "personality state"). This is not a simple terminological or even conceptual debate, but an ontological problem worsened by the ambiguity of the concepts. According to Locke's philosophy, personal identity consists of consciousness and memory [22]. In other words, the defining characteristics of a person are the capacity to retain a set of memories, and the external expression of consciousness. Thus, a person is not something or someone, but two particular features, perhaps temporary, which appear to belong to that something or someone. The understanding of a person as the expression of memory and

consciousness underlies current debates on dissociation, since subjectivity (i.e., in this context, the mind), at least from Descartes, is generally viewed as something clear and distinct from the body. Moreover, according to this view, subjectivity is the radical essence of what a person is. And what is subjectivity, according to Descartes? "I take the word 'thought' to cover everything that we are aware of as happening within us, and it counts as 'thought' because we are aware of it. That includes not only understanding, willing, and imagining, but also sensory awareness" [23]. Interestingly, this position is compatible with both monist and dualist conceptions of the human being. Whereas the former interprets subjectivity as some shallow and temporary event that happens to the human body, the latter assumes that subjectivity is separable from a body to the extent that the relation between mind and body may appear problematic.

The influence of Lockean philosophy on the description of DID in the manuals may be implicit, albeit unquestionable. Disruptions of memory and self-consciousness are at the core of the DID diagnosis. According to the DSM-5 (and also to previous versions), the disorder can be identified by two clusters of symptoms which follow this Lockean conception of identity or person, namely: (1) alterations in the sense of self and agency; and (2) recurrent amnesias. Moreover, the DSM-5 description indicates that while the symptoms related to the first criterion are subjective, they typically have an external manifestation.

According to further developments of Locke's proposal, a living being (i.e., a human) could start being a person at some point (for example, when a baby starts expressing memory and consciousness), and consequently could stop being a person before death (for example, when memory and consciousness are affected by dementia). A non-trivial question that arises from this interpretation of the term "person" is as follows: what degree of consciousness and memory is required for a human being to be considered a person, or for a non-human animal to be considered a person at some point of their lives? The Australian philosopher Peter Singer leads this discussion to an extreme position. He proposes *personism*, which consists on defending the rights of some animals as persons, and denying them to infants, for example [24]. In our opinion, John Locke's view is relevant for a proper understanding of the evolution of DID diagnosis. In turn, consciousness and memory have been important factors to determine the degree of independence of the alter personalities in DID patients. As we mentioned in previous sections, the third version of DSM considered that alters should have full consciousness and memory to diagnose DID; however, Nijenhuis and van de Hart [6] proposed minimum requirements (situatedness, phenomenal now, and transparency). Furthermore, the criteria to evaluate consciousness and memory in these patients have varied greatly between the different versions of the manuals, as well as among theorists. As we commented above, according to ICD-10 and earlier versions of DSM, a nearly total independence between alters (sharing the same body) was required to diagnose DID. In our opinion, this is no less extreme than Singer's personism, in as much as alters could be understood as "independent persons" by expressing their own memory sets and self-consciousness. However, more recent proposals such as those of Nijenhuis and Van der Hart only demand a minimal degree of self-consciousness to the dissociated parts.

Thus, the main message of these introductory paragraphs is that, according to Lockean philosophy, personal identity is defined as the expression of memory and consciousness; and this view is common in theoretical approaches to DID from both philosophy and psychiatry.

In our opinion, this is the *primary dissociation*: the sharp conceptual dissociation between a person (understood as certain mental features) and his or her body. Please note that the *primary dissociation* refers to the conceptual starting point that most psychiatrists and philosophers have accepted when discussing on dissociative disorders.

This could be the reason why DID has aroused such a great interest among certain philosophers: just the very possibility of this disease would prove that the person is independent from the body, in as much as different "persons" (i.e., expressions of consciousness and memory) can "be connected with" the same body. The kind of

"connection" between the persons and the body should be clarified, and this is where different philosophical interpretations are proposed. A prototypical example of what we refer to as primary dissociation is the excerpt included at the beginning of this chapter, extracted from the classical book by Morton Prince "The Dissociation of a Personality". He describes how the different alters of his patient had a complete existence that ran in parallel, up to the point of having different attentional spans, verbal comprehension, and interests. This extreme account is nonetheless fully in line with the DSM-III description of the disorder, as it includes that each personality is fully integrated with unique memories, behavior patterns, and social relationships, and subpersonalities may actively perceive all that is going on. When such conceptions of DID are held, it is certainly assumed that there are different fully-formed psychological persons within the same body. DSM-5, while still embedded in this current of thought, it has nevertheless softened the requirements of independence and completeness of the identities. On the one hand, the necessity of identities to be fully formed is not the most important element for diagnosis anymore. It has been substituted for the relevance of disruptions in the subjective feeling of agency and self. In addition, these should be accompanied by other alterations in any other cognitive, behavioral, or sensory-motor function. On the other hand, as discussed above, it does not include any control requirements of the *alters*.

From a multidisciplinary point of view, there are some problematic consequences when the *primary dissociation* is assumed. Here, we will mention two: the defining characteristics of the person, and the consideration of dissociation as pathological. Concerning the former, we have explained that the starting point is to define the person independently from the body. According to Locke, the defining characteristics of the person are thus memory and consciousness. Consciousness is problematic itself, because it is not something static and clearly defined in a specific point of time (such as *awareness*). In fact, it does not belong to time but is the condition for its articulation. In this way, consciousness allows us to integrate temporal events and to understand experienced events as a unity across time. With respect to memory, the fact of having a set of memories that one recognizes as his or her own would be the defining characteristic of a person. If one accepts this view, a dissociation of memories would clearly entail the appearance of a new person in the same body. Interestingly, the third version of DSM fully accepted this Lockean interpretation of the person, which has been extensively revised in later versions of this manual. In fact, in addition to this philosophical reflection, empirical data contradict in part the primary dissociation. For example, switching between personality states is not as overt, frequent, or clearly observable as previously thought. Similarly, the body control of some alters at the expense of others is also an obscure topic. Furthermore, many of the most common symptoms of the disorder are related to detachment rather than compartmentalization, and hence they do not support the plausibility of a real primary dissociation [25].

Concerning the latter consequence of accepting the *primary dissociation*, that is, the problem of considering dissociation as pathological, different persons could share the same body precisely because that body is not an intrinsic part of them. If this is so, which among the different persons has the right to claim the body? From the therapist's point of view, which of them, if any, must be preserved or suppressed? Moreover, if the *primary dissociation* is accepted, one could push it to the limit. If consciousness is one of the main features of a person, and it is interrupted during sleeping, we could accept that different persons live in the same body temporarily, as it happens in DID according to some descriptions. Several identities, personalities, or personality states occupy the same body in a serial way.

Against the Primary Dissociation: Dynamic Identity and Person as Unifying Force

In our opinion, the *primary dissociation* is radically problematic because it tears the concepts of identity and person away from the reality of

human beings. In this last chapter, we will present some philosophical reflections to suggest that both identity and person can be understood from a different perspective, mainly active, normative, and inseparable from the body.

The identity of the person is one of the problems involved in the understanding of DID, and perhaps of dissociative disorders in general. According to Christine Korsgaard, there are two possible interpretations of identity: a person is "both active and passive, both an agent and a subject of experiences. Utilitarian and Kantian moral philosophers, however, characteristically place a different emphasis on these two aspects of our nature" [26]. She clarifies that whereas the utilitarian interpretation focuses on the passive side of human nature, the Kantian highlights human agency. At a moral level, the former wonders what should be done for people, whereas according to the latter each person wonders what she or he should do. Korsgaard believes that personal identity is centered on agency. This turn is mainly based on two facts: "First, the need for identification with some unifying principle or way of choosing is imposed on us by the necessity of making deliberative choices, not by the metaphysical facts. Second, the metaphysical facts do not obviously settle the question: I must still decide whether the consideration that some future person is "me" has some special normative force for me. It is practical reason that requires me to construct an identity for myself; whether metaphysics is to guide me in this or not is an open question" [26]. Therefore, we could synthesize Korsgaard's position by stating that identity is developed through action, and thus it goes beyond memory and consciousness. Other authors, in the context of dissociation, have defended the normative character of identity as well. However, in our opinion, they go too far in two aspects: (1) stressing the social and extrinsic nature of this constraint [27], and (2) separating identity from nature, and hence their relation within the living being remains unclear [28].

This view is supported by other authors. Remarkably, Spaemann states that it is hard to find an adequate definition of identity if we separate it from the fact of "being identical": it is a process, something dynamic where being itself is at stake [29]. In a similar way, Charles Taylor defends the notion that one's "identity is defined by the commitments and identifications which provide the frame or horizon within which I can try to determine from case to case what is good, or valuable, or what ought to be done, or what I endorse or oppose" [30]. Using the term person instead of identity, Paul Ricoeur stresses the dynamic feature of the person by insisting on its relational aspect, as Taylor did. According to Ricoeur, "self-constancy is for each person that manner of conducting himself or herself so that others can count on that person" [31].

The main purpose of these philosophical brushstrokes is to expose the fact that complex terms, such as identity and person, go far beyond a simple fulfillment of criteria such as expressing memory or consciousness. A possible framework to understand identity, and in our opinion the most adequate, is under the scope of action and commitment with other persons. Within this framework, the *activity* of a human being should not be torn apart from corporeity. As Aristotle wrote, "for living beings, to be alive is to be" [32]. Being a person is being a *living being* of a special kind. In the case of humans, this implies that we are a living body, and that our consciousness and our body are referred to the same "thing", whose reality is more than just consciousness or the body. We all recognize this assumption in day-to-day life. When seeing a picture of ourselves when we were kids, we do not say "that is my body when I was a child" or "this is the body that later produced me". We say "this is *me* when I was a child". Something within us, what makes us persons, our identity in a dynamical sense is present in childhood as it is in adulthood. Spaemann wonders whether, at some point, we are ever just exactly what we are. He answers as follows [29]:

> The possibility of role-play depends on the fact that as persons, we are always playing a role. Our identity is, on the one hand, simply the identity of a natural thing, an organism, and as such we can at any time be recognized by others as one and the same with ourselves. But this basic natural identity contains only a set of directions for the way, and on that way we must look for our identity—or construct it. The person is neither the product of this

> construction, nor the end-point on the way. The person is the way itself, the whole biography anchored in biological identity. Persons are not roles, but they are role-players, who *stylize* themselves in one or another manner.

Therefore, Spaemann gives a richer and more holistic definition of identity and person than that implicit in the texts analyzed in previous sections: a person is the way to construct an identity, which in turn involves our biography and the interaction with others. Spaemann himself contributes to the field of DID stating the following: "What appears within the drama as two subjects, substantially distinct, is in reality only two aspects of one subject, though qualitatively so disparate that it seems for the moment impossible they should ever be integrated. But even in this case, to integrate them is the task we face" [29].

These definitions are in sharp contrast with those based in Locke's philosophy, according to which the concept of person was simply characterized by the expression of consciousness and memory.

What are the implications of our interpretation of these concepts for DID and other dissociative disorders? How does this contribute to the current debates? Due to its dynamic character, identity is normative: it is directed to the best possible development. Let's suppose that identity, understood as a dynamic growth, is a single vertical line. The characterization of DID by the manuals, including DSM-5, assume that it is possible for a person to have a double vertical line. The debates arise when the therapist has to consider whether that twofold identity should be treated, or which line should prevail. According to our approach, there is always a single line and the dissociation is manifested as a horizontal movement, coursing alternatively between left and right; this pendulous swinging prevents the normal development of identity, and therefore we can speak of "dissociative identity disorder". However, there is just one person (one single vertical line) who *never* suffers a primary dissociation: his or her subjectivity (i.e., mental qualities) can never be dissociated from corporeity. Assuming this, it is impossible for various persons to live in the same body, because they could not be defined independently from the body that they are supposedly sharing.

Conclusion

In the present chapter, we have studied in depth the dialogue between philosophy and psychiatry in the case of dissociative disorders, and in particular DID. These are our main conclusions, which emerge from the dialogue between the two disciplines and are intended to be helpful for the understanding of these disorders within both standpoints. (1) Dissociative disorders and DID have been extensively revised across the different versions of the medical reference guides. (2) Updates have been focused on the independence of multiple personalities and their control on the person's behavior. (3) Although DSM-5 is less strict in these criteria than earlier versions, it considers a notion of person with a clear Lockean influence: a person is characterized by the expression of consciousness and a set of memories. (4) These interpretations of DID and other dissociative disorders entail a *primary conceptual dissociation* between person and body. (5) The notion of person has a dual character: (5a) the self and (5b) the living being that the self is. Dissociating this dual character would imply, again, the primary dissociation, and therefore it should be avoided. (6) However, identity has a dynamic and imperative character: it is given, but it also has to be reached and developed through the behavior of each human being.

In conclusion, a deep study of identity should always consider its dynamic nature and understand the person as a mind–body unity. We hope our interdisciplinary reflection will be useful for philosophers and psychiatrists in addressing dissociative disorders and DID with new insights.

References

1. Prince M. The dissociation of a personality: a biographical study in abnormal psychology. New York: Longmans, Green, and Co; 1908. p. 575.
2. Ross C, Duffy C, Ellason J. Prevalence, reliability and validity of dissociative disorders in an inpatient setting. J Trauma Dissociation. 2002;3(1):1–17.

3. Spiegel D, Lewis-Fernández R, Lanius R, Vermetten E, Simeon D, Friedman M. Dissociative disorders in DSM-5. Annu Rev Clin Psychol. 2013;9(1): 299–326.
4. International Society for the Study of Trauma and Dissociation. Guidelines for treating dissociative identity disorder in adults, third revision: summary version. J Trauma Dissociation. 2011;12(2):188–212.
5. Dorahy MJ, Brand BL, Sar V, Krüger C, Stavropoulos P, Martínez-Taboas A, et al. Dissociative identity disorder: an empirical overview. Aust N Z J Psychiatry. 2014;48(5):402–17.
6. Nijenhuis ERS, van der Hart O. Dissociation in trauma: a new definition and comparison with previous formulations. J Trauma Dissociation [Internet]. 2011;12(4):416–45. Available from: http://www.tandfonline.com/doi/abs/10.1080/15299732.2011.570592.
7. Braude S. The conceptual unity of dissociation: a philosophical argument. In: Dissociation and the dissociative disorders: DSM-V and beyond. New York: Routledge; 2009. p. 27–36.
8. van der Hart O, Dorahy M. History of the concept of dissociation. Dissociation and the dissociative disorders: DSM-V and beyond. New York: Routledge; 2009. p. 3–26.
9. Lynn SJ, Lilienfeld SO, Merckelbach H, Giesbrecht T, van der Kloet D. Dissociation and dissociative disorders: challenging conventional wisdom. Curr Dir Psychol Sci. 2012;21(1):48–53.
10. Piper A, Merskey H. The persistence of folly: critical examination of dissociative identity disorder. Part II. The defence and decline of multiple personality or dissociative identity disorder. Can J Psychiatry. 2004;49(10):678–83.
11. Piper A, Merskey H. The persistence of folly: a critical examination of dissociative identity disorder. Part I. The excesses of an improbable concept. Can J Psychiatry. 2004;49(9):592–600.
12. Brand BL, Loewenstein RJ, Spiegel D. Dispelling myths about dissociative identity disorder treatment: an empirically based approach. Psychiatry. 2014 Jan;77(2):169–89.
13. Cardeña E. The domain of dissociation. In: Lynn SJ, Rhue JW, editors. Dissociation: clinical and theoretical perspectives. New York: Guilford Press; 1994. p. 15–31.
14. Cherry EC. Some experiments on the recognition of speech, with one and with two ears. J Acoust Soc Am. 1953;25(5):975.
15. Spitzer C, Barnow S, Freyberger HJ, Grabe HJ. Recent developments in the theory of dissociation. World Psychiatry. 2006;5(2):82–6.
16. Brown RJ. Commentary on "dissociation in trauma: a new definition and comparison with previous formulations" by Nijenhuis and Van der Hart. J Trauma Dissociation. 2011;12(4):450–3.
17. Bowman ES. Nijenhuis and Van der Hart: one view of the elephant. J Trauma Dissociation. 2011;12(4):446–9.. discussion 469–73
18. Butler LD. Must dissociation be unusual? J Trauma Dissociation. 2011;12(4):454–6.
19. Butler LD. Normative dissociation. Psychiatr Clin North Am. 2006;29(1):45–62.
20. Waller N, Putnam FW, Carlson EB. Types of dissociation and dissociative types: a taxometric analysis of dissociative experiences. Psychol Methods. 1996;1(3):300–21.
21. Maldonado J, Spiegel D. Trauma, dissociation and hypnotizability. In: Marmar R, Bremmer D, editors. Trauma: memory and dissociation. Washington, DC: American Psychiatric Press; 1998. p. 57–106.
22. Locke J. Essay concerning human understanding. Ware (Hertfordshire): Wordsworth Editions; 1998.
23. Descartes R. Principia philosophiae. In: Adam C, Tannery P, editors. Oeuvres de Descartes. Paris: J. Vrin; 1964.
24. Singer P. Practical ethics. Cambridge: Cambridge University Press; 1990.
25. Spiegel D, Loewenstein RJ, Lewis-Fernández R, Sar V, Simeon D, Vermetten E, et al. Dissociative disorders in DSM-5. Depress Anxiety. 2011;28(12):E17–45.
26. Korsgaard C. Personal identity and the unity of agency: a Kantian response to Parfit. Philos Public Aff. 1989;18(2):103–31.
27. Radden J. Multiple selves. In: Gallagher S, editor. The Oxford handbook of the self. New York: Oxford University Press; 2011. p. 547–70.
28. Rovane C. The bounds of agency. Princeton: Princeton University Press; 1998.
29. Spaemann R. Persons. Oxford: Oxford University Press; 2006.
30. Taylor C. Sources of the self: the making of the modern identity. Harvard: Harvard University Press; 1989.
31. Ricoeur P. Oneself as another. Chicago: University of Chicago Press; 1992.
32. Aristotle. On the soul. New York: Penguin Books; 1986.

Part II

From Basic Neurosciences to Human Brain

Effects of Emotional Stress on Astrocytes and Their Implications in Stress-Related Disorders

10

Christian Luis Bender, Gastón Diego Calfa, and Víctor Alejandro Molina

Abstract

Stress is a major risk factor in the etiology of several psychiatric diseases, such as anxiety disorders and depression. On the other hand, a growing body of evidence has demonstrated that astrocytes play a pivotal role in the normal functioning of the nervous system. Hence, understanding the effects of stress on astrocytes is crucial for a better comprehension of stress-related mental disorders. Here, we describe the evidence showing astrocyte changes induced by stress in animals and how this plasticity could operate to induce behavioral sequelae. In addition, human data linking astrocytes with psychiatric disorders related to stress are also discussed. Altogether, the data indicate that both chronic and acute stressors are capable of changing the morphology and function of astrocytes in the brain areas that are known to play a critical role in emotional processing, such as the prefrontal cortex, hippocampus, and amygdala. Furthermore, different lines of evidence suggest that astrocyte plasticity may contribute to the behavioral consequences of stress.

Keywords

Astrocytes • Chronic stress • Acute stress • Plasticity • Anxiety • Depression

Abbreviations

AQP4	Aquaporin 4
ATP	Adenosine triphosphate
CUS	Chronic unpredictable stress
Cx43	Connexin 43
FGF2	Astrocytic fibroblast growth factor
GABA	Gamma aminobutyric acid
GFAP	Glial fibrillary acidic protein

C.L. Bender, PhD (✉) • G.D. Calfa • V.A. Molina
IFEC-CONICET, Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina
e-mail: christianbender@gmail.com

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_10

GLAST	Glutamate aspartate transporter, also known as excitatory amino acid transporter 1 (EAAT1)
GLT-1	Glutamate transporter-1, also known as excitatory amino acid transporter 2 (EAAT2)
GS	Glutamine synthetase
IP3	Inositol triphosphate receptors
S100β	Calcium-binding protein β

Introduction

Stress is a relevant issue in neuroscience and has been the subject of intense research over many decades. A wide body of evidence has shown that the neurotransmitters, neuromodulators, and hormones released during exposure to stress reshape the brain in the long term. For instance, acute and chronic stress alter the morphology of neurons, leading to changes in spine density and dendritic length and complexity [1–3]. These changes induced by stress can help to explain the development of pathologic endophenotypes. For example, stress promotes an increase of dendritic spines in the amygdala (which is a brain region of particular interest for emotional processing) associated with increased anxiety-like behavior [4]. Hence, prior exposure to stress makes the amygdala more responsive, producing the emotional hyper-reactivity that is a hallmark of anxiety disorders [5, 6].

Surprisingly, even though across different brain tissues and different species the ratio of glia to neurons is approximately one [7–8], most of the research on the neurobiology of stress has focused exclusively on neurons. This bias was presumably generated by the misconception that glial cells were merely supportive cells. This obsolete view has been completely revised since the birth of the tripartite synapse more than two decades ago [9]. However, the study of the effect of stress on astrocytes and other types of glial cells is still in its infancy [10–12].

Here, we describe briefly the anatomy and function of astrocytes, which lays the groundwork for understanding the multiple ways that make astrocytes an obvious candidate for the alterations in brain functioning that may underlie stress-related pathologies. Then, we summarize the current evidence of the astrocyte alterations induced by stress from studies that used classical protocols to induce stress in mature animals. Then we explore the possible functional implications of astrocyte alterations induced by stress and the mechanisms that could be altered in the tripartite synapse. Finally, we bridge those findings in animals with the literature on humans with a focus on depression, since this is the pathology related to stress that has received more attention.

The Tripartite Synapses Concept

The two main subtypes of astrocytes are the protoplasmic and fibrous ones. Protoplasmic astrocytes are found throughout all gray matter and exhibit several stem branches that give rise to many finely branching processes. In contrast, fibrous astrocytes are found in white matter and exhibit many long, fiberlike processes. The fine processes of protoplasmic astrocytes envelop synapses, whereas the processes of fibrous astrocytes contact nodes of Ranvier, with both types of astrocytes forming gap junctions between the distal processes of neighboring astrocytes [13, 14]. From studies performed on rodent hippocampus and cortex, many finely branching processes from a single astrocyte are estimated to contact several hundred dendrites from multiple neurons and to envelop 100,000 or more synapses [15, 16]. The main stem processes of astrocytes, which have glial fibrillary acidic protein (GFAP) as their main constituent, represent around 15% of the total astrocyte volume. These processes ramify progressively to finally generate a dense matrix of thin elaborate terminal processes that associate with neuropil elements and in particular with the synapses. These fine astrocytic processes account for 70–80% of the astrocytic plasma membrane and are devoid of GFAP. It is important to point out that even perisynaptic processes are found in all brain regions, although the proportion of synapses having these

and the level of synaptic coverage vary significantly between areas and within the same area [17].

A striking fact is that human protoplasmic astrocytes were found to be 2.6 times larger, and more complex (103 more primary processes), than rodent astrocytes. In addition, their larger diameter and more numerous processes imply that human protoplasmic astrocytes occupy a 16.5-fold greater volume than their mouse counterparts, and cover up to two million synapses [18]. In an interesting experiment, mice were engrafted with human glial progenitor cells, and upon maturation the recipient brains exhibited large numbers of human astrocytes. The engrafted human glia were coupled to host astroglia through gap junctions, yet retained the size and pleomorphism of hominid astroglia. Notably, the human glial chimeric mice showed enhanced learning and long-term potentiation, effects attributed to an increase in glia-released gliotransmitters [19].

The knowledge about the pivotal role that astrocytes play as fundamental units of the synaptic function began more than two decades ago [8, 20–22]. This revaluation started with the tripartite synapse concept, which incorporated the astrocytes as the third functional component of the synapse to the classic pre- and postsynaptic elements. This conceptual framework is based on the following aspects. First, astrocytes control the synaptic microenvironment through transporters, channels, and enzymes, with several of these highly or exclusively expressed by these glial cells. For instance, GLAST (glutamate aspartate transporter also known as EAAT1) and GLT-1 (glutamate transporter-1 also known as EAAT2), which remove extracellular glutamate. Furthermore, glutamine synthetase (GS), an enzyme that converts glutamate to glutamine is the precursor to synthetase glutamate and gamma aminobutyric acid (GABA). Second, astrocytes respond to the neurotransmitters released by neurons through membrane receptors, and in fact, most of the receptors present in neurons are also present in astrocytes. Third, they release substances termed "gliotransmitters", which in turn can affect neuronal activity. Specifically, they have been shown to be capable of releasing glutamate, D-serine, adenosine triphosphate (ATP), adenosine, GABA, tumor necrosis factor alpha, prostaglandins, atrial natriuretic peptide and brain-derived neurotropic factor, among other candidates.

Astrocytes communicate with each other through calcium waves, which are believed to represent for astrocytes what action potentials do for neurons [22]. These calcium signals are mainly due to the release of internal stores by activation of inositol triphosphate receptors (IP3), and the calcium waves propagate to neighboring astrocytes through gap junctions, where connexin 43 (Cx43) is an important constituent of the channels. These waves can be observed in vivo by two-photon microscopy after different sensory stimulations after 3–10 s, but even faster responses have also been reported. Calcium waves are implicated in gliotransmitter release and probably play an important role in other astrocyte functions. For instance, using whole-cell-path clamp combined with selective activation of astrocytes has shown that calcium waves can trigger an increase in the excitatory or inhibitory postsynaptic potential frequencies and, in more selected cases, also increase the amplitudes of excitatory potentials. These are transient effects that occur 20–60 s after stimulation [22, 23].

Emotional Stress and the Effects on Astrocyte Structure and Function

Stress is an adaptative physiological response that prepares the organism to face events that represent a physical and/or psychological threat. Hence, it is essential for survival and dealing with situations that require rapid "flight or fight" responses. However, when the stressors are overwhelming or are repeated over time they can eventually lead to pathology, especially when the predictability, control, and coping mechanisms are perceived as being insufficient to deal with the demands placed on them [24–26]. In fact, stress is considered to be one of the main risk factors for the development of psychiatric disorders such as anxiety-related disorders, depression and

drug addiction [27–30]. In general, brief and intensive aversive situations can provoke symptoms of anxiety [31, 32] while chronic mild stress tends to induce a more depression-related phenotype [33, 34].

Taking into account that morphological change in neurons is a hallmark of chronic stress effects with resulting increases, or reductions (depending on the brain structure) in both dendrite branches and spine density [1], it could be expected that astrocytes, which are in a close relationship at the synaptic level, could show structural plasticity. However, as mentioned above, the complexity of the astrocyte structures and the thickness of the perisynaptic processes have precluded an extensive morphological analysis of the intact brain after stress using the most common microscopy setups.

In-vitro evidence has clearly shown that astrocyte morphology is very dynamic, with highly motile astrocytic filopodia-like processes moving or growing over a time course of only a few minutes or even in seconds [17, 35]. Using organotypic hippocampal slices, a preparation that retains the three-dimensional architecture of astrocyte–synapse interactions, it has been demonstrated that astrocytes can rapidly extend and retract fine processes to engage or disengage postsynaptic dendritic spines [36]. Studies on intact brain also indicate that mature astrocytes are able to elongate or retract their perisynaptic processes and also to alter the whole shape of these cells [17, 37, 38]. One of the pioneering examples of astrocyte structural plasticity was shown in the paraventricular nucleus of the hypothalamus, which regulates the release of oxytocin, a hormone necessary for milk ejection from mammary glands. During lactation, astrocyte perisynaptic processes retract from the synapses, with the consequence (among other coordinated mechanisms) that astrocytes decrease the removal of glutamate from the synaptic cleft, thereby increasing the action of the neurotransmitter. At the time of weaning, the astrocytes then elongate again into the synapses and the oxytocin release returns to normal levels [39].

Most that we know about stress-induced morphological plasticity came from investigations that have used the gold standard marker of astrocyte GFAP detected by immunohistochemistry. GFAP is an intermediate filament protein present in the astrocyte cytoskeleton, but only expressed in the main processes; hence, it does not stain the perisynaptic processes that emanate from the principal astrocyte branches. In addition, changes in GFAP probably not only reflect a structural, but also a functional consequence for the astrocyte physiology, since this protein has been implicated in cell-to-cell communication, anchoring of proteins, and the reaction to brain insults [14]. For instance, cells lacking GFAP proteins do not develop perisynaptic processes with neurons [40], and have a reduction in the trafficking of the astrocytic glutamate transporter GLAST [41].

One of the pioneering studies that revealed astrocyte changes induced by stress was performed by Czéhet et al. [42]. In this study, adult male tree shrews were subjected to 5 weeks of psychosocial stress, and the number of cells (measured using stereological methods) showed a 25% reduction in the number of GFAP-positive cells in the hippocampus. Moreover, this work showed that the somatic volume of astrocytes was reduced by 25% in stressed animals. Even though GFAP is not a good marker for somas, since it is a protein exclusively present in the main processes, the changes reported are suggestive of an astrocyte process rearrangement. In support of this hypothesis, recent work carried out an extensive analysis of GFAP staining after chronic restraint described that stress induces a reduction in both the number and shortening of main processes [43].

In another seminal work performed by Banasr and coworkers [44], a chronic unpredictable stress (CUS) induced a 19% reduction in the number of GFAP-positive cells in the rat infralimbic cortex. These types of observations have been replicated and extended in a number of studies in rats, resulting in one of the most consistently reproducible results in the field [43, 45–49]. However, since GFAP is not present in all astrocytes, these studies are not conclusive. Another important limitation

of these pioneering works was the lack of measurement of the total cell number with other markers. Hence, it was unclear if there were fewer GFAP-positive cells because they had died or maybe had stopped producing GFAP at detectable levels for immunohistochemistry.

Subsequent findings by Gosselin and coworkers [50] represented an important step forward in this issue. This research analyzed some broader areas in Wistar Kyoto rats, which are more responsive to stressors and manifest more anxiety and depressive-like behavior compared to Sprague Dawley rats. In this model, GFAP-positive cells were again found to be reduced in hippocampus, prefrontal cortex, and amygdala, but not in the other cortical areas evaluated. However, when astrocytes were counted using calcium-binding protein β (s100β) marker which stains astrocyte somas, there were no differences observed, suggesting that the astrocytes were not degenerating, but instead that the expression of GFAP was being downregulated. In fact, when the protein level was assessed by western blot, GFAP levels were found to be decreased in the prefrontal cortex and amygdala. In addition, since there were no differences between rat strains in terms of the number of nuclei quantified with DAPI (which stain all cell types) or with the neuronal marker NeuN, then this strongly suggests that there was no loss of astrocytic cells (or neurons).

Unfortunately, we do not know if the differences reported in Wistar Kyoto rats in stress sensitivity are a cause or a consequence of astrocyte differences. However, similar results were obtained with the chronic restraint stress model, using a similar staining approach in the prefrontal cortex [43], suggesting that stress induce astrocyte plasticity. Regardless of the limitation that Nissl staining was used to identify astrocytes, Kassem et al. [51] also did not find any differences in the astrocyte number in CA1, amygdala, or retrosplenial cortex after chronic restraint. On the other hand, using the CUS model, a decrease in the GFAP level was reported in the hippocampus detected by western blot [45, 52], and also at the RNA level in the hippocampus [45, 49] and prefrontal cortex [49, 53], indicating that downregulation operates at the transcription level.

A quite different result has been reported by other authors who used the chronic restraint model, in which they found an increase of GFAP-positive cells and protein level in hippocampus [54, 55]. Using this model, but analyzing other areas, a GFAP downregulation in the periaqueductal and the raphe nucleus was reported [56, 57]. Thus, unlike the CUS and psychosocial stress paradigms, the chronic restraint model has produced more variability in the results of GFAP measurements, with differences in the predictability and controllability in those models probably accounting for the differences reported following stress exposure [25].

Another marker of astrocytes that has been studied is s100β. This is a protein that acts as a calcium sensor, which when activated, interacts with several other proteins and thus affects broad cellular functions. Moreover, it is secreted and induces cellular activities by acting in autocrine, paracrine, and endocrine manners [58]. As mentioned above, although the number of astrocytes expressing this protein does not change after stress, the level of s100β has been reported to be increased in the prefrontal cortex [43] and the hippocampus after CUS [47; however, see 59]. This implies that calcium waves may be altered by stress, but as far as we are aware there are no publications that have measured calcium waves after stress protocols.

As mentioned above, an important aspect of astrocytes is that they are highly interconnected through gap junctions which are the substrate for calcium-wave propagation. Accordingly, some authors have explored whether the substrate for this communication is disrupted after CUS, and found that the intra–infralimbic diffusion of a permeable dye, which was preferentially spreading among astrocytes through gap junctions, was notably decreased after CUS. Furthermore, alterations in astrocyte gap junctions were confirmed at electron microscopy level, and were associated with downregulation of Cx43 [48].

A more direct functional measurement of astrocytes after CUS was performed based on

infusion of (2-13C) acetate, which has been shown to be preferentially metabolized in astrocytes. The findings of this experiment showed that after stress, the animals had a reduction in the marked glutamate, glutamine, and GABA, indicating a slowing in the astrocyte metabolism [53]. In the same series of experiments, other proteins that are involved in glutamatergic transmission and preferentially expressed by astrocytes, such as GLT-1, GLAST, or GS, were found to be unchanged in the prefrontal cortex after CUS, at least at the mARN level [53].

All the above results were performed in chronic stress paradigms; hence, an important question not answered in those works is how much stress is necessary to observe changes in astrocytes. However, a couple of studies have explored astrocyte proteins after acute stressors that give a partial answer to this question. There was no change in GFAP immunostaining in the hippocampus from rats that were restrained for 2 h with the additional stress of being submerged in water [60]. However, when a presumably "stronger stressor" was used (the combination of restrain, forced swimming test, and ether exposure in an acute sequential session), there was a reduction of hippocampal GFAP expression in the hippocampus [61]. On the other hand, investigations that observed a downregulation of GFAP in the periaqueductal area and raphe nucleus in the chronic restraint model did not observe these changes during a shorter stress session (3 day/6 h compared to the standard 21 day/6 h), suggesting that changes in GFAP in these areas require exposure to chronic stress [56, 57].

In a predator paradigm in which rats were exposed for 5 min to the sight and smell of a cat, the s100β content was enhanced in cerebrospinal fluid, but not in the hippocampus or cerebral cortex, 1 h after the stressful experience [62]. A similar result was found in the restraint model [63], suggesting that s100β is rapidly released from astrocytes after acute stress. Other experiments have further shown an increase in the number of astrocytes expressing the inflammatory protein interleukin 1β in the hippocampus, hypothalamus, amygdala, and periaqueductal gray [60]. In addition, 3 h of restraint induced an increase of astrocytic fibroblast growth factor (FGF2) which was associated with an enhancement in hippocampal neurogenesis, suggesting a beneficial effect of astrocyte release FGF2 induced by stress [64]. In lateral/basolateral amygdala samples from rats subjected to 15 footshocks over a 93-min period, many astrocyte-enriched genes were either upregulated or downregulated in the stressed animals, and these seemed to be long lasting changes since measurements were taken 22 days after stress [65]. For example, an upregulation of GLAST and downregulation of serine racemase, which synthetizes the gliotransmitter D-serine, were detected in stressed animals.

Taken together, these data indicate that acute stress is able to induce changes in astrocytes, which suggests that these cells are rapidly sensing and responding to hormones and/or neurotransmitter released during the stress response. Furthermore, those changes could be long-lasting and more pronounced after chronic stress. However, a not-answered issue in most publications cited above, either after acute or chronic paradigms, is whether these astrocyte changes are reversible after a time of recovery. This is important, since more permanent changes are most probably related to the physiopathological changes that underlie long-lasting maladaptive behavioral effects of stress, such as anxiety and depression.

The Role That Stress-Induced Astrocyte Plasticity May Be Playing in the Behavioral Sequelae of Stress

A long tradition in neuroscience research has shown that stress can induce depressive and anxiety-like behavior in animals [24, 66]. For instance, the CUS model induces anhedonic-like effects, operationally defined as a decrement in sucrose consumption, and also hopelessness measured by a forced swimming test and active avoidance paradigms [53, 67]. On the other hand, as described in this review, there is extensive evidence that chronic and acute stress are capable of inducing changes in astrocyte morphology or

functionality, which are presumed to be deleterious for brain functioning and eventually form a part of the physiopathology of stress-related disorders. Therefore, an important question arises: does stress-induced astrocyte plasticity play any role in the behavioral sequelae induced by stress?

Several of the studies presented above using stress chronic models have also shown that antidepressant drugs, e.g., fluoxetine and clomipramine, which normalize stress-induced behavioral changes, prevented stress-induced astrocyte changes [42, 45, 48]. This strongly suggests that astrocytes are involved in the behavioral consequences of stress. The question about their sufficiency, however, is not simple to address, but the use of gliotoxins and transgenic animals has indicated that astrocytes may indeed play a causal role in the long-lasting effects induced by stressful experiences. One of the first experimental findings supporting this proposal came from experiments performed by Banasr and coworkers [44]. By applying L-alpha-aminoadipic acid microinjections into the rat prefrontal cortex, which selectively decreased the number of GFAP positive cells by 23% (but not neurons), anhedonia and hopelessness were induced in the short term. This type of experiment has been subsequently replicated and extended using other gliotoxins [48, 68–70]. Moreover, gliotoxin-induced depressive behavior was prevented by systemic antidepressant drugs [68].

Transgenic mice with an alteration in the nitric oxide synthetase 2 (which is predominantly expressed in glial cells) produce high levels of nitric oxide in astrocytes. This astrocytic alteration render the animals more susceptible to acute stress, as evidenced by higher anxiety-like behavior, increased acoustic startle responses, and higher plasma corticosterone levels compared to wild-type mice after predator scent exposure [71]. Another mousee which had a reduction in the ATP secreted from astrocytes showed a depressive phenotype, which was similar to the one observed after chronic stress paradigms that also decreased the release of ATP [67], Furthermore, in another transgenic mouse line, the release of ATP from astrocytes was increased after injection of a specific ligand, which induced antidepressant-like effects in the forced swimming test and in the chronic social defeat stress model [67]. Thus, selective alterations of the astrocyte machinery were sufficient to either trigger stress-like effects or give protection from the behavioral consequences of stress. However, these findings have been challenged by a recent paper which did not find any behavioral alteration in several emotional and cognitive tasks in similar transgenic mice that were knockout for astrocytic IP3R2, which is a critical receptor for triggering calcium wave signals [72].

Current advances in more selective and less invasive ways of activating or silencing astrocyte activity, such as optogenetic and designer receptors exclusively activated by designer drugs [73], will be critical for understanding how astrocytes contribute to the emergence of the behavioral aberrations associated to stress exposure.

Pathophysiological Changes in the Tripartite Synapse That Could Underlie Behavioral Sequelae of Stress

As mentioned before, the retraction of astrocytes from synapses in the hypothalamus has been shown to be critical to increase the glutamate effects as a result of a reduction in the removal of this transmitter, which is mainly taken up by astrocyte transporters [39]. In the same direction, the retraction of astrocytes (Bergmann glia) in cerebellar cortex enhances the excitatory postsynaptic current amplitude of Purkinje cells [38]. In hippocampus also, a mutation that makes astrocytes to retract from the synapses facilitates glutamate spillover and increases the NMDA currents in pyramidal neurons after burst stimulation [74]. On the other hand, acute and chronic stress has been associated with increases in glutamate release/content in the synaptic cleft, and excitotoxicity has been claimed as an important mechanism to produce cellular effects that underlie morphological and behavioral disturbances induced by stress [75]. Hence, the reduction in astrocyte processes induced by stress

could be a mechanism by which enhancement in excitability or even excitotoxicity is produced or increased. In fact, administration of GLT-1 blocker in PFC induced anhedonia-like behavior in rats [70]; and systemic injection of rulizole, a drug that facilitates glial cell glutamate uptake and decreases presynaptic release, prevented both the behavioral and astrocyte sequelae of chronic stress [53].

Another way in which stress-induced astrocyte alterations could affect behavior is through modulation of the GABAergic system. GABAergic synapses play a pivotal role in both anxiety disorders and emotional disturbance induced by stress [5, 76]. On the other hand, recent findings suggest that astrocytes release GABA and regulate GABA extrasynaptic content, which in turn is responsible for tonic GABA-A receptor-mediated currents [77]. Interestingly, chronic stress exposure induced a loss of tonic (but not phasic) inhibition in amygdala, an effect blocked by glucocorticoid synthesis inhibitor and mimicked by corticosterone [78]. Moreover, a study performed in slices from thalamus has shown that astrocytes also release a peptide that mediates a benzodiazepine-mimicking effect, and treatment with a gliotoxin reduced the effective inhibitory charge of GABA-A mediated spontaneous inhibitory postsynaptic currents [79]. Thus, the retraction of astrocytes from synapses or impairment in their function after chronic stress could account for the loss of tonic inhibition and consequent excitability of amygdala which is the hallmark of anxiety disorders such as posttraumatic stress disorder [27].

Mechanisms That Could Underlie Stress-Induced Astrocyte Plasticity

An important issue in this context is whether astrocytes can express receptors for stress-related hormones and neurotransmitters (e.g., glucocorticoids, norepinephrine) that allow them to directly respond to stress chemical mediators. Immunohistochemical studies have shown that beta receptors are extensively present in the lateral amygdala astrocytes [80] and alpha receptors in the prefrontal cortex [81]. It has long been established in astrocyte cultures that they show a morphological change (called stellation) in response to adrenergic beta receptor stimulation [37]. On the other hand, it is known that glucocorticoid and mineralocorticoid receptors are widely expressed in astrocytes and other glial cells [82, 83]. Recent postmortem studies in human tissue revealed the presence of glucocorticoid receptors in amygdala [84], hippocampus, and cortex [85]. Interestingly, experiments in vitro have demonstrated that corticosterone, at stress-relevant concentrations of 0.1–1 μM [86], induces an increase in the velocity of calcium waves and in gliotransmitter release [87]. Taken together, these findings indicate that astrocytes can directly sense and possibly change their morphology or functionality in response to chemicals released during the stress response.

Interestingly, corticosterone administration to rats (5 days or 4 months) caused a reduction in GFAP content in hippocampus and cortex [88] which, as after chronic stress, operates at the transcription level [89]. Norepinephrine is also able to modulate astrocyte activity. Using 2-photon microscopy in mice that express a Ca2+ indicator in astrocytes, it was shown that alpha adrenoceptor antagonists inhibited the activation of astrocyte networks that are triggered by the arousal associated to locomotion [90]. This effect seems to be specific for norepinephrine, since it was abolished by chemical depletion of norepinephrine but not by antagonists of serotonergic, muscarinic, metabotropic glutamate, or cannabinoid receptors [90]. In the same direction, when the locus coeruleus output was triggered by an air-puff startle response, it produced astrocyte calcium waves in prefrontal cortex that were suppressed by cortical administration of alpha adrenergic receptor antagonists or chemical depletion of norepinephrine [91]. Another way that norepinephrine could affect astrocytes is through phosphorylation of GFAP [92], which is believed to regulate the structural plasticity of glial filaments [93].

The molecular cascades that are triggered by stress in astrocytes and how they orchestrate the stress-induced astrocyte plasticity is essentially unknown, but clearly the astrocytes possess the machinery to sense and response to norepinephrine and glucocorticoids and probably other stress-released mediators.

Evidence of Astrocyte Alterations in Human Psychiatric Disorders Associated with Stress

Research related to this topic is strongly limited by the lack of non-invasive techniques that allow discriminating cell types in the intact brain. As a result, the only direct way of visualizing astrocytes in human brain is through postmortem studies. As far as we know, the principal psychiatric illness strongly associated to stress that has been studied in humans and focused on glial cells is depression. Related to this, several cell-counting studies have reported decreases in the packing density or number of the Nissl-stained populations of glial cells in subjects diagnosed with major depression, compared to non-psychiatric controls. These types of changes have been observed in fronto-limbic brain regions, including the dorsolateral prefrontal cortex, orbitofrontal cortex, subgenual cortex, anterior cingulate cortex and amygdala [94]. Another approach has been to study astrocyte morphology using the Golgi staining method, which allows the identification of scattered cells, permitting a 3D reconstruction of the whole individual cell. This technique was applied by Torres-Plata et al. [95] in the anterior cingulate cortex from suicidal depressive patients, and compared to matched control samples. These authors found an increase in the volume of the cell body and the number and length of the fibrous astrocyte processes located in the white matter adjacent to the anterior cingulate cortex, but not in the cortex itself.

Immunohistochemistry with antibodies against astrocyte specific proteins applied to postmortem tissue enables a more direct assessment of the astrocyte contribution to glial alterations in depressive subjects. Müller and coworkers [96] observed a reduction of GFAP immunoreactivity in the hippocampal areas CA1 and CA2, with the caveat that an observational criterion was used. A lower level of coverage of GFAP staining as well as a reduction in the number of GFAP-positive cells in the dorsolateral prefrontal cortex were found in young depressed patients, but not in older patients [97]. Similar findings were obtained in the orbitofrontal cortex using the western blot technique and fraction area of immunostaining [98]. In another study which used the s100β marker instead, a decrease in the number of astrocytes was also found in depressed and bipolar patients compared to matched controls [99]. By studying the amygdala postmortem tissue belonging to different psychiatric patients, another investigation found a reduction in GFAP-positive astrocytes, but only in depressive disorder [100; however, see 101]. In contrast, glucocorticoid receptors in amygdala astrocytes were increased in depressive patients compared to healthy controls or bipolar disorder patients [84]. This may be a compensatory response to high levels of glucocorticoids usually associated to depression.

Other measurements that have been applied to human postmortem tissue include in-situ hybridization and quantitative real-time PCR, which allow the detection and quantification of the mRNA present in brain sections and dissected dissolved tissue, respectively. Using this approach in the locus coeruleus [102], it was found that several transcripts for astrocyte proteins were altered in major depression but not in bipolar disorder. Specifically a reduction of GLT-1, GLAST, GS, GFAP, s100β, AQP4 (aquaporin 4, a water channel), Cx43, and connexin 30 (another gap-junction protein) was observed. A decrease in the expression of GLT-1, GLAST, and GS mRNAs has also been described in the anterior cingulate and dorsolateral prefrontal cortices [103]. Correspondingly, some of these transcripts have been also found to be reduced at the protein expression level in other areas. For instance, Cx43 [104], AQP4 [105], GLT-1, and GLAST [98] were decreased in the orbitofrontal cortex of depressive patients. Glutamate astrocytic transporters were also reduced in the amygdala of alcoholic individuals [106] which, according to these authors, could increase amygdala activity and the expression of associative memories and anxiety which underlie continued drug-seeking and chronic relapse.

Since s100β is secreted into the blood stream, this protein makes it possible to perform serum measurements in living patients, making it possible to investigate alterations of this astrocyte-related protein in different illnesses. Several studies have used this approach in psychiatric

populations, and a meta-analysis has been performed by Schroeter and coworkers [107] indicating that serum levels of s100β are consistently elevated during acute episodes of depression, with an increase with respect to control of 2.57 ± 0.70 (mean ± SD) fold. It is important to note that this increase is not specific to depression, as bipolar patients have also revealed increases in serum 100β. On the other hand, as s100β is also expressed in oligodendrocytes and some other body cells, then the respective contribution of these cells to the blood concentration is uncertain.

A big limitation in almost all human studies with depressive patients cited above is that most of the subjects were under antidepressant or other kind of psychopharmacology treatment that could affect the astrocyte measurement performed. However, some of these studies took account of this issue and made statistical comparison between persons under treatment vs. no medicated patients. For instance, in the study of Miguel-Hidalgo et al. [98] when subjects with depression that had antidepressant medication detected in the postmortem toxicology screening were compared to those without antidepressant, no differences in GLT-1, GLAST, GS, or GFAP levels were detected. Similarly, no medication effects were observed by Gos et al. [99], Rajkowska et al. [105], Wang et al. [84], and Miguel-Hidalgo et al. [104]. Interestingly, studies involving serum s100β measurement before and after successful treatment with antidepressive drugs indicated that s100β levels (which are larger than in control subjects) were reduced after treatment [107]. Even though the effect was small, this meta-analysis found a significant positive correlation between clinical treatment effects and serological treatment effects of s100β, suggesting that antidepressant could act to reduce s100β release from glia.

Investigations in non-psychiatric populations presumed to be exposed to robust stressors have also suggested that stress affect astrocytes. In this sense, serum s100β measurements taken 2 days after cardiac surgery along with Spielberger's anxiety inventory performed in cardiologic patients indicated that individuals with elevated s100β had higher levels of state anxiety and trait anxiety [108]. In the same way, s100β, as well as serum cortisol, were significantly increased in soldiers during combat training compared to at rest period, being concomitant to greater stress, anxiety, and depression levels assessed by psychological questionnaires [109].

Taken together, the human data from depressive patients showed a reduction of astrocyte markers in the dorsolateral prefrontal cortex, orbitofrontal cortex, hippocampal CA1 and CA2, and amygdala. Based on the results obtained in animals, it is possible to speculate that these changes might be caused by the effects of being exposed to chronic stress. However, while the animal data indicate that stress induced a downregulation of astrocyte markers without inducing "astrodegeneration", human studies have revealed a reduction in the number of the glial population stained with Nissl or s100β, suggesting that they degenerate or that the proliferation was reduced. Undoubtedly, depression is a multifactorial disease that is not only dependent on stress, and as referred above, there is evidence that reduction of GFAP could be an early manifestation that "disappears" at more advanced stages of the illness. On the other hand, studies on individuals that underwent a significant stress exposure, revealed clear changes in the astrocyte-related proteins 100β, which could be detected even at the blood level, suggesting a strong involvement of astrocytes in response to stress. Clearly, additional human studies are still necessary to fully understand the impact of stress on astrocytes functioning and the neurobiological and behavioral consequences.

Conclusions and Remarks

Stress effects on neuron morphology and function have been the subject of numerous investigations, which has been crucial for a better understanding of the mechanisms through which stress induces deleterious effects on brain functioning and on behavior. As shown in this review, different approaches in animals and humans have indicated that astrocytes are also an important target of stress, with both chronic and acute stressors being able to alter the morphology or the expression of several astrocyte

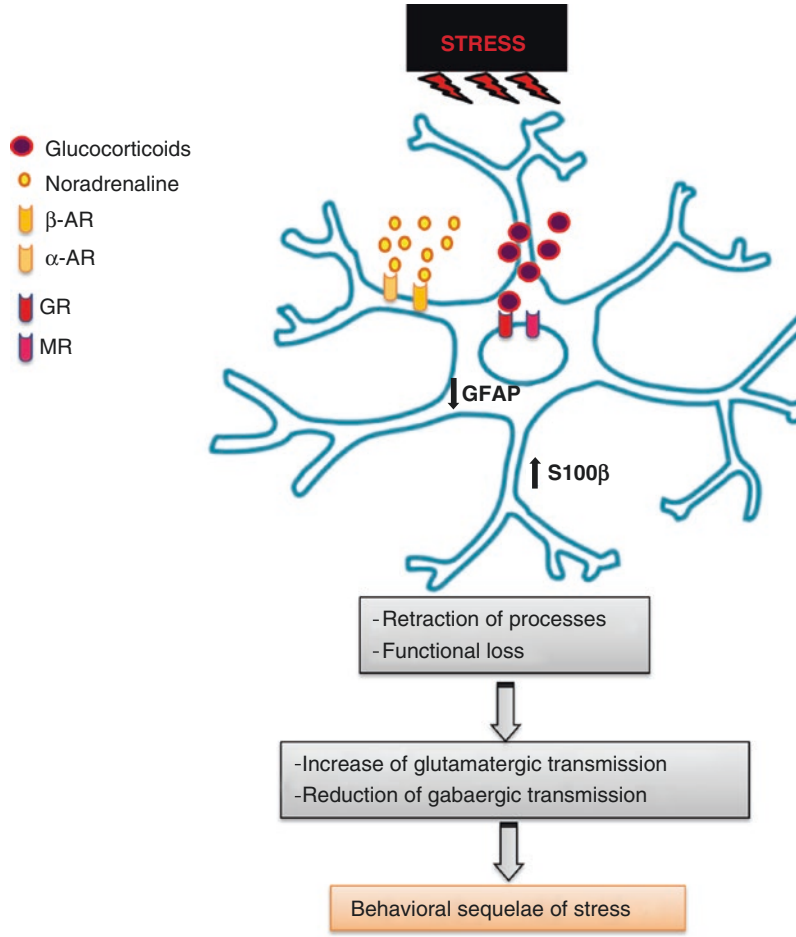


Fig. 10.1 Model of stress effects on astrocytes. Stress hormones (e.g., glucocorticoids) and neurotransmitters (e.g., norepinephrine) released during the stress response activate the receptors located in astrocytes and initiate intracellular cascades (including a decrease of GFAP levels and increase in S100b release) that ultimately produce changes in the morphology/physiology of astrocytes, which alters the normal functioning of tripartite synapses in a pathophysiological direction that is known to drive behavioral sequelae of stress, such as increases of glutamate transmission and/or reduction of GABAergic transmission

specific proteins in brain areas that are known to play a critical role in emotional processing, such as the prefrontal cortex, hippocampus, and amygdala. Furthermore, different lines of evidence have suggested that these changes may underlie the behavioral consequences of stress. First, astrocyte cellular effects induced by stress were prevented by the administration of drugs that averted the behavioral sequelae of stress. Second, astrocyte-specific toxins induced similar behaviors to those observed after stress exposure. Third, astrocyte-specific alterations in transgenic mice were able to emulate stress effects. Human data from psychiatric populations also support the notion that astrocytes are affected in mental disorders, with there being a remarkable agreement indicating that astrocyte-specific proteins are decreased in major depression, an illness strongly associated to stress. All together, the data suggest that stress hormones (e.g., glucocorticoids) and neurotransmitters (e.g., norepinephrine) through their receptors located in astrocytes directly induce intracellular cascades that ultimately introduce changes in the morphology/physiology of astrocytes, which alters the normal functioning of tripartite synapses in a pathophysiological direction that is known to drive behavioral sequelae of stress, such as increases of glutamate transmission and/or reduction of GABAergic transmission (see Fig. 10.1).

Acknowledgments This research was supported by grants from MinCyT-Cordoba, SECYT-UNC, CONICET, and Agencia Nacional de Promoción Científica y Tecnológica–FONCYT (Argentina) to Victor A Molina and SECYT-UNC, CONICET, and Agencia Nacional de Promoción Científica y Tecnológica–FONCYT (Argentina) to Gaston Calfa.

References

1. Christoffel DJ, Golden SA, Russo SJ. Structural and synaptic plasticity in stress-related disorders. Rev Neurosci. 2011;22(5):535–49.
2. Davidson RJ, McEwen BS. Social influences on neuroplasticity: stress and interventions to promote well-being. Nat Neurosci. 2012;15(5):689–95.
3. Giachero M, Calfa GD, Molina VA. Hippocampal structural plasticity accompanies the resulting contextual fear memory following stress and fear conditioning. Learn Mem. 2013;20(11):611–6.
4. Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S. Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. Proc Natl Acad Sci U S A. 2005;102(26):9371–6.
5. Martijena ID, Molina VA. The influence of stress on fear memory processes. Braz J Med Biol Res. 2012;45(4):308–13.
6. Etkin A, Wager TD. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry. 2007;164(10):1476–88.
7. Hilgetag CC, Barbas H. Are there ten times more glia than neurons in the brain? Brain Struct Funct. 2009;213(4–5):365–6.
8. Herculano-Houzel S. The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. Glia. 2014;62(9):1377–91.
9. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. 1999;22(5):208–15.
10. Walker FR, Nilsson M, Jones K. Acute and chronic stress-induced disturbances of microglial plasticity, phenotype and function. Curr Drug Targets. 2013;14(11):1262–76.
11. Delpech JC, Madore C, Nadjar A, Joffre C, Wohleb ES, Layé S. Microglia in neuronal plasticity: influence of stress. Neuropharmacol. 2015;96(Pt A):19–28.
12. Edgar N, Sibille E. A putative functional role for oligodendrocytes in mood regulation. Transl Psychiatry. 2012;2:e109.
13. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. Acta Neuropathol. 2010;119(1):7–35.
14. Middeldorp J, Hol EM. GFAP in health and disease. Prog Neurobiol. 2011;93(3):421–43.
15. Halassa MM, Fellin T, Takano H, Dong JH, Haydon PG. Synaptic islands defined by the territory of a single astrocyte. J Neurosci. 2007;27(24):6473–7.
16. Ogata K, Kosaka T. Structural and quantitative analysis of astrocytes in the mouse hippocampus. Neuroscience. 2002;113(1):221–33.
17. Bernardinelli Y, Muller D, Nikonenko I. Astrocyte-synapse structural plasticity. Neural Plast. 2014;2014:232105.
18. Oberheim NA, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M. Uniquely hominid features of adult human astrocytes. J Neurosci. 2009;29(10):3276–87.
19. Han X, Chen M, Wang F, Windrem M, Wang S, Shanz S, Xu Q, Oberheim NA, Bekar L, Betstadt S, Silva AJ, Takano T, Goldman SA, Nedergaard M. Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. Cell Stem Cell. 2013;12(3):342–53.
20. Barres BA. The mystery and magic of glia: a perspective on their roles in health and disease. Biol Psychiatry. 2008;64(10):863–70.
21. Kimelberg HK. Functions of mature mammalian astrocytes: a current view. Neuroscientist. 2010;16(1):79–106.
22. Perea G, Araque A. GLIA modulates synaptic transmission. Brain Res Rev. 2010;63(1–2):93–102.
23. Haydon PG, Nedergaard M. How do astrocytes participate in neural plasticity? Cold Spring Harb Perspect Biol. 2014;7(3):a020438.
24. McEwen BS, Gianaros PJ. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. Ann N Y Acad Sci. 2010;1186:190–222.
25. Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flügge G, Korte SM, Meerlo P, Murison R, Olivier B, Palanza P, Richter-Levin G, Sgoifo A, Steimer T, Stiedl O, van Dijk G, Wöhr M, Fuchs E. Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav Rev. 2011;35(5):1291–301.
26. Franklin TB, Saab BJ, Mansuy IM. Neural mechanisms of stress resilience and vulnerability. Neuron. 2012;75(5):747–61.
27. Shin LM, Liberzon I. The neurocircuitry of fear, stress, and anxiety disorders. Neuropsychopharmacology. 2010;35(1):169–91.
28. Guimaraes F, Joca SR, Padovani CM, Molina VA. Mood disorders. In: Neurobiology of mental disorders. Nova Science Publishers: New York, 2006, p. 95–124.
29. Edwards S, Baynes BB, Carmichael CY, Zamora-Martinez ER, Barrus M, Koob GF, Gilpin NW. Traumatic stress reactivity promotes excessive alcohol drinking and alters the balance of prefrontal cortex-amygdala activity. Transl Psychiatry. 2013;3:e296.
30. Papp M, Gruca P, Lason-Tyburkiewicz M, Litwa E, Willner P. Effects of chronic mild stress on the development of drug dependence in rats. Behav Pharmacol. 2014;25(5–6):518–31.
31. Bazak N, Kozlovsky N, Kaplan Z, Matar M, Golan H, Zohar J, Richter-Levin G, Cohen H. Prepubertal stress exposure affects adult behavioral response in association with changes in circulating corticosterone and brain-derived neurotrophic factor. Psychoneuroendocrinology. 2009;34(6):844–58.
32. Bignante A, Paglini G, Molina VA. Previous stress exposure enhances both anxiety-like behaviour and p 35 levels in the basolateral amygdala complex: modulation by midazolam. Eur Neuropsychopharmacol. 2010;20(6):388–97.
33. Elizalde N, García-García AL, Totterdell S, Gendive N, Venzala E, Ramirez MJ, Del Rio J, Tordera

RM. Sustained stress-induced changes in mice as a model for chronic depression. Psychopharmacology. 2010;210(3):393–406.
34. Zhu S, Shi R, Wang J, Wang JF, Li XM. Unpredictable chronic mild stress not chronic restraint stress induces depressive behaviours in mice. Neuroreport. 2014;25(14):1151–5.
35. Reichenbach A, Derouiche A, Kirchhoff F. Morphology and dynamics of perisynaptic glia. Brain Res Rev. 2010;63(1–2):11–25.
36. Haber M, Zhou L, Murai KK. Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. J Neurosci. 2006;26(35):8881–91.
37. Rodnight RB, Gottfried C. Morphological plasticity of rodent astroglia. J Neurochem. 2013;124(3):263–75.
38. Saab AS, Neumeyer A, Jahn HM, Cupido A, Šimek AA, Boele HJ, Scheller A, Le Meur K, Götz M, Monyer H, Sprengel R, Rubio ME, Deitmer JW, De Zeeuw CI, Kirchhoff F. Bergmann glial AMPA receptors are required for fine motor coordination. Science. 2012;337(6095):749–53.
39. Theodosis DT, Poulain DA, Oliet SH. Activity-dependent structural and functional plasticity of astrocyte–neuron interactions. Physiol Rev. 2008;88(3):983–1008.
40. Weinstein DE, Shelanski ML, Liem RK. Suppression by antisense mRNA demonstrates a requirement for the glial fibrillary acidic protein in the formation of stable astrocytic processes in response to neurons. J Cell Biol. 1991;112(6):1205–13.
41. Hughes EG, Maguire JL, McMinn MT, Scholz RE, Sutherland ML. Loss of glial fibrillary acidic protein results in decreased glutamate transport and inhibition of PKA-induced EAAT2 cell surface trafficking. Brain Res Mol Brain Res. 2004;124(2):114–23.
42. Czéh B, Simon M, Schmelting B, Hiemke C, Fuchs E. Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology. 2006;31(8):1616–26.
43. Tynan RJ, Beynon SB, Hinwood M, Johnson SJ, Nilsson M, Woods JJ. Walker FR Chronic stress-induced disruption of the astrocyte network is driven by structural atrophy and not loss of astrocytes. Acta Neuropathol. 2013;126(1):75–91.
44. Banasr M, Duman RS. Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biol Psychiatry. 2008;64(10):863–70.
45. Liu Q, Li B, Zhu HY, Wang YQ, Yu J, Wu GC. Clomipramine treatment reversed the glial pathology in a chronic unpredictable stress-induced rat model of depression. Eur Neuropsychopharmacol. 2009;19(11):796–805.
46. Liu Q, Li B, Zhu HY, Wang YQ, Yu J, Wu GC. Glia atrophy in the hippocampus of chronic unpredictable stress-induced depression model rats is reversed by electroacupuncture treatment. J Affect Disord. 2011;128(3):309–13.
47. Ye Y, Wang G, Wang H, Wang X. Brain-derived neurotrophic factor (BDNF) infusion restored astrocytic plasticity in the hippocampus of a rat model of depression. Neurosci Lett. 2011;503(1):15–9.
48. Sun JD, Liu Y, Yuan YH, Li J, Chen NH. Gap junction dysfunction in the prefrontal cortex induces depressive-like behaviors in rats. Neuropsychopharmacology. 2012;37(5):1305–20.
49. Li LF, Yang J, Ma SP, Qu R. Magnolol treatment reversed the glial pathology in an unpredictable chronic mild stress-induced rat model of depression. Eur J Pharmacol. 2013;711(1–3):42–9.
50. Gosselin RD, Gibney S, O'Malley D, Dinan TG, Cryan JF. Region specific decrease in glial fibrillary acidic protein immunoreactivity in the brain of a rat model of depression. Neuroscience. 2009;159(2):915–25.
51. Kassem MS, Lagopoulos J, Stait-Gardner T, Price WS, Chohan TW, Arnold JC, Hatton SN, Bennett MR. Stress-induced grey matter loss determined by MRI is primarily due to loss of dendrites and their synapses. Mol Neurobiol. 2013;47(2):645–61.
52. Araya-Callís C, Hiemke C, Abumaria N, Flugge G. Chronic psychosocial stress and citalopram modulate the expression of the glial proteins GFAP and NDRG2 in the hippocampus. Psychopharmacology. 2012;224(1):209–22.
53. Banasr M, Chowdhury GM, Terwilliger R, Newton SS, Duman RS, Behar KL, Sanacora G. Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. Mol Psychiatry. 2010;15(5):501–11.
54. Jang S, Suh SH, Yoo HS, Lee YM, Oh S. Changes in iNOS, GFAP and NR1 expression in various brain regions and elevation of sphingosine-1-phosphate in serum after immobilized stress. Neurochem Res. 2008;33(5):842–51.
55. Kwon MS, Seo YJ, Lee JK, Lee HK, Jung JS, Jang JE, Park SH, Suh HW. The repeated immobilization stress increases IL-1beta immunoreactivities in only neuron, but not astrocyte or microglia in hippocampal CA1 region, striatum and paraventricular nucleus. Neurosci Lett. 2008;430(3):258–63.
56. Imbe H, Kimura A, Donishi T, Kaneoke Y. Chronic restraint stress decreases glial fibrillary acidic protein and glutamate transporter in the periaqueductal gray matter. Neuroscience. 2012;223:209–18.
57. Imbe H, Kimura A, Donishi T, Kaneoke Y. Effects of restraint stress on glial activity in the rostral ventromedial medulla. Neuroscience. 2013;241:10–21.
58. Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, Tubaro C, Giambanco I. S100B's double life: intracellular regulator and extracellular signal. Biochim Biophys Acta. 2009;1793(6):1008–22.
59. Rong H, Wang G, Liu T, Wang H, Wan Q, Weng S. Chronic mild stress induces fluoxetine-reversible decreases in hippocampal and cerebrospinal fluid levels of the neurotrophic factor S100B and its specific receptor. Int J Mol Sci. 2010;11(12):5310–22.
60. Sugama S, Takenouchi T, Sekiyama K, Kitani H, Hashimoto M. Immunological responses of astro-

glia in the rat brain under acute stress: interleukin 1 beta co-localized in astroglia. Neuroscience. 2011;192:429–37.
61. Xia L, Zhai M, Wang L, Miao D, Zhu X, Wang W. FGF2 blocks PTSD symptoms via an astrocyte-based mechanism. Behav Brain Res. 2013;256:472–80.
62. Margis R, Zanatto VC, Tramontina F, Vinade E, Lhullier F, Portela LV, Souza DO, Dalmaz C, Kapczinski F, Gonçalves CA. Changes in S100B cerebrospinal fluid levels of rats subjected to predator stress. Brain Res. 2004;1028(2):213–8.
63. Scaccianoce S, Del Bianco P, Pannitteri G, Passarelli F. Relationship between stress and circulating levels of S100B protein. Brain Res. 2004;1004(1–2): 208–11.
64. Kirby ED, Muroy SE, Sun WG, Covarrubias D, Leong MJ, Barchas LA, Kaufer D. Acute stress enhances adult rat hippocampal neurogenesis and activation of newborn neurons via secreted astrocytic FGF2. Elife. 2013;2:e00362.
65. Ponomarev I, Rau V, Eger EI, Harris RA, Fanselow MS. Amygdala transcriptome and cellular mechanisms underlying stress-enhanced fear learning in a rat model of posttraumatic stress disorder. Neuropsychopharmacology. 2010;35(6):1402–11.
66. Daskalakis NP, Yehuda R, Diamond DM. Animal models in translational studies of PTSD. Psychoneuroendocrinology. 2013;38(9):1895–911.
67. Cao X, Li LP, Wang Q, Wu Q, Hu HH, Zhang M, Fang YY, Zhang J, Li SJ, Xiong WC, Yan HC, Gao YB, Liu JH, Li XW, Sun LR, Zeng YN, Zhu XH, Gao TM. Astrocyte-derived ATP modulates depressive-like behaviors. Nat Med. 2013;19(6):773–7.
68. Domin H, Szewczyk B, Woźniak M, Wawrzak-Wleciał A, Śmiałowska M. Antidepressant-like effect of the mGluR5 antagonist MTEP in an astroglial degeneration model of depression. Behav Brain Res. 2014;273:23–33.
69. Lee Y, Son H, Kim G, Kim S, Lee DH, Roh GS, Kang SS, Cho GJ, Choi WS, Kim HJ. Glutamine deficiency in the prefrontal cortex increases depressive-like behaviours in male mice. J Psychiatry Neurosci. 2013;38(3):183–91.
70. John CS, Smith KL, Van't Veer A, Gompf HS, Carlezon Jr WA, Cohen BM, Öngür D, Bechtholt-Gompf AJ. Blockade of astrocytic glutamate uptake in the prefrontal cortex induces anhedonia. Neuropsychopharmacology. 2012;37(11):2467–75.
71. Abu-Ghanem Y, Cohen H, Buskila Y, Grauer E, Amitai Y. Enhanced stress reactivity in nitric oxide-synthase type 2 mutant mice: findings in support of astrocytic nitrosative modulation of behavior. Neuroscience. 2008;156(2):257–65.
72. Petravicz J, Boyt KM, McCarthy KD. Astrocyte IP3R2-dependent Ca(2+) signaling is not a major modulator of neuronal pathways governing behavior. Front Behav Neurosci. 2014;8:384.
73. Li D, Agulhon C, Schmidt E, Oheim M, Ropert N. New tools for investigating astrocyte-to-neuron communication. Front Cell Neurosci. 2013;7:193.
74. Tanaka M, Shih PY, Gomi H, Yoshida T, Nakai J, Ando R, Furuichi T, Mikoshiba K, Semyanov A, Itohara S. Astrocytic Ca2+ signals are required for the functional integrity of tripartite synapses. Mol Brain. 2013;6:6.
75. Popoli M, Yan Z, McEwen BS, Sanacora G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat Rev Neurosci. 2011;13(1):22–37.
76. Rodriguez Manzanares P, Nora I, Carrer H, Molina VA. Prior stress facilitates fear memory, attenuates GABAergic inhibition and increases synaptic plasticity in the rat basolateral amygdala. J Neurosci. 2005;25(38):8725–34.
77. Yoon BE, Lee CJ. GABA as a rising gliotransmitter. Front Neural Circuits. 2014;8:141.
78. Liu ZP, Song C, Wang M, He Y, Xu XB, Pan HQ, Chen WB, Peng WJ, Pan BX. Chronic stress impairs GABAergic control of amygdala through suppressing the tonic GABAA receptor currents. Mol Brain. 2014;7:32.
79. Christian CA, Huguenard JR. Astrocytes potentiate GABAergic transmission in the thalamic reticular nucleus via endozepine signaling. Proc Natl Acad Sci U S A. 2013;110(50):20278–83.
80. Farb CR, Chang W, Ledoux JE. Ultrastructural characterization of noradrenergic axons and Beta-adrenergic receptors in the lateral nucleus of the amygdala. Front Behav Neurosci. 2010;4:162.
81. Aoki C, Venkatesan C, Go CG, Forman R, Kurose H. Cellular and subcellular sites for noradrenergic action in the monkey dorsolateral prefrontal cortex as revealed by the immunocytochemical localization of noradrenergic receptors and axons. Cereb Cortex. 1998;8(3):269–77.
82. Bohn MC, Howard E, Vielkind U, Krozowski Z. Glial cells express both mineralocorticoid and glucocorticoid receptors. J Steroid Biochem Mol Biol. 1991;40(1–3):105–11.
83. Cintra A, Bhatnagar M, Chadi G, Tinner B, Lindberg J, Gustafsson JA, Agnati LF, Fuxe K. Glial and neuronal glucocorticoid receptor immunoreactive cell populations in developing, adult, and aging brain. Ann N Y Acad Sci. 1994;746:42–61.
84. Wang Q, Verweij EW, Krugers HJ, Joels M, Swaab DF, Lucassen PJ. Distribution of the glucocorticoid receptor in the human amygdala; changes in mood disorder patients. Brain Struct Funct. 2014;219(5):1615–26.
85. Wang Q, Van Heerikhuize J, Aronica E, Kawata M, Seress L, Joels M, Swaab DF, Lucassen PJ. Glucocorticoid receptor protein expression in human hippocampus; stability with age. Neurobiol Aging. 2013;34(6):1662–73.
86. Komatsuzaki Y, Hatanaka Y, Murakami G, Mukai H, Hojo Y, Saito M, Kimoto T, Kawato S. Corticosterone induces rapid spinogenesis via synaptic glucocorticoid receptors and kinase networks in hippocampus. PLoS One. 2012;7(4):e34124.
87. Chatterjee S, Sikdar SK. Corticosterone treatment results in enhanced release of peptidergic vesicles

in astrocytes via cytoskeletal rearrangements. Glia. 2013;61(12):2050–62.
88. O'Callaghan JP, Brinton RE, McEwen BS. Glucocorticoids regulate the synthesis of glial fibrillary acidic protein in intact and adrenalectomized rats but do not affect its expression following brain injury. J Neurochem. 1991;57(3):860–9.
89. Nichols NR, Osterburg HH, Masters JN, Millar SL, Finch CE. Messenger RNA for glial fibrillary acidic protein is decreased in rat brain following acute and chronic corticosterone treatment. Brain Res Mol Brain Res. 1990;7(1):1–7.
90. Paukert M, Agarwal A, Cha J, Doze VA, Kang JU, Bergles DE. Norepinephrine controls astroglial responsiveness to local circuit activity. Neuron. 2014;82(6):1263–70.
91. Ding F, O'Donnell J, Thrane AS, Zeppenfeld D, Kang H, Xie L, Wang F, Nedergaard M. α1-Adrenergic receptors mediate coordinated Ca2+ signaling of cortical astrocytes in awake, behaving mice. Cell Calcium. 2013;54(6):387–94.
92. Mobley PL, Combs DL. Norepinephrine-mediated protein phosphorylation in astrocytes. Brain Res Bull. 1992;29(3–4):289–95.
93. Takemura M, Gomi H, Colucci-Guyon E, Itohara S. Protective role of phosphorylation in turnover of glial fibrillary acidic protein in mice. J Neurosci. 2002;22(16):6972–9.
94. Rajkowska G, Stockmeier CA. Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. Curr Drug Targets. 2013;14(11):1225–36.
95. Torres-Platas SG, Hercher C, Davoli MA, Maussion G, Labonté B, Turecki G, Mechawar N. Astrocytic hypertrophy in anterior cingulate white matter of depressed suicides. Neuropsychopharmacology. 2011;36(13):2650–8.
96. Müller MB, Lucassen PJ, Yassouridis A, Hoogendijk WJ, Holsboer F, Swaab DF. Neither major depression nor glucocorticoid treatment affects the cellular integrity of the human hippocampus. Eur J Neurosci. 2001;14(10):1603–12.
97. Miguel-Hidalgo JJ, Baucom C, Dilley G, Overholser JC, Meltzer HY, Stockmeier CA, Rajkowska G. Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. Biol Psychiatry. 2000;48(8):861–73.
98. Miguel-Hidalgo JJ, Waltzer R, Whittom AA, Austin MC, Rajkowska G, Stockmeier CA. Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. J Affect Disord. 2010;127(1–3):230–40.
99. Gos T, Schroeter ML, Lessel W, Bernstein HG, Dobrowolny H, Schiltz K, Bogerts B, Steiner J. S100B-immunopositive astrocytes and oligodendrocytes in the hippocampus are differentially afflicted in unipolar and bipolar depression: a postmortem study. J Psychiatr Res. 2013;47(11):1694–9.
100. Altshuler LL, Abulseoud OA, Foland-Ross L, Bartzokis G, Chang S, Mintz J, Hellemann G, Vinters HV. Amygdala astrocyte reduction in subjects with major depressive disorder but not bipolar disorder. Bipolar Disord. 2010;12(5):541–9.
101. Hamidi M, Drevets WC, Price JL. Glial reduction in amygdala in major depressive disorder is due to oligodendrocytes. Biol Psychiatry. 2004;55(6):563–9.
102. Bernard R, Kerman IA, Thompson RC, Jones EG, Bunney WE, Barchas JD, Schatzberg AF, Myers RM, Akil H, Watson SJ. Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. Mol Psychiatry. 2011;16(6):634–46.
103. Choudary PV, Molnar M, Evans SJ, Tomita H, Li JZ, Vawter MP, Myers RM, Bunney Jr WE, Akil H, Watson SJ, Jones EG. Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. Proc Natl Acad Sci U S A. 2005;102(43):15653–8.
104. Miguel-Hidalgo JJ, Wilson BA, Hussain S, Meshram A, Rajkowska G, Stockmeier CA. Reduced connexin 43 immunolabeling in the orbitofrontal cortex in alcohol dependence and depression. J Psychiatr Res. 2014;55:101–9.
105. Rajkowska G, Hughes J, Stockmeier CA, Javier Miguel-Hidalgo J, Maciag D. Coverage of blood vessels by astrocytic endfeet is reduced in major depressive disorder. Biol Psychiatry. 2013;73(7):613–21.
106. Kryger R, Wilce PA. The effects of alcoholism on the human basolateral amygdala. Neuroscience. 2010;167(2):361–71.
107. Schroeter ML, Abdul-Khaliq H, Krebs M, Diefenbacher A, Blasig IE. Serum markers support disease-specific glial pathology in major depression. J Affect Disord. 2008;111(2–3):271–80.
108. Bergh CD, Bäckström M, Axelsson K, Jönsson H, Johnsson P. Protein S100B after cardiac surgery: an indicator of long-term anxiety? Scand Cardiovasc J. 2007;41(2):109–13.
109. Li X, Wilder-Smith CH, Kan ME, Lu J, Cao Y, Wong RK. Combat-training stress in soldiers increases S100B, a marker of increased blood-brain-barrier permeability, and induces immune activation. Neuro Endocrinol Lett. 2014;35(1):58–63.

Role of the Glia and the Neural Crest in Central Nervous System Health and Disease

11

Jorge B. Aquino

Abstract

Glia were until recently regarded as the glue cells of the nervous system. In this chapter, new and unexpected roles of main glia subtypes are discussed, including learning, memory, fear conditioning, long-term potentiation and some complex neurocognitive functions. Different mechanisms have been involved, at the cellular and systemic levels, at least partially explaining these features and usually involving glia–neuron and glia–glia interactions, and suggesting that human brain evolution required a concomitant specialization of both neural types. In addition, evidence involving glial cells in the origin as cause or effectors of different psychiatric pathologies and/or some of their symptoms is also considered. The neural crest is a subpopulation of cells that delaminate from dorsal regions of the neural tube and contribute to many structures of the body, including all the peripheral nervous system. They were shown to migrate toward the rostral regions of the embryonic brain, and this was found to induce the formation of the forebrain from which the neocortex originates. Interestingly, some studies involved the neural crest in certain types of autism and schizophrenia. Moreover, after traumatic central nervous system injury as well as in the context of demyelinating diseases, Schwann-like cells (one of neural crest derivatives) were found to invade and/or remyelinate some axons, thus playing a role in nervous system regeneration and myelin reconstitution. While after injury some peripheral nerves can contribute with these cells through invasion of peripheral nerve components, some of the Schwann-like cells appearing in the affected areas could originate from progenitors

J.B. Aquino, PhD
Developmental Biology and Regenerative Medicine Laboratory, IIMT CONICET-Universidad Austral, Av. Presidente Perón 1500, B1629AHJ Derqui-Pilar, Buenos Aires, Argentina

CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Buenos Aires, Argentina
e-mail: jaquino@austral.edu.ar

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_11

of the central nervous system. Finally, the growing spectrum of Schwann cell progenitor derivatives is also herein discussed, with evidence suggesting a developmental plasticity and differentiation potential much broader than expected in neural committed cells.

Keywords
CNS • Astrocytes • Oligodendrocyte precursor cell • Psychiatry • Brain • Schwannosis • Spinal cord injury • Schwann cell precursors

The Glia and the Brain

The human brain has developed to its actual size as a result of the expansion of the neocortex and the increase in subcortical white matter. Its tissue comprises neurons and glia. The latter, until recently merely regarded as neural non-excitable supporting cells, interact extensively with neurons, both physically (i.e., through gap junctions) and chemically (through interchange of neurotransmitters and trophic and cytokine/chemokine factors, of either origin). Glial cells of the central nervous system (CNS; including the encephalon—brain—and the spinal cord) mainly comprise astrocytes, oligodendrocytes, and microglia, while they are represented by satellite cells and Schwann cells in the peripheral nervous system (PNS). In certain areas of the brain, glial cells are far more abundant than neurons. On average, the glia-to-neuron ratio in the human cerebrum is approximately 4:1; nevertheless, similar proportions of both cell types could be found within the grey matter [1]. Interestingly, in the human prefrontal and frontal cortex (areas 4, 9 L, 32 and 44), there are 50% more glia than neurons [2]. By mean of stereological studies in human, Pelvig et al. [3] reported that males have more glial cells than females, and that the relative composition of glia subtypes in the neocortex is as follows: 17.3/20.2% astrocytes; 75.6/74.6% oligodendrocytes, and 6.5/5.2% microglia (males/females).

Astrocytes are essential for brain homeostasis [4]. As reviewed by Steardo et al. [5], they regulate pH, ion balance, blood flow, and oxidative stress. In addition, astrocytes contribute to synaptogenesis, modulate signal transmission, and regulate neuronal and synaptic plasticity. They feed neurons by making glucose/lactate available to them, and provide them with oxygen. They allow axonal excitability by keeping ionic balance and through the uptake of K^+. Astrocytes express receptors for most of the known neurotransmitters and neuromodulators, as well as ion channels, and their required intracellular signaling molecules [6]. They are able to release gliotransmitters into the synapses they participate in [7]. Interestingly, neurotransmitters induce fluctuations in calcium intracellular levels in astrocytes, which can eventually be transmitted as waves to other astrocytes that are interconnected, forming a cellular network [8–10]. Although calcium waves seems to be propagated by simple diffusion through gap junctions, this process is faster in human astrocytes than in rodent ones [11]. Properties of astrocytes with regard to the extent and speed of calcium-wave propagation differ according to brain areas [12].

Astrocytes, as a third part of the synapse, have been found by many studies to be functionally involved in learning and memory [6], as well as in long-term potentiation and in complex neurocognitive functions such as sleep [13, 14]. For instance, as reviewed by Moraga-Amaro et al. [6], excitatory stimulation was found to induce calcium waves in hippocampal astrocytes, and application of endocannabinoids was shown to increase calcium intracellular levels able to trigger astrocyte glutamate release and the subsequent activation of neuronal N-methyl-D-aspartate (NMDA) receptors. Moreover, glutamate release from astrocytes caused by cholinergic signalling-mediated intracellular calcium increase was shown to be required for cholinergic-induced synaptic plasticity. Astroglial CB1 receptor activation was found to modulate hippocampal long-term depression after cannabinoid administration, thus involving

astrocytes in processes of working memory. Spatial learning has been found associated in rat with an increase in astrocyte numbers within the CA3 region of the hippocampus. Blockage of gliotransmitter-release from basolateral amygdala astrocytes prior to training, through connexin 43 hemichannels, was found to affect memory consolidation, being able to induce complete amnesia. Moreover, overexpression of S100b, a $Ca2^{+}$ binding protein expressed by astrocytes, in transgenic mice was shown to impair long-term potentiation (LTP) and spatial learning [15].

Diamond and collaborators [16] compared values of the neuron-to-glia ratio between 11 human male brain samples (average age: 64) and those dissected from Albert Einstein (age: 76). Samples were obtained from two brain areas (9 and 39) from both right and left hemispheres. These areas correspond to the prefrontal cortex—superior frontal gyrus on the dorsal lateral surface—and to the inferior parietal lobule, including the anterior lip of the angular gyrus surrounding the termination of the superior temporal sulcus, respectively. Interestingly, while no differences were found in the other three areas, the neuron/glia fraction was significantly reduced in the left area 39 in Albert Einstein's brain. It is worth noting that lesions in the inferior parietal lobule were associated with loss in versatility of imagery and in the ability for complex thinking. Interestingly, $GFAP^{+}$ intralaminar astrocytic processes were larger in Einstein's cortex when compared with the other four aged-matched samples obtained from human beings without known neurological or psychiatric disease [17].

Furthermore, Han et al. [18] found that the engraftment of human $A2B5^{+}$/PSA-$NCAM^{-}$ glial progenitor cells, in-vitro-primed to differentiate into astrocytes, into the cortex of neonatal athymic mice resulted in an increased performance in the Barnes maze assay, object-location memory, contextual fear-conditioning and tone fear-conditioning assays, when compared to engraftment of similar mice progenitor cells. In these chimeric humanized mice, human-derived $GFAP^{+}$ astrocyte processes were larger and with greater architectural complexity, as well as being morphologically more diverse than mouse counterparts, resembling typical human intralaminar ones. Human-derived astrocytes established connections with mouse ones, although retaining their physiological original properties (i.e., being able to propagate calcium waves more rapidly). Interestingly, human-derived astrocytes were found to probably enhance LTP in the adult mice hippocampus through the release of the cytokine TNF-α.

Oligondendrocytes are by far the most abundant cells in neocortex white matter, and are found normally largely in contact with some neuronal axons, surrounding them and forming the central myelin. Myelination not only makes axonal saltatory conduction possible but also provides neuronal processes with trophic and metabolic support [19]. Oligodendrocytes express receptors to neurotransmitters and are able to signal back to neurons [7]. For instance, a mechanism of neurotransmitter-induced exosome delivery from oligodendrocytes to neurons was recently reported, with involvement for example of glial ionotropic NMDA receptors [20]. Energy deprivation induces over-activation of AMPA/kainate receptors expressed in oligodendrocytes and in myelinated axons. This is followed by increasing levels of intracellular calcium and excitotoxicity [7]. In the adult, new oligodendrocytes can be generated from the so called oligodendrocyte progenitor cells (OPCs), which express the NG2 proteoglycan (and therefore OPCs are also known as NG2-glia) and comprise 4–8% of cells in the adult brain [21]. The OPCs are multipotent progenitors which mostly differentiate into oligodendrocytes in adulthood, although they can also give rise to astrocytes, piriform neurons, and Schwann cells in vitro and in vivo, depending on different environmental or pathological conditions [21, 22]. It was speculated that approximately 50% of OPCs divide every 3 days in the adult [21]. OPCs of different developmental origins show similar proliferation rates, cell cycle length, and membrane properties [21]. Nevertheless, there seem to be more important dissimilarities between OPCs with regard to whether they are located in the white or grey matter: i.e., white-matter OPCs have shorter cell cycle length, proliferate under stimulation with PDGF, differentiate better into mature myelinating oligodendrocytes, and express different voltage- and ligand-dependent ion channels [21]. These cells receive direct excitatory and inhibitory synapses in all brain regions, and

synaptic activity modulates OPC proliferation, oligodendrogenesis, and/or myelination properties, resulting in behavioral improvements [21, 23]. OPCs show synchronized activity with neurons and were shown to be involved in LTP, perhaps through changes in NG2 expression levels, thus regulating information processing and plasticity at neuronal synapses [21]. They maintain functional synapses while undergoing proliferation [21]. Excitatory synapses of grey-matter OPCs are characterized by their: (i) small amplitudes, (ii) rapid kinetics, and (iii) high sensitivity to AMPA receptor antagonists [21]. Interestingly, some OPCs in the subventricular zone do not receive synapses, a feature which might be related to their condition as premigratory cells [21].

Microglia are yolk-sac-derived innate immune cells located in the CNS [24]. They normally show a stellate morphology with thin and highly ramified processes, which do not overlap with processes of other glial cells [7]. They get activated after injury, and then produce and release pro- or anti-inflammatory molecules as well as remove debris [7]. They were found to establish contact to synapses, in a dynamic way and for short-time, through tiny processes, and express receptors for several neurotransmitters [7]. Some evidence suggests that microglia would probably modulate synapse activities, and based on that some reports use the term *quadripartite synapse* (the structure made by pre-synaptic and post-synaptic neurons, astrocytes, and microglia) [7]. Microglia have well-known functions in synaptic pruning, by responding to neuronal activity and neurotransmitters [25, 26], as well as in synaptic maturation [24]. Nevertheless, little is known with regard to the significance of microglia contribution to processes such as learning, memory, or other cognitive functions.

The Glia and Psychiatry

After brain injury, astrocytes have been found to trigger neuroinflammation; and this, as well as microglial production of reactive oxygen species, can exacerbate the damage and cause astrocyte dysfunction. In some cases, these events might induce neurodegeneration, including the development of Alzheimer's disease [5, 14, 27]. S100β, among factors which are mainly expressed by astrocytes, has been found to be increased with age and with progression of Alzheimer's disease-like dementia, Parkinson's disease, paranoid schizophrenics and in patients with brain trauma. Furthermore, S100β upregulation probably precedes the occurrence of neuritic plaques [5, 14]. Interestingly, another astrogliosis marker, myo-inositol, has been found upregulated in brain areas of patients with mild cognitive impairment and with Alzheimer's disease [5]. Induction of the glial HMOX1 gene would result in abnormal iron deposition, increasing oxidative cellular damage and Alzheimer's/Parkinson's disease or schizophrenia [28].

If the negative effect of stress, anxiety, or other negative emotions on hippocampal structure and function could not be sufficiently reduced by learning and/or positive proactive attitudes (i.e., environmental enrichment), capillary vascularization would be affected, thus resulting in the lack of adequate nutrient provision. A lower availability of energy to brain cells would then negatively affect the number of glia and/or their required function in maintaining/activating synapses, all features involved in learning and memory, resulting in cognitive impairment with age [14].

Interestingly, it is now established knowledge that depression or chronic stress can be linked to a reduced neurogenesis at the hippocampus. Nevertheless, less consideration has been shown to the fact that it also negatively influences gliogenesis at the medial prefrontal cortex (mPFC) of rodents, which corresponds to the dorsolateral prefrontal cortex (dlPFC) of human beings, areas which are involved in stress regulation. It is worth noting that fluoxetine treatment has been shown to significantly reverse both features [14]. In addition, depression can probably be causally linked to a downregulation in astroglial production of neurogliotrophins, such as brain-derived neurotrophic factor (BDNF). Treatment with fluoxetine has been shown to restore the ability of astrocytes to produce BDNF and to increase their use of glucose and release of lactate [14]. Either stress or depression would cause alterations in astrocyte expression profile, which might possibly explain why patients

relapse when treatment with antidepressant medications is stopped too early [14]. Finally, significant changes in neuronal activation in affected brain areas, which are frequently induced as a result of astrocyte inefficient function, were shown to induce microglia activation, thus influencing working memory and further reducing the capacity to deal with stress or depression [14].

Interestingly, fluoxetine was also found to reverse the effect of stress by inducing an increase in OPCs in the left mPFC (the dominant hemisphere), resulting in the normalization of progenitors numbers [14]. Similarly, corticosterone and electroconvulsive therapy significantly affect OPCs abundance [14]. Interestingly, chronic stress would result in increased numbers of oligodendrocytes at the expense of an increase in neurogenesis; this might endure connections to the amygdala, making the inhibitory control of the amygdala by the PFC less functional, and possibly causing posttraumatic stress disorders [14].

Studies made in mouse models of the Rett syndrome suggest that it can be caused by defects in astroglial MeCP2 (methyl CpG binding protein 2 (Rett syndrome)) gene expression, probably through their failing to promote extensive neuronal dendritic arborisation and normal spine density [28]. Interestingly, oligodendrocytes defective in MeCP2 have been shown to result instead in a milder Rett-like phenotype which develops later than in MeCP2 null mice [28]. Consistently, restoration of MeCP2 expression specifically in oligodendrocytes was shown to partially ameliorate the disease phenotype, through, for example, rescuing their myelin basic protein (MBP) expression. Finally, some studies have also involved MeCP2-defective microglia in the development of Rett-like phenotype [28].

Schizophrenia is considered a neurodevelopmental disorder with polygenic and environmental influences [28]. It arises because of abnormal neuronal communication; however, it is not known whether this feature is epiphenomenal, i.e., secondary to axon–glial or glial defects [29, 30]. Schizophrenia would probably be caused by environmental factors rather than genetic ones, probably involving epigenetic changes through repeated events over time [31]. For instance, it has been suggested that stress might epigenetically inhibit myelination in a critical early-age period of development [30]. Schizophrenia has been associated with reduced numbers of glia in the brain, and treatment with anti-psychotics was found to increase such numbers [14]. Moreover, suspension of antipsychotic treatment was shown to be followed by a reduction in astrocyte-enwrapping capacity which can eventually explain relapses in symptoms [14]. In a large group of Scottish families, a disruption in the expression of DISC1 and DISC2 genes was found to be related to the disease: interestingly, a dysfunction of DISC1 was involved in abnormal oligodendrocyte development [28]. It is worthy of note that astrocytes expressing a mutant form of DISC1 were found to cause dysfunction of the NMDA receptor, a feature previously involved in schizophrenia, through a diminished production of D-serine by these cells [28].

Independently of this, alterations in the expression or function of neuregulin 1 (NRG1), ErbB3 (expressed by glia) and ErbB4 (enriched in neurons and expressed by glia) signaling were also associated with schizophrenia as well as with other psychiatric disorders [28, 32]. A reduction in NRG1 and ErbB4 expression levels in mouse was described to result in a phenotype resembling schizophrenia [28]. SynCAM1, which plays a significant role in astrocyte–astrocyte or astrocyte–neuron adhesive communication, was found to be functionally related to ErbB4, and both were shown to be co-expressed by astrocytes. Interestingly, schizophrenia-like symptoms were seen to develop in animals with oligodendrocytes expressing a dominant negative form of ErbB4. Moreover, oligodendrocytes defective for ErbB3 expression showed deficits in social interaction and working memory [28]. In addition, a dysregulation in other oligodendrocyte-expressed proteins has also been involved in schizophrenia-like behaviors [28, 33]. Furthermore, psychotic symptoms have been reported in cases in which normal myelin development and/or integrity were interrupted [29, 34]. Both reduction in cortical oligodendrocyte numbers and in the expression levels of myelin genes have been found in some patients with schizophrenia and in others with bipolar disorders [32]. Consistently, results from the analyses of

Cnp1$^{+/-}$ mice phenotype also suggest an involvement of oligodendrocytes in the origin of schizophrenia [30]. Cnp1 is a myelin protein expressed in oligodendrocytes which is not crucial for myelin formation but for axon function and survival. These animals show catatonia and depression-like symptoms when they get relatively old. Interestingly, in these and other mutants the first pathological signs are axonal swellings, a feature shared with axonopathies caused by mitochondria disease [30].

In the two-hit inflammatory model for psychosis, it is speculated that a first hit (which could consist on either an in-utero infection or stress) is able to generate genetic vulnerability through priming of microglia; nevertheless, only after a second hit would neuronal dysfunctions be triggered by the activation of the previously primed microglia, resulting in the development of the disease [35]. Finally, it has been suggested that microglia might probably affect mood in bipolar disorders by influencing serotonin neurotransmitter signaling through inflammatory mechanisms [36]. Therefore, considerable evidence suggests that multifactorial and diverse events are probably linked to the development of psychiatric disorders, most of them involving glia as primary cause or effectors or as part of the mechanism itself.

The Neural Crest and the Healthy and Diseased Brain

The neural crest is a population of cells which arise from the dorsal neural tube through a process of epithelial-to-mesenchymal transition and subsequent delamination. They are highly motile, and have been found to contribute to diverse neural and non-neural cell types in very different tissues.

Some cranial neural crest cells, known as facial neural crest cells (FNCCs), are originated from the neural tube in between the posterior diencephalon and rhombomere 2 levels, and have been shown to lack Hox genes expression and to express Six proteins (Six1, Six2 and Six4) [37]. FNCCs have been found to give rise to the mesenchymal progenitors which originate the facial bones, and to the meninges of the forebrain, as well as to the perivascular smooth muscle cells and pericytes of the face and forebrain [38–40]. Interestingly, it was recently suggested that head ectomesenchyme is derived from E-cadherin^{+} non-neural ectoderm cells, placed adjacent to neural ectoderm, which start to express Sox9 and delaminate before neuroectodermal cells; however, although showing different properties both neural- and non-neural-ectoderm-derived delaminating cells could be regarded as neural crest cells (NCCs) [41]. FNCCs migrating rostrally were found to be required for the generation of telencephalic vesicles as well as of the thalamic and pre-tectal nuclei. Their ablation was shown to disrupt brain organizers, resulting in the loss of Fgf8 expression in the anterior neural ridge (ANR) and loss of dorsal Wnt and ventrolateral expansion of Shh domains [38]. Some of the molecular mechanisms involved in FNCC-mediated induction of the forebrain have been recently described: FNCCs express Dkk1 and Cerberus, two modulators of Wnt1, which under the control of Smad1 (also expressed by FNCCs) stimulate Foxg1 expression in the forebrain (a transcriptional factor required for telencephalic development) and regulate Otx2 and Foxa2 balance at the diencephalic/mesencephalic boundary [38]. FNCC-derived mesenchymal cells require Wnts to proliferate, and mesenchymal cell expansion has been found to induce cortical midline invagination and lateral ventricle formation [39]. Thus, alterations in Foxg1 and Otx2 expression caused by deficiencies in FNCCs would probably be involved in the generation of some atypical Rett syndromes and other related disorders [38].

It is worth noting that Nakajima and collaborators, by using Wnt1-cre and Wnt1-GAL4 double transgenic mice, were able to find a psychiatric abnormal behavior phenotype (i.e., increased locomotor activity, reduced social interaction, and impaired short-term spatial memory and nest-building activity) in animals with defects in NCCs [42]. These mice show irregularities in the trajectory of cholinergic and glutamatergic fibers connecting the medial habenula nucleus of the thalamus with the interpeduncular nucleus of the midbrain tegmentum. Interestingly, the habenula has been involved in the pathogenesis of schizophrenia [42].

Schizophrenia, considered to be a disease originated in a deficient formation of forebrain neuronal circuits rather than a degenerative one, shows frequent association with craniofacial as well as

limb and heart malformations in selected adult patient populations suffering from velo-cardio-facial syndrome (VCFS), with involvement of retinoic acid (RA) signaling [43, 44]. The retinoic acid produced by the frontonasal NCC-dependent mesenchyme has been found to be required for proper forebrain development [43]. Thus, a deficiency in NCC migration and in the lack of required RA levels might explain some defects in facial appearance as well as in neural development, and in the establishment of neuronal connections in the forebrain of those patients (Fig. 11.1). Previous findings suggest that deficiencies in NCCs might cause or be involved in the origin of certain cases of autism and schizophrenia.

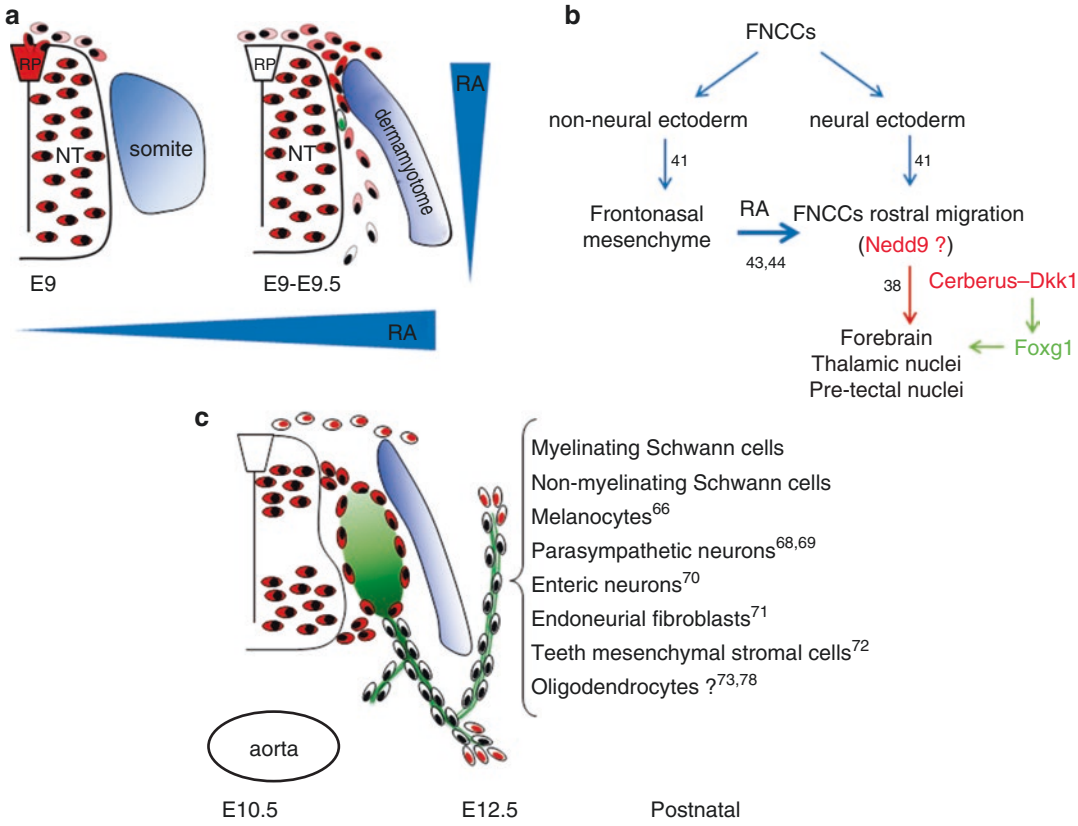


Fig. 11.1 Neural crest migration and schizophrenia. Schematic figure showing the dynamics in Nedd9 expression in trunk premigratory and migratory neural crest cells (*NCCs*) which, being induced by dermamyotome-derived retinoic acid *(RA)*, is required for their efficient migratory behavior (**a**). Note that Nedd9 is transiently expressed in the neural tube roof (before NCC delamination), and later on is confined to multipotent premigratory and migratory NCCs (see cells with their cytoplasm depicted in *red*, outside of the neural tube). Nedd9 is sharply downregulated when migrating NCCs become restricted to the neuronal lineage (see a cell with nucleus depicted in *green*, expressing Brn3a and Ngn2) at E9-E9.5 in mouse embryos and equivalent stage in chicken. (**b**) Facial NCCs (FNCCs) are composed by early delaminated non-neural ectoderm cells which give rise to the frontonasal mesenchyme. This structure produces RA, which is required for neural ectoderm-derived FNCCs, cells which might also depend on Nedd9 expression for their motility. Deficiencies in FNCC migration would result in decreased Foxg1 levels (the master transcriptional regulator in forebrain development) and in the ventralization of the most rostral part of the brain, thus affecting the thalamic and pre-tectal nuclei. Such features could be involved in some schizophrenia cases. (**c**) At E10.5 and equivalent stages in chicken, Nedd9 is expressed in the boundary cap and in the multipotent Sox2+ NCCs located at the periphery of the dorsal root ganglia. It is downregulated as soon as NCCs become restricted to the neuronal or glial lineages. From E12.5 on, neural crest-derived cells no longer express Nedd9, although some glia progenitors named as Schwann cell precursors (SCPs) continue migrating through growing peripheral nerves. Some SCPs detach from nerves, and shortly afterward they turn on MITF expression (a master transcriptional factor regulator of melanocytes development): see cells depicted with *red nuclei*. Note that some late neural crest cells migrating through the dorsolateral route are also a source of melanocytes. Derivatives of SCPs as they are known today are listed

The Retinoid Acid and the Migratory Capacity of Neural Crest Cells

The motile capacity of NCCs has been shown to depend on the RA-dependent induction of Nedd9 expression [45]; (Fig. 11.1). Nedd9 (also known as HEF1 and Cas-L; a name which stands for *neural precursor cell expressed, developmentally down-regulated 9*) is a scaffolding protein, member of the Crk-associated substrate (CAS) family, involved in the beta1-integrin signaling pathway, and frequently localized in focal adhesions and found associated with FAK and the Abl kinase. In addition, it has been found expressed in the centrosome and in the mitotic spindle during mitosis. It has been suggested that this protein may play a role in cell adhesion and migration, apoptosis, and cell cycle.

Nedd9 was first reported by Kumar et al. [46] by performing subtraction cloning experiments with the aim of identifying genes highly expressed in the brain during development. Nedd9 was shown to induce upregulation of matrix metalloproteinases, ephrin ligands and receptors, and the NRG receptor ErbB2; nevertheless, both its pathway–target specificity and how those genes are activated remain unknown [47]. Cell adhesion was found to trigger the conversion of Nedd9 isoforms from p105 into p115, which reflects the serine/threonine phosphorylation state and is associated with integrin receptor activation and cytoskeleton organization [47].

In embryonic stem cells, Nedd9 promoter was found to be co-occupied by Sox2 and Nanog, which would suggest its involvement in stem-cell behavior [47]. By means of a screening based on a degenerate PCR approach looking at a homeobox-sequence containing proteins highly enriched in the neural tube and the DRG at intermediate stages of mouse development, Aquino et al. [48] were able to clone a 498 bp corresponding to a fragment in the C-terminal domain of Nedd9. Interestingly, by in-situ hybridization and immunohistochemistry analyses Nedd9 was found to be expressed in multipotent progenitors (able to give rise to all common tissue-specific derivatives) of diverse tissues, including the CNS and the PNS [48].

Moreover, during early CNS development, Nedd9 is expressed in the ventricular zone of the neural tube, from the diencephalon to the caudal spinal cord axial levels [48]. Nedd9 expression is further upregulated in dorso-ventral neural tube domains of multipotent cells, giving rise to $Ngn2^{+}$ neuronal progenitors in the CNS. Interestingly, these regions are those which show further growth at early neural tube morphogenesis (E10-E12 in mouse; Fig. 11.1). Considering the described role of Nedd9 in the migratory and/or mitosis behavior, whether or not Nedd9 higher expression in such progenitor domains could be associated with tissue morphogenesis remains to be addressed. It is worth noting that $Ngn2^{+}$ progenitors are among the first neuronal precursors to be born in the CNS. Finally, considering that RA-mediated Nedd9 upregulation makes NCCs responsive to integrin as well as to other pro-migratory signals, it might probably be involved in the onset of their frontward migration within the head.

With regard to the PNS, Nedd9 is induced in neural tube roof by the time of neural crest delamination as well as at the onset of their migration through the sclerotome. Consistent with recent reports confirming the multipotency of the majority of premigratory and migratory neural crest cells [49], most of them were found to co-express Sox10 and Nedd9 [45]. Thus, maintenance of multipotency in NCCs is probably required for their migratory behavior (Fig. 11.1).

It is worth noting that Nedd9 is downregulated as soon as cells become restricted to any of neural lineages in the CNS as well as in the PNS, and consistent with that it was not found to be expressed in the NCC lineage at E12.5 and thereafter. Gain of function of Nedd9 in chicken, by forced expression of its full length, enhances the migratory behavior of NCCs, whereas Nedd9 knockdown was found to inhibit their emigration from the neural tube and the migratory capacity of NCCs once delaminated. Similar results were

obtained in boundary cap neural crest stem cells (bNCSCs) by performing in-vitro assays. The reduced Sox10/Nedd9 expression levels found in first $Ngn2^{+}$/$Brn3a^{+}$ migrating NCCs, which correspond to neuronal precursors biased to the sensory fate, might suggest a role for Nedd9 downregulation in the formation of the DRG through a reduction in their migratory properties.

Nedd9 function in the migratory behavior of multipotent NCCs was found to be dependent on integrin ligands, since NCCs were only able to efficiently spread in laminin, and Nedd9 siRNA caused a significant reduction in cell spreading area when compared to control. Consistently, while the majority of scrambled siRNA-targeted NCCs showed a migratory phenotype, cells became mostly stationary when Nedd9 was knocked-down [45]. In addition and as was expected, Nedd9 loss-of-function resulted in decreased frequency of focal complexes and actin filaments and the rare appearance of stress fibers in NCCs, which are all signs of their hypomorphic migratory phenotype.

In-vivo quantification analyses of Nedd9 expression levels during NCCs development suggested that it is induced by a signal derived from the dermamyotome, since a peak in Nedd9 immunoreactivity was found in migrating cells located close to the dermamyotome dorsal lip, and these levels decreased in cells which have migrated further down this landmark (Fig. 11.1). Indeed, when neural tube explants were incubated for 6 h in culture medium supplemented with different inducible factor candidates and/or their antagonists/inhibitors (all-trans retinoic acid at low and high levels, citral, BMP4, Noggin, FGF8, Shh, Wnt3a, Wnt3a plus BMP4, Wnt5a or none—control) RA was found to be the only factor likely able to induce Nedd9 expression. Several experimental outcomes support this statement: (1) the highest Nedd9 expression levels were obtained in our in-vitro model system when explants were cultured in presence of high retinoic acid, (2) upon citral treatment (an inhibitor of retinoic acid biosynthesis), Nedd9 was down-regulated in NCCs when compared to control condition, and (3) retinoic acid injection in pregnant mothers up-regulated Nedd9 expression in trunk neural crest of E9.5 mouse embryos after 6 h of treatment. A minor increase in Nedd9 expression was seen for FGF8, Wnt3a, and Shh treatments when compared to controls; however, this could eventually be explained by direct or indirect Nedd9 expression regulation and/or a role played by these factors in maintaining the multipotent status of NCCs in vitro. Results suggesting retinoic acid induction of Nedd9 in NCCs are also consistent with dynamic changes in in-vivo Nedd9 expression patterns [47]. It is known that the expression of Raldh2, the main enzyme synthesizing retinoic acid in the trunk, increases in a posterior-to-anterior fashion in somites and peaks at the level of somite dissociation into dermamyotome and sclerotome. And consistently, and since Nedd9 is involved in NCC motility, first NCCs migrating down through the rostral half of the somite do so at this very same trunk level.

In conclusion, a retinoic acid regulation of Nedd9 expression probably gives multipotent NCCs the competence to respond to extracellular signals and to initiate migration through the sclerotome in an integrin-dependent manner. Nedd9 might exert its effects in NCCs through regulation of focal complexes and actin filaments, required for cell adhesion and spreading and for the development of traction forces which altogether drive motility.

Schwann Cells in the Injured Central Nervous System

Schwann cells are one of the NCC–glia derivatives which can be found associated with one or several axons, depending on whether they correspond to a myelinating or to a non-myelinating phenotype, respectively. Apart from their supportive role to axons and myelin production, Schwann cells have also been shown to be able to: (1) present antigens and produce immunologically-relevant cytokines (in certain cases); (2) modulate neuromuscular synapse formation, actively respond to neurotransmitters, and repair the neuromuscular

junction; (3) modulate pain, and (4) regulate the activity of haemopoietic stem cells [50]. Interestingly, Schwann-like cells were shown to contribute to remyelination in the CNS in demyelinating injury models and in multiple sclerosis. Moreover, some of the Schwann-like cells were found to originate from OPCs (the NG2-glia; [51]). In addition, neural progenitor cells from the subventricular zone might possibly give rise to some $Sox10^{+}$/$GFAP^{+}$ Schwann like-cells, an issue which requires further examination [52]. In fact, a week after injury some $Sox2^{+}$/$Sox10^{+}$ cells were found within the ependymal layer of the spinal cord central channel, which could consist of progenitors capable of originating Schwann-like cells (47; Fig. 11.2). Interestingly, 9 days after weight-drop injury many $Sox10^{+}$/$Sox2^{+}$ cells could be found rostrally to the injury core (47; Fig. 11.2). CNS-derived Schwann cells show properties which differ from those of NCC-derived ones, and resemble those of OPCs, such as: (1) they express higher levels of O4/A2B5 and lower levels of S100 expression; (2) they display prominent outward rectifier KD currents, and (3) their K^{+} currents are more efficiently inhibited by broad-spectrum potassium channel blockers (TEA, $Ba2^{+}$; [53]). Some of them might also correspond to previously reported aldynoglial Schwann cells [54].

Schwannosis is a very frequent feature after contusion, transection/hemisection or photochemical insult of the spinal cord, and it is characterized by the invasion and growth of peripheral nerve elements, including axons and Schwann cells [55, 56]. In such injuries, in the long-term, CNS axons are mainly remyelinated by Schwann cells, while oligodendrocytes remain largely unable to do it in the absence of astrocytes [57, 58]. It has been shown that Schwann cells can myelinate CNS axons only when they are demyelinated or growing [59]. Moreover, $p75^{+}$ Schwann cells, which can interact and ensheath axons in a 1:1 relationship, have been observed filling the epicentre of the injury after grafting of olfactory ensheathing cells, exogenous Schwann cells, and bone marrow stromal cells [47, 56]. In such cases, central axons were found to be partially enwrapped by $P0^{+}$ myelin (Fig. 11.2). Interestingly, in a weight-drop injury experimental model in rat and after grafting of exogenous bNCSC-derived Schwann cells, endogenous Schwann cells were found to invade the epicenter of the injury in its anterior border and then to grow and migrate toward the posterior stump of the injury core, concomitantly to regenerating axons (47, 56; Fig. 11.2). Moreover, recruitment of endogenous $p75^{+}$/ $Sox10^{+}$/ $Sox2^{-}$/ $S100^{+/low}$ Schwann-like cells toward the dorsal funiculus rostrally to, and within the injury core was enhanced in animals treated with bNCSC-Schwann cells when compared to vehicle-controls, and this was found to be associated with a reduction in $OX42^{+}$-activated macrophages and with a less atrophic dorsal funiculus (Fig. 11.2). These cells were found to increase in numbers over time, thus being able to reach the distal stump by 2 weeks after bNCSC-Schwann cells grafting. By then, few $Sox2^{+}$/$Sox10^{-}$ astrocyte-like cells appear in association with $p75^{+}$ cells at the middle of the injury core. Finally, few neurofilament $(NF)^{+}$ / $PGP9.5^{+}$ axons were found to be in contact with p75+ while most of them lack PGP9.5 expression, which is probably a sign that only few peripheral axons invade the spinal cord or that most of axons belong to cortical motorneurons which have regenerated though the injury core in association with Schwann-like cells.

Whether or not some of these endogenous Schwann cells could have originated from CNS progenitors at the ependymal layer or from OPCs in the context of traumatic injuries remains to be addressed. In this regard, Barnabé-Heider and collaborators performed lineage tracing studies using different transgenic mice, identifying contribution of cells to the injury site originated from the ependymal layer (Foxj1-CreER mice), astrocytes (Cx30-CreER mice) or OPCs (Olig2-CreER mice) [60]. However, since the authors removed part of the dorsal funiculus without affecting root entry/exit zones (thus avoiding formation of cystic cavities) and they used markers which could be also expressed in CNS-derived Schwann-like cells, such as NG2, new studies are required to address the extent of Schwannosis vs CNS origin of glial subtypes, including Schwann-like cells, repopulating injured spinal cord areas in a more common scenario.

Kaneko and collaborators have shown that long-term inhibition of semaphorin3A (Sema3A) was

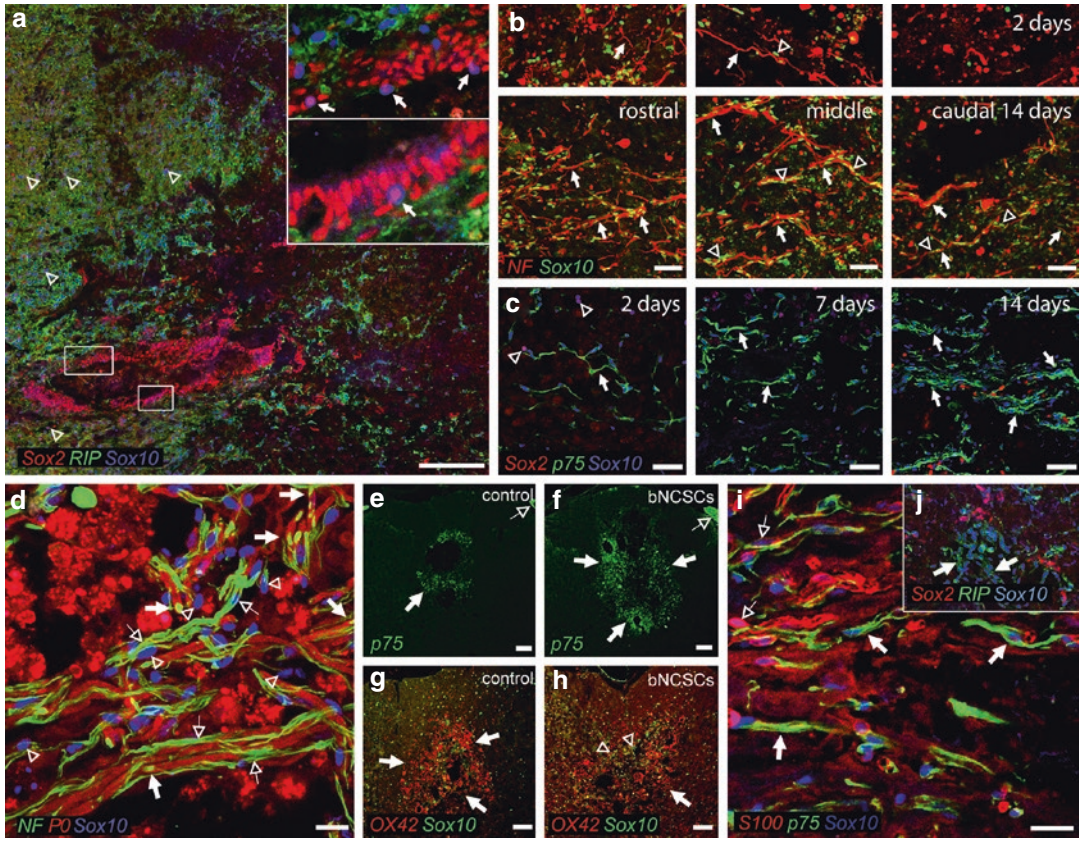


Fig. 11.2 Schwann/Schwann-like cells in spinal cord regeneration. These representative microphotographs show changes in cellular subpopulations as well as axonal regeneration through the injured area, in a rat weight-drop model. Unless stated, pictures were taken from saggital sections of the spinal cord. Most of them correspond to animals treated with mouse Rosa26 boundary cap neural crest stem cell-derived Schwann cells (bNCSCs). In most cases, grafted cells die shortly after transplantation. Nevertheless, only when injected alive were they able to induce changes resulting in significant improvements in BBB locomotor score studies. (**a**) A composite image showing rostral aspects of the injury zone (9 days after injury and 2 days after bNCSC grafting). Note the significant increase in the density of $Sox2^+/Sox10^+$ oligodendrocyte progenitor-like cells (*empty arrowheads*) within the spinal cord parenchyma at less affected and anatomically more conserved areas of the spinal cord. In addition, $Sox2^+/Sox10^+$ cells are found within the ependymal layer (*arrows*, a layer which is disorganized after injury; see higher magnifications images of insets at the *upper-right corner*). RIP is a marker of oligodendrocytes. *Scale bar*: 200 μm. (**b**) Changes in endogenous cellular populations in between two (*upper panel*) and 14 (*lower panel*) days after cellular transplantation in rostral (*left*), middle (*central*) and caudal (*right*) regions within the injury core. Note that the density of axons (*arrows*, immunolabeled using antibodies against neurofilament -NF- 160KDa and 200 KDa epitopes) and of associated $Sox2^-/Sox10^+$ glia (*empty arrowheads*) are increased at all regions analyzed with time. In addition, while few axons could be observed at the middle and only debris are seen at distal/caudal regions of the injury zone at early time points analyzed, they are increased in numbers and appear forming bundles at both regions at later time points, suggestive of spinal cord regeneration. *Scale bars*: 50 μm. (**c**) Representative figures showing endogenous $Sox2^-/ Sox10^+/p75^+$ glial cells (*arrows*) at central aspects of the injury core at 2, 7, and 14 days after cellular transplantation. Note the increase in the density of these cells with time, and their organization in bundle-like structures (consistent with **b**). These cells are probably derived from a Schwannosis process, although some of them might originate from spinal cord progenitors (see **a**), a matter that requires further studies. *Scale bars*: 50 μm). (**d**) Many Sox10+ cells at the middle of the injury core (14 days after bNCSC grafting) were also $P0^+$ (and thus peripheral Schwann cells which have invaded the spinal cord) and were able to remyelinate axons (*arrows*) or served as scaffolds for axonal regrowth (*empty arrows*; *in green*). Note also the presence of $Sox10^+/P0^-$ cells (*empty arrowheads*) which could in part be derived from spinal cord progenitors. *Scale bar*: 20 μm). (**e**, **f**) Pictures from transversal sections of the spinal cord proximal (rostrally) to the injury zone immunostained for p75. Note the increase in the abundance of Schwann/Schwann-like cells in the dorsal funiculus of bNCSC-treated animals (*arrows*). *Scale bars*: 100 μm. (**g**, **h**) The increase in $Sox10^+/p75^+$ glia in the dorsal funiculus at similar axial levels was accompanied by a reduction in $OX42^+$-activated microglia/macrophages. *Scale bars*: 100 μm. (**i**) Glia invading the center of the injury core after bNCSC grafting are $S100^{+/low}$ (*empty* or *filled arrows*, respectively). *Scale bar*: 20 μm. (**j**) Some of the $Sox2^-/Sox10^+$ glia express RIP at low levels (*arrows*) and few of them are associated with $PGP9.5^+$ axons

able to enhance spinal cord regeneration after complete spinal cord transection at the Th8 lamina level in rat [61]. The Sema3A inhibitor used, SM-216289, was found to enhance axonal regeneration (mainly of neuropilin-1$^+$ axons, including serotoninergic raphe–spinal tract ones) and to reduce cavity volume in the injured spinal cord. Interestingly, most of the regenerated axons in the experimental group were remyelinated by cells with peripheral-type properties, i.e., presence of Schwann cells, perineuria, and basal lamina, a feature rarely seen in control animals. Thus, and with support from in-vitro assays, the authors concluded that this treatment was able to enhance Schwann cell migration all through the spinal cord injury zone. Thus, the invasive behavior of Schwann cells and/or Schwann cell precursors (SCPs; [62]), as a natural common phenomenon after spinal cord injury, might probably play a significant role for future therapeutic strategies with the aim of enhancing CNS regeneration. Nevertheless, undesired side-effects of promoting such processes, such as the formation of Schwannomas or the possible development of neuropathic pain, would require further analyses [56].

The Multipotency of Schwann Cell Precursors

One of the latest discoveries in the neural crest research field, the unexpected multipotency and high plasticity of the so-called SCPs, has greatly increased our knowledge on developmental biology and opened new ways for regenerative medicine. SCPs are the first stage in Schwann cell differentiation lineage of some of the postmigratory NCCs. They are found in association with growing axons, which correspond to recently born peripheral sensory neurons and spinal cord motorneurons, and at their growth cone tip [63]. They are characterized by novel expression of proteolipid protein (PLP), brain fatty acid-binding protein (BFABP), $P_{0,}$ and Cadherin-19, among other markers. Interestingly, such postmigratory committed neural crest cells coexpressing P0 and PMP22 obtained for instance from the dorsal root ganglia were found to be able to differentiate in vitro into neurons and glia [64]. SCPs and satellite glial cells (another peripheral glia subtype, found in association with peripheral neuronal soma in ganglia), from postmigratory neural crest cells or from boundary cap neural crest cells (localized at the neural tube-nerve root entry/exit zone), would probably differ from each other in the factors specifying them [65].

By performing mouse genetic lineage-tracing experiments as well as nerve ablation and in vivo chicken electroporation studies, Adameyko and collaborators showed that SCPs are also a source of a significant proportion of skin melanocytes [66]. Interestingly, shortly after SCP detachment from growing axon tips and if they do not contact neuronal projections shortly after again, they would start expressing MITF and undergo melanocytic differentiation (Fig. 11.1). Thus, in the absence of axonal NRG1 signals (required for subsequent steps of Schwann cell differentiation, [67] SCPs become melanoblasts, provided that they find sufficient levels of survival growth factors [such as insulin growth factor-like 1 (IGF-1), platelet-derived growth factor, PDGF, or hepatocyte growth factor, HGF]. In the same article, the authors wonder themselves whether after injury myelinating Schwann cells might regain the capacity to undergo dedifferentiation and subsequently generate pigment cells in the absence of NRG1. With this aim, they performed right sciatic nerve axotomy on Krox20-Cre-YFP mice, with ligation of the proximal stump nerve end in order to avoid regeneration. They also dissected out a 0.5 cm fragment distally to the axotomy and sutured it to the underlying muscle. Two months later, animals were sacrificed, and many YFP$^+$ were seen in the nerve fragment and in the dermis of the ipsilateral experimental animals flank, thus confirming their hypothesis.

A few years later, two independent groups reported that parasympathetic neurons (which constitute one of the two divisions of the autonomic nervous system) originate from FOXD3$^+$/ p75NTR$^+$/ ErbB3$^+$/ Cadherin-19$^+$/ PLP$^+$/ Phox2B$^+$ SCPs [68, 69]. And even more recently, SCPs recruited to the gut through pelvic nerve innervation were shown to be the source of postnatally born neurons of the enteric nervous system [70]. Most of these SCP-derived neurons are calretinin$^+$

and are present in both the myenteric and submucosal ganglia of the large intestine. Moreover, SCP-specific *Ret* ablation causes oligoganglionosis of the terminal region of the colon, which might require higher levels of Ret expression for their survival and development. Such results likely suggest a role for SCPs in Hirschsprung disease-related disorders.

Interestingly, SCPs were also shown to originate endoneural fibroblasts and mesenchymal stromal cells in the teeth [65, 71, 72], thus expanding the multipotency of these cells toward some mesoderm-like derivatives (Fig. 11.1).

Neurospheres obtained from different peripheral organs were shown to be able to originate CNS cell types, including myelinating oligodendrocytes [73]. Interestingly, $p75^+$/ $Sox10^+$/ $Nestin^+$ human skin progenitor cells with competence to originate neural lineages were recently shown to express SCP markers [74]. Thus, SCP plasticity might eventually help in the future to explain certain features previously reported in developmental biology and, for example, the multipotency/pluripotency-like properties of certain stem-like cells [75]. Finally, it remains to be addressed whether or not the differentiation potential of SCPs might depend on their cranial/peripheral NCC origin [76], and in the latter it might vary according to whether they are derived from boundary cap/no boundary cap cells [67, 77] and/or if they are close/distant to the CNS/DRG [78]. In this regard, during embryonic development, boundary cap neural crest-derived cells were recently shown to migrate along peripheral nerves and to contribute to $p75^+$/ $Sox10^+$/ $Nestin^+$ human skin progenitor cells [79].

Concluding Remarks

Thanks to advancements in technology as well as in the developmental biology, molecular biology, and neuroscience fields we can now understand better the important role played by the glia in the CNS health and disease. Our new knowledge, which also includes crucial aspects of stable changes in gene activity through epigenetic modifications, introduce much more complexity in brain function and at the same time it gives us more tools to be able to uncover it. In addition, even the neural crest, which was previously disregarded in considering affections of the CNS, has been found to be crucial in no less than the development of the cerebral cortex and of some thalamic and pre-tectal nuclei. Therefore, defects in facial neural crest migration, probably involving a reduction in RA-mediated Nedd9 expression levels through insufficient development of neuronal circuits, are now considered as playing a role in some cases of autism and schizophrenia. Moreover, the Schwann cells (a subtype of neural crest-derived glia) which could also possibly be generated from CNS progenitors invade the spinal cord after traumatic injury. Furthermore, experimental evidence suggests that if this process called Schwannosis is enhanced, significant behavioral improvements as well as regeneration rates could be achieved. Finally, during normal embryonic and/or adult development, Schwann cell precursors have recently been shown to also give rise to endoneural fibroblasts, pigment cells of the skin, parasympathetic neurons, teeth mesenchymal stem cells, and enteric neurons, and eventually to other cell types. Thus, new ways are being opened for better understanding pathological CNS origin and states and for using neural cells, for example, maybe from the adult skin, as a source of multipotent progenitors with unexpected plasticity, in new regenerative medicine approaches.

References

1. Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, et al. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J Comp Neurol. 2009;513(5):532–41.
2. Sherwood CC, Stimpson CD, Raghanti MA, Wildman DE, Uddin M, Grossman LI, et al. Evolution of increased glia–neuron ratios in the human frontal cortex. Proc Natl Acad Sci U S A. 2006;103(37):13606–11.
3. Pelvig DP, Pakkenberg H, Stark AK, Pakkenberg B. Neocortical glial cell numbers in human brains. Neurobiol Aging. 2008;29(11):1754–62.
4. Verkhratskii AN, Butt A. Glial physiology and pathophysiology. Chichester: Wiley; 2013.

5. Steardo Jr L, Bronzuoli MR, Iacomino A, Esposito G, Steardo L, Scuderi C. Does neuroinflammation turn on the flame in Alzheimer's disease? Focus on astrocytes. Front Neurosci. 2015;9:259.
6. Moraga-Amaro R, Jerez-Baraona JM, Simon F, Stehberg J. Role of astrocytes in memory and psychiatric disorders. J Physiol Paris. 2014;108(4–6): 240–51.
7. Gundersen V, Storm-Mathisen J, Bergersen LH. Neuroglial transmission. Physiol Rev. 2015;95(3):695–726.
8. Charles AC, Merrill JE, Dirksen ER, Sanderson MJ. Intercellular signaling in glial cells: calcium waves and oscillations in response to mechanical stimulation and glutamate. Neuron. 1991;6(6): 983–92.
9. Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ. Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. Science. 1990;247(4941):470–3.
10. Haustein MD, Kracun S, Lu XH, Shih T, Jackson-Weaver O, Tong X, et al. Conditions and constraints for astrocyte calcium signaling in the hippocampal mossy fiber pathway. Neuron. 2014;82(2):413–29.
11. Oberheim NA, Goldman SA, Nedergaard M. Heterogeneity of astrocytic form and function. Methods Mol Biol. 2012;814:23–45.
12. Scemes E, Giaume C. Astrocyte calcium waves: what they are and what they do. Glia. 2006;54(7):716–25.
13. Sun W, McConnell E, Pare JF, Xu Q, Chen M, Peng W, et al. Glutamate-dependent neuroglial calcium signaling differs between young and adult brain. Science. 2013;339(6116):197–200.
14. Woodbury-Farina MA. The importance of glia in dealing with stress. Psychiatr Clin North Am. 2014;37(4):679–705.
15. Gerlai R, Wojtowicz JM, Marks A, Roder J. Overexpression of a calcium-binding protein, S100 beta, in astrocytes alters synaptic plasticity and impairs spatial learning in transgenic mice. Learn Mem. 1995;2(1):26–39.
16. Diamond MC, Scheibel AB, Murphy Jr GM, Harvey T. On the brain of a scientist: Albert Einstein. Exp Neurol. 1985;88(1):198–204.
17. Colombo JA, Reisin HD, Miguel-Hidalgo JJ, Rajkowska G. Cerebral cortex astroglia and the brain of a genius: a propos of A. Einstein's. Brain Res Rev. 2006;52(2):257–63.
18. Han X, Chen M, Wang F, Windrem M, Wang S, Shanz S, et al. Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. Cell Stem Cell. 2013;12(3):342–53.
19. Nave KA. Myelination and the trophic support of long axons. Nat Rev Neurosci. 2010;11(4):275–83.
20. Rajendran L, Bali J, Barr MM, Court FA, Kramer-Albers EM, Picou F, et al. Emerging roles of extracellular vesicles in the nervous system. J Neurosci Off J Soc Neurosci. 2014;34(46):15482–9.
21. Dimou L, Gallo V. NG2-glia and their functions in the central nervous system. Glia. 2015;63(8):1429–51.
22. Crawford AH, Stockley JH, Tripathi RB, Richardson WD, Franklin RJ. Oligodendrocyte progenitors: adult stem cells of the central nervous system? Exp Neurol. 2014;260:50–5.
23. Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS, et al. Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. Science. 2014;344(6183):1252304.
24. Salter MW, Beggs S. Sublime microglia: expanding roles for the guardians of the CNS. Cell. 2014;158(1): 15–24.
25. Schafer DP, Lehrman EK, Stevens B. The "quadpartite" synapse: microglia-synapse interactions in the developing and mature CNS. Glia. 2013;61(1): 24–36.
26. Tremblay ME, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A. The role of microglia in the healthy brain. J Neurosci Off J Soc Neurosci. 2011;31(45):16064–9.
27. Nguyen L, Lucke-Wold BP, Mookerjee SA, Cavendish JZ, Robson MJ, Scandinaro AL, et al. Role of sigma-1 receptors in neurodegenerative diseases. J Pharmacol Sci. 2015;127(1):17–29.
28. Yamamuro K, Kimoto S, Rosen KM, Kishimoto T, Makinodan M. Potential primary roles of glial cells in the mechanisms of psychiatric disorders. Front Cell Neurosci. 2015;9:154.
29. Bernstein HG, Steiner J, Guest PC, Dobrowolny H, Bogerts B. Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. Schizophr Res. 2015;161(1):4–18.
30. Nave KA, Ehrenreich H. Myelination and oligodendrocyte functions in psychiatric diseases. JAMA Psychiatry. 2014;71(5):582–4.
31. Gebicke-Haerter PJ. Engram formation in psychiatric disorders. Front Neurosci. 2014;8:118.
32. Haroutunian V, Katsel P, Roussos P, Davis KL, Altshuler LL, Bartzokis G. Myelination, oligodendrocytes, and serious mental illness. Glia. 2014;62(11): 1856–77.
33. Roussos P, Haroutunian V. Schizophrenia: susceptibility genes and oligodendroglial and myelin related abnormalities. Front Cell Neurosci. 2014;8:5.
34. Mighdoll MI, Tao R, Kleinman JE, Hyde TM. Myelin, myelin-related disorders, and psychosis. Schizophr Res. 2015;161(1):85–93.
35. Bergink V, Gibney SM, Drexhage HA. Autoimmunity, inflammation, and psychosis: a search for peripheral markers. Biol Psychiatry. 2014;75(4):324–31.
36. Watkins CC, Sawa A, Pomper MG. Glia and immune cell signaling in bipolar disorder: insights from neuropharmacology and molecular imaging to clinical application. Transl Psychiatry. 2014;4:e350.
37. Garcez RC, Le Douarin NM, Creuzet SE. Combinatorial activity of Six1-2-4 genes in cephalic neural crest cells controls craniofacial and

brain development. Cell Mol Life Sci: CMLS. 2014;71(11):2149–64.
38. Aguiar DP, Sghari S, Creuzet S. The facial neural crest controls fore- and midbrain patterning by regulating Foxg1 expression through Smad1 activity. Development. 2014;141(12):2494–505.
39. Choe Y, Zarbalis KS, Pleasure SJ. Neural crest-derived mesenchymal cells require Wnt signaling for their development and drive invagination of the telencephalic midline. PLoS One. 2014;9(2):e86025.
40. Yamanishi E, Takahashi M, Saga Y, Osumi N. Penetration and differentiation of cephalic neural crest-derived cells in the developing mouse telencephalon. Dev Growth Differ. 2012;54(9):785–800.
41. Lee RT, Nagai H, Nakaya Y, Sheng G, Trainor PA, Weston JA, et al. Cell delamination in the mesencephalic neural fold and its implication for the origin of ectomesenchyme. Development. 2013;140(24): 4890–902.
42. Nakajima M, Mori H, Nishikawa C, Tsuruta M, Okuyama S, Furukawa Y. Psychiatric disorder-related abnormal behavior and habenulointerpeduncular pathway defects in Wnt1-cre and Wnt1-GAL4 double transgenic mice. J Neurochem. 2013;124(2):241–9.
43. LaMantia AS. Forebrain induction, retinoic acid, and vulnerability to schizophrenia: insights from molecular and genetic analysis in developing mice. Biol Psychiatry. 1999;46(1):19–30.
44. Murphy KC. Schizophrenia and velo-cardio-facial syndrome. Lancet. 2002;359(9304):426–30.
45. Aquino JB, Lallemend F, Marmigere F, Adameyko II, Golemis EA, Ernfors P. The retinoic acid inducible Cas-family signaling protein Nedd9 regulates neural crest cell migration by modulating adhesion and actin dynamics. Neuroscience. 2009;162(4):1106–19.
46. Kumar S, Tomooka Y, Noda M. Identification of a set of genes with developmentally down-regulated expression in the mouse brain. Biochem Biophys Res Commun. 1992;185(3):1155–61.
47. Aquino JB. Neural crest in development and repair. Stockholm: Karolinska Institutet; 2007.
48. Aquino JB, Marmigere F, Lallemend F, Lundgren TK, Villar MJ, Wegner M, et al. Differential expression and dynamic changes of murine NEDD9 in progenitor cells of diverse tissues. Gene Expr Patterns: GEP. 2008;8(4):217–26.
49. Baggiolini A, Varum S, Mateos JM, Bettosini D, John N, Bonalli M, et al. Premigratory and migratory neural crest cells are multipotent in vivo. Cell Stem Cell. 2015;16(3):314–22.
50. Armati PJ, Mathey EK. An update on Schwann cell biology—immunomodulation, neural regulation and other surprises. J Neurol Sci. 2013;333(1–2):68–72.
51. Crawford AHFRJM. Neural stem cells and demyelinating disease. In: Kuhn HGaE AJ, editor. Neural stem cells in development, adulthood and disease. Stem Cell Biology and Regenerative Medicine: Elsevier; 2015. p. 125–53.
52. Xing YL, Roth PT, Stratton JA, Chuang BH, Danne J, Ellis SL, et al. Adult neural precursor cells from the subventricular zone contribute significantly to oligodendrocyte regeneration and remyelination. J Neurosci Off J Soc Neurosci. 2014;34(42): 14128–46.
53. Kegler K, Imbschweiler I, Ulrich R, Kovermann P, Fahlke C, Deschl U, et al. CNS Schwann cells display oligodendrocyte precursor-like potassium channel activation and antigenic expression in vitro. J Neural Transm. 2014;121(6):569–81.
54. Imbschweiler I, Seehusen F, Peck CT, Omar M, Baumgartner W, Wewetzer K. Increased p75 neurotrophin receptor expression in the canine distemper virus model of multiple sclerosis identifies aldynoglial Schwann cells that emerge in response to axonal damage. Glia. 2012;60(3):358–71.
55. Norenberg MD, Smith J, Marcillo A. The pathology of human spinal cord injury: defining the problems. J Neurotrauma. 2004;21(4):429–40.
56. Zhang SX, Huang F, Gates M, Holmberg EG. Role of endogenous Schwann cells in tissue repair after spinal cord injury. Neural Regen Res. 2013;8(2):177–85.
57. Jasmin L, Ohara PT. Remyelination within the CNS: do Schwann cells pave the way for oligodendrocytes? Neuroscientist Rev J Bringing Neurobiol, Neurol Psychiatry. 2002;8(3):198–203.
58. Salgado-Ceballos H, Guizar-Sahagun G, Feria-Velasco A, Grijalva I, Espitia L, Ibarra A, et al. Spontaneous long-term remyelination after traumatic spinal cord injury in rats. Brain Res. 1998;782(1–2): 126–35.
59. Dusart I, Marty S, Peschanski M. Demyelination, and remyelination by Schwann cells and oligodendrocytes after kainate-induced neuronal depletion in the central nervous system. Neuroscience. 1992;51(1):137–48.
60. Barnabe-Heider F, Goritz C, Sabelstrom H, Takebayashi H, Pfrieger FW, Meletis K, et al. Origin of new glial cells in intact and injured adult spinal cord. Cell Stem Cell. 2010;7(4):470–82.
61. Kaneko S, Iwanami A, Nakamura M, Kishino A, Kikuchi K, Shibata S, et al. A selective Sema3A inhibitor enhances regenerative responses and functional recovery of the injured spinal cord. Nat Med. 2006;12(12):1380–9.
62. Agudo M, Woodhoo A, Webber D, Mirsky R, Jessen KR, McMahon SB. Schwann cell precursors transplanted into the injured spinal cord multiply, integrate and are permissive for axon growth. Glia. 2008; 56(12):1263–70.
63. Wanner IB, Mahoney J, Jessen KR, Wood PM, Bates M, Bunge MB. Invariant mantling of growth cones by Schwann cell precursors characterize growing peripheral nerve fronts. Glia. 2006;54(5):424–38.
64. Hagedorn L, Suter U, Sommer L. P0 and PMP22 mark a multipotent neural crest-derived cell type that displays community effects in response to TGF-beta family factors. Development. 1999;126(17):3781–94.

65. Woodhoo A, Sommer L. Development of the Schwann cell lineage: from the neural crest to the myelinated nerve. Glia. 2008;56(14):1481–90.
66. Adameyko I, Lallemend F, Aquino JB, Pereira JA, Topilko P, Muller T, et al. Schwann cell precursors from nerve innervation are a cellular origin of melanocytes in skin. Cell. 2009;139(2):366–79.
67. Aquino JB, Hjerling-Leffler J, Koltzenburg M, Edlund T, Villar MJ, Ernfors P. In vitro and in vivo differentiation of boundary cap neural crest stem cells into mature Schwann cells. Exp Neurol. 2006;198(2):438–49.
68. Dyachuk V, Furlan A, Shahidi MK, Giovenco M, Kaukua N, Konstantinidou C, et al. Neurodevelopment. Parasympathetic neurons originate from nerve-associated peripheral glial progenitors. Science. 2014;345(6192):82–7.
69. Espinosa-Medina I, Outin E, Picard CA, Chettouh Z, Dymecki S, Consalez GG, et al. Neurodevelopment. Parasympathetic ganglia derive from Schwann cell precursors. Science. 2014;345(6192):87–90.
70. Uesaka T, Nagashimada M, Enomoto H. Neuronal differentiation in Schwann cell lineage underlies postnatal neurogenesis in the enteric nervous system. J Neurosci Off J Soc Neurosci. 2015;35(27):9879–88.
71. Jessen KR, Mirsky R, Lloyd AC. Schwann cells: development and role in nerve repair. Cold Spring Harb Perspect Biol. 2015;7(7):a020487.
72. Kaukua N, Shahidi MK, Konstantinidou C, Dyachuk V, Kaucka M, Furlan A, et al. Glial origin of mesenchymal stem cells in a tooth model system. Nature. 2014;513(7519):551–4.
73. Binder E, Rukavina M, Hassani H, Weber M, Nakatani H, Reiff T, et al. Peripheral nervous system progenitors can be reprogrammed to produce myelinating oligodendrocytes and repair brain lesions. J Neurosci Off J Soc Neurosci. 2011;31(17):6379–91.
74. Etxaniz U, Perez-San Vicente A, Gago-Lopez N, Garcia-Dominguez M, Iribar H, Aduriz A, et al. Neural-competent cells of adult human dermis belong to the Schwann lineage. Stem Cell Rep. 2014;3(5):774–88.
75. Fiore EJ, Mazzolini G, Aquino JB. Mesenchymal stem/stromal cells in liver fibrosis: recent findings, old/new caveats and future perspectives. Stem Cell Rev. 2015;11(4):586–97.
76. Wong CE, Paratore C, Dours-Zimmermann MT, Rochat A, Pietri T, Suter U, et al. Neural crest-derived cells with stem cell features can be traced back to multiple lineages in the adult skin. J Cell Biol. 2006;175(6):1005–15.
77. Jacob C. Transcriptional control of neural crest specification into peripheral glia. Glia. 2015.
78. Adameyko I, Lallemend F, Furlan A, Zinin N, Aranda S, Kitambi SS, et al. Sox2 and Mitf cross-regulatory interactions consolidate progenitor and melanocyte lineages in the cranial neural crest. Development. 2012;139(2):397–410.
79. Gresset A, Coulpier F, Gerschenfeld G, Jourdon A, Matesic G, Richard L, et al. Boundary caps give rise to neurogenic stem cells and terminal glia in the skin. Stem Cell Rep. 2015;5(2):278–90.

12 Nitric Oxide Pathways in Neurotoxicity from Glutamate-Induced Apoptosis: Emerging Mitochondrial Role

Walter Manucha

Abstract

Glutamate is a key neurotransmitter in the central nervous system; however, excessive levels may produce neurotoxicity and the development of neurodegenerative diseases. Multiple mechanisms underlying glutamate-induced neurotoxicity have been discussed recently. Apoptosis is also a regulated process inherent to normal cellular brain development and/or maintenance. Nevertheless, a clear deregulation of the mitochondrial respiratory mechanism has been described in patients with neurodegeneration. Thus, a growing body of evidence suggests involvement of oxidative stress, inflammation, and apoptosis in neurodegenerative diseases. To highlight this, nitric oxide, an atypical neurotransmitter synthesized and released on demand by post-synaptic neurons, has many important implications for nerve cell survival and differentiation. Moreover, apoptosis induction or inhibition by nitric oxide may be explained by several mechanisms involving the expression/localization of the enzymatic precursors, bioavailability, and/or possible protein interaction. Consequently, synaptogenesis, synapse elimination, and neurotransmitter release are modulated by nitric oxide. Finally, of particular interest to current understanding, an emergent role of nitric oxide pathways has been discussed in relation to neurotoxicity from glutamate-induced apoptosis. These findings suggest that nitric oxide pathway modulation could prevent oxidative damage to neurons by apoptosis inhibition. This chapter discusses the emergent aspects of nitric oxide–mediated signaling in the brain, and how they can be related to neurotoxicity and the development of neurodegenerative diseases.

W. Manucha, PhD
Institute of Medical and Experimental Biology of Cuyo, National Scientific and Technical Research Council (IMBECU-CONICET), Mendoza, Argentina

Pharmacology Area, Pathology Department, Medical Sciences College, National University of Cuyo, Mendoza, CP5500, Argentina
e-mail: wmanucha@yahoo.com.ar

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_12

Keywords
Glutamate • Neurotoxicity • Neurodegenerative diseases • Mitochondria • Nitric oxide • Oxidative stress • Apoptosis

Introduction

In 1950, Awapara et al. discovered the gamma aminobutyric acid (GABA), which is also usually an inhibitory neurotransmitter. GABA acts like a brake to the excitatory neurotransmitters that lead to anxiety. Thus, by managing GABA transmission, medicine has a tool to treat anxiety. On the other hand, glutamate (discovered by Ikeda in 1907) is an excitatory relative of GABA. It is the most common neurotransmitter in the central nervous system—as much as half of all neurons in the brain—and is especially important with regard to memory. It is also of interest that glutamate is actually toxic to neurons, and an excess will kill them [1]. Brain damage or a stroke sometimes lead to an excess and cause many more brain cells to die than the original trauma. Many researchers believe that it may also be responsible for quite a variety of diseases of the nervous system, and they are looking for ways to minimize its effects. Glutamate was discovered by Ikeda of Tokay Imperial University in 1907; however, it took decades for Usherwood to identify glutamate as a neurotransmitter in locusts.

Multiple mechanisms underlying glutamate-induced neurotoxicity have been proposed recently. With regard to this, current evidence highlights decoupling in the mitochondrial respiratory chain [2, 3]. This is consistent, since it is known that glutamate transmission is strongly dependent on calcium homeostasis and on mitochondrial function [4]. Moreover, apoptosis is a regulated process inherent to normal cellular brain development and/or maintenance. Nevertheless, a clear deregulation of the mitochondrial respiratory mechanism has been described in patients with neurodegeneration associated to an increase of oxidative stress [5–7]. In relation to this, several hypotheses have been proposed for neurotoxicity. These suggest mitochondrial dysfunctions and oxidative stress linked to glutamate-mediated excitotoxicity [8, 9]. Accordingly, glutamate excitotoxicity, oxidative stress, and mitochondrial dysfunctions are common features leading to neuronal death in cerebral ischemia, traumatic brain injury, Parkinson's disease, Huntington's disease, Alzheimer's disease, and amyotrophic lateral sclerosis [10]. In addition, a growing set of observations points to mitochondrial dysfunction, oxidative damage, and chronic inflammation as common pathognomonic signs of a number of neurodegenerative diseases [11]. However, mitochondrial disease may be a primary event in neurodegeneration, contributing to oxidative stress and apoptosis, or it may be caused by other cellular processes.

Particularly relevant for neurodegenerative processes is the relationship between mitochondria and nitric oxide (NO). NO, a common but short living product of nitrogen metabolism, is now understood to participate as a regulatory factor in a diverse array of physiological functions, from the control of vascular resistance up to acting as a neurotransmitter mediating inflammatory processes [12]. Regulation of cell number is a crucial property of multicellular organisms. Every moment billions of cells die to secure the functionality of the whole organism. Apoptosis is essential to normal development as well as to physiological cell turnover. The excess and/or defect can manifest across different pathology types. NO is a factor involved in apoptosis modulation, but that has produced controversy. Principal mechanisms would be cytoprotective stress protein, cGMP-dependent protein kinase, caspase activity and cytochrome C release. The accumulated data indicate that physiologically relevant levels of NO contribute to apoptosis balance. The decision for a cell to undergo apoptosis is the result of a shift in the balance between the antiapoptotic and proapoptotic forces within a cell [13]. Thus, in an original study from Sorokina

et al., they demonstrate the ability for NO to oxidize unsaturated fatty acids and the ability of serum albumin to bind them after their hydrolytic removal, and suggested that the serum albumin-induced potentiation of glutamate neurotoxicity resulted from exacerbation of the toxic effects of NO and other trace radicals on the neuronal membranes [14]. In addition, NO alone or in cooperation with superoxide anion and peroxynitrite is emerging as a predominant effect of neurodegeneration [10]. These and other more recent studies have proposed novel neuroprotective strategies with selective NO neuronal modulators. These findings suggest that NO pathway modulation could prevent oxidative damage to neurons by apoptosis inhibition. Moreover, growing evidence suggests that mitochondrial dysfunction linked to apoptosis bears the key responsibility in neurodegenerative diseases [15, 16]. Given the fact that mitochondria participates in diverse cellular processes, including energetics, metabolism, and death, the consequences of mitochondrial dysfunction in neuronal cells are inevitable.

Finally, the etiology of main neurodegenerative diseases is still unknown, but increasing evidence suggests that glutamate and mitochondria are two prominent players in the oxidative stress process that underlie these illnesses [17]. Moreover, an emergent role of NO pathways linked to mitochondrial dysfunction has been discussed. This is of particular interest to current understanding. Its role appears to be related to the neurotoxicity from glutamate-induced apoptosis

Nitric Oxide in the Central Nervous System: A Key Player

NO, a ubiquitous gaseous signaling molecule, participates in the regulation of a variety of physiological and pathological processes. Since it was first identified (1998 Medicine Nobel Prize), growing evidence points to it playing an important role in relaxation of blood vessels [18]. Furthermore, NO has been demonstrated to regulate many biological processes [19–23], especially in the central nervous system (CNS) [24]. Of the three types of enzymes that produce NO in humans, the neuronal type is found almost exclusively in the nervous system. The original evidence of NO synthesis in the CNS was the finding that N-methyl-D-aspartate (NMDA) receptor agonists caused the release of a substance similar to endothelium-derived relaxing factor [25]. Later, this was followed by the demonstration of neuronal nitric oxide synthase (nNOS) in rat brain [26].

NO is an atypical neurotransmitter, which maintains the activities of neural cells and regulates the normal functions of brain. It promotes the transfer of nerve signals from one neuron to another, maintaining the synaptic strength. Also, NO is a relevant regulator on neurogenesis and synaptogenesis, producing positive or negative effects upon different signal pathways or cellular origins and locations [27].

In 1990, Dr. Bredt and collaborators described localization of nNOS indicating a neural role for NO [28]. They demonstrated nNOS in the brain exclusively associated with discrete neuronal populations. This was the case of the neural innervation of the posterior pituitary, the autonomic nerve fibers in the retina, the cell bodies and nerve fibers in the myenteric plexus of the intestine, the adrenal medulla, and the vascular endothelial cells. Therefore, these transcendental findings provide the first conclusive evidence for a strong association of NO with neuronal functioning. In addition, several observations suggested that the Ca_2^+-dependent postsynaptic release of NO may be important in the formation and function of the vertebrate nervous system.

NO release is critically related to synaptic plasticity, control of cerebral blood flow, and the establishment and activity-dependent refinement of axonal projections during the later stages of development [29].

At the present time, it is well known that NO participates in the regulation of a variety of physiological and pathological processes. Generally, low concentrations of NO are neuroprotective and mediate physiological signaling whereas higher concentrations mediate neuroinflammatory actions and are neurotoxic (Fig. 12.1).

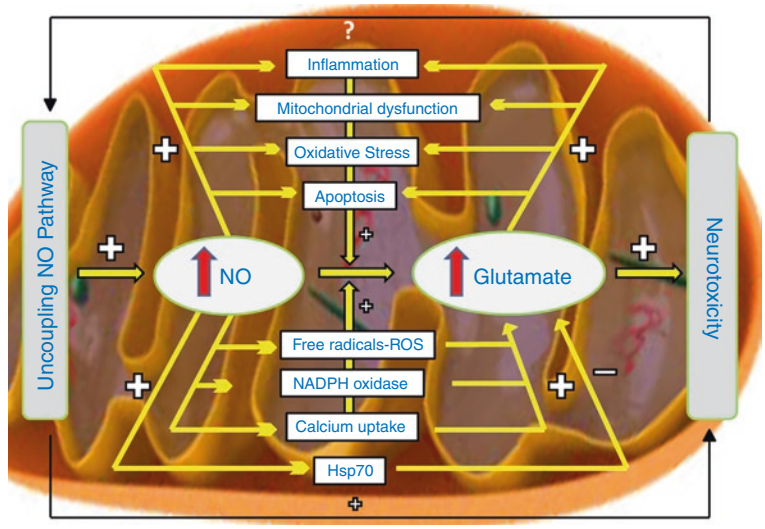


Fig. 12.1 Nitric oxide and glutamate linked to neurotoxicity. A representative overview of the interaction between nitric oxide and glutamate in the central nervous system: The proposed interaction may occur throughout the mitochondria

In relation to neurotoxic effects, some recent studies have implicated NO as a critical regulator of neuroinflammation, thus suggesting a possible role in the pathophysiology of major depressive disorder. Also, NO has long been considered part of the neurotoxic insult caused by neuroinflammation in the Alzheimer's brain, although this idea is changing. Therefore, this has highlighted a compensatory, neuroprotective role for NO that protects synapses by increasing neuronal excitability. Here, a potential mechanism for augmentation of excitability by NO via modulation of voltage-gated potassium channel activity has been suggested [30]. In addition, a low production of NO is linked to the pathogenesis of schizophrenia. So, an increase in the production of NO might constitute a potential treatment for schizophrenia. NO donors might be a promising class of compounds for the treatment of schizophrenia. Moreover, current analysis shows that both NO donors and NOS inhibitors are involved in object recognition memory, and suggests that cognition impairments might be a promising target for NO [31, 32]. In this context, an interesting pharmacological application supporting evidence for the neuroprotective actions of d-arginine (NO donor) has recently been discussed. This strategy may be used to avoid neurotoxicity induced by high levels of glucocorticoids in the CNS. This might be a novel way of neutralizing the neurotoxic effects of glucocorticoids without compromising their positive peripheral actions [33]. However, the potential neurotoxicity and the slight therapeutic window of NO donors would add a note of caution.

Nitric Oxide Linked to Neurotoxicity from Glutamate: Mitochondrial Emerging Role

Glutamate is one of the 20 amino acids forming part of proteins. It is critical for cell function and is not an essential nutrient, because in man it can be synthesized from other compounds. It is the classic excitatory neurotransmitter in the human cortex. Its role as a neurotransmitter is mediated by the stimulation of specific receptors, called glutamate receptors, which are classified into ionotropic (ion channel) and metabotropic receptors (seven transmembrane G protein coupled domains). All neurons contain glutamate, but only a few use it as a neurotransmitter. Glutamate is potentially excitotoxic (Fig. 12.1). Whereas a variety of neurotransmitters could potentially trigger excitotoxic cell injury, glutamate is thought to be the primary contributor because of its potent effect on increasing intracellular calcium through ionotropic receptors [34]. Therefore, a complex machinery to regulate levels

is active. In this regard, and of special interest, the central role played by NO in the CNS has been emphasized in the current literature.

In CNS, NO can be originated from at least four different sources: the endothelium of cerebral vessels, the immunostimulated microglia and astrocytes, the nonadrenergic noncholinergic nerve, and the glutamate neuron [35]. It should be noted that the highest stimulus for the release of NO is the activation of NMDA receptors by glutamate. Also, the release of NO can also be elicited by non-NMDA receptors for glutamate, as well as receptors for acetylcholine, angiotensin, bradykinin, serotonin (5-hydroxytryptamine; 5-HT), neurotensin, and endothelin [36].

An original report by Dawson et al. established that NO mediates the neurotoxicity of glutamate [37]. The authors proposed free radical formation linked to neurotoxicity, and NO is a reactive free radical. According to this, a growing body of evidence suggests involvement of oxidative stress, inflammation, and apoptosis in neurodegenerative diseases [38–41] (Fig. 12.1). Moreover, apoptosis is a regulated process inherent to the normal cellular brain development and/or maintenance; nevertheless a clear deregulation of the mitochondrial respiratory mechanism has been described in patients with neurodegeneration associated to an increase of oxidative stress [42–44].

Toxicity mediated by NO has been controversial. In this sense, Dr. Kiedrowski suggested that the neuroprotective properties of a NO donor such as sodium nitroprusside (SNP) on glutamate- and NMDA-induced neurotoxicity are not due to the release of NO and activation of guanylate cyclase, but are determined by the ferrocyanide portion of the SNP molecule [45]. NO was demonstrated to afford protection from NMDA receptor-mediated neurotoxicity. This pathway for NO regulation of physiological function is not via cGMP, but instead involves reactions with membrane-bound thiol groups on the NMDA receptor-channel complex [46].

NO can react with superoxide to yield peroxynitrate, which is extremely reactive [47]. In models of macrophage-mediated cytotoxicity, NO can complex with the iron–sulfur center of enzymes to inactivate them [48]. Because several of these enzymes are in the mitochondrial electron-transport complex, NO can inhibit mitochondrial respiration, diminishing the ability of the cells to deal with oxidative stress. Specifically, high concentrations of NO irreversibly inhibit complexes I, II, III, IV, and V in the mitochondrial respiratory chain (Fig. 12.1), whereas physiological levels of NO reversibly reduce cytochrome oxidase [49]. Also, further evidence was found in a study on manganese neurotoxicity. Manganese is sequestered in mitochondria, where it inhibits oxidative phosphorylation. The exposure to manganese results in important changes. They include decreased uptake of glutamate. Increased densities of binding sites for the "peripheral-type" benzodiazepine receptor may also be observed. This is a class of receptor localized in the mitochondria of astrocytes, and involved in oxidative metabolism and mitochondrial proliferation. An increased uptake of L-arginine, a precursor of NO, together with increased expression of the inducible form of NOS (iNOS) has also been reported. Accordingly, potential consequences include failure of energy metabolism, production of reactive oxygen species (ROS), and increased extracellular glutamate concentration with excitotoxicity effects [50] (Fig. 12.1).

The mechanisms of neurotoxicity involve activation of NMDA receptors by glutamate, production of NO by nNOS and iNOS, oxidative injury to DNA, and activation of the DNA damage-sensing enzyme poly (ADP-ribose) polymerase (PARP). In this sense, the translocation of a mitochondrial protein apoptosis-inducing factor (AIF) from mitochondria to the nucleus depends on PARP activation, and plays an important role in excitotoxicity-induced cell death [51]. In addition, the accumulation of calcium into mitochondria may play a key role as a trigger to mitochondrial pathology. In the case of calcium overload in neurons, the neurotoxicity of glutamate depends on mitochondrial calcium uptake, but the toxicity to mitochondria also requires the generation of NO. The calcium increase mediated by NMDA receptor activation is thus associated with NO, and the combination leads to the collapse of mitochondrial membrane potential followed by cell death [52].

It is clear that glutamate neurotoxicity is mediated, at least in part, by NO and mitochondrial damage. However, recently a closely related new finding has been postulated. These reports indicate that heat shock protein 70 (Hsp70) upregulation may provide protection in depression by downregulation of iNOS protein expression through suppression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation [53]. This was validated by Liu et al., who used an in-vitro spinal cord injury model induced by glutamate treatment. Here, treatment with allicin (an organosulfur compound obtained from garlic) significantly attenuated glutamate-induced lactate dehydrogenase (LDH) release, loss of cell viability, and apoptotic neuronal death. Allicin decreased the expression of iNOS following glutamate exposure. Moreover, allicin treatment significantly increased the expression of Hsp70 [54].

Heat shock proteins (HSP) are a shock-induced family of proteins, whose most prominent members are a group of molecules dedicated to maintaining the function of other proteins. Interestingly, after being exposed to heat shock, typical proinflammatory agonists modify the heat shock-induced transcriptional program and expression of HSP genes, suggesting a complex reciprocal regulation between the inflammatory pathway and that of the heat shock response. The specific task of Hsp70, the most widespread and highly conserved HSP, is to protect against inflammation through multiple mechanisms. Hsp70 modulates inflammatory response, as well as downregulating the nuclear factor kappa-light-chain-enhancer of activated B cells. Also, a decreased expression of renal Hsp70 may contribute to the activation of the toll-like receptor 4-initiating inflammatory signal pathway. In addition, several studies have revealed that Hsp70 is involved in the regulation of angiotensin II, a peptide with proinflammatory activity. Increased inflammatory response is generated by nicotinamide adenine dinucleotide phosphate oxidase (NADPH), following activation by angiotensin II. Also, Hsp70 protects the epithelium by modulation of NADPH, a fundamental step in the pro-inflammatory mechanism [55].

Inflammation is present in many diseases, such as diabetes, obesity, metabolic syndrome, impaired glucose tolerance, hypertension, cardiac disease, and CNS disease [19, 56]. Inflammation is connected to mitochondrial dysfunction, overproduction of oxidants, and an over-activation of the renin–angiotensin system linked to NADPH oxidase activity [57]. In addition, NO is also associated with inflammation linked to mitochondrial dysfunction. Moreover, and as mentioned above, reduced NO release induces Hsp70 expression [54], mediating beneficial effects against oxidative stress injury, inflammation, and apoptosis [19, 58]. Curiously, 15 years ago, an elevated expression of the genes encoding Hsp70 linked to apoptosis or necrosis induced by glutamate, was proposed [59]. Later, Hsp70 was suggested as a molecular marker of neurotoxicity [60]. Accordingly, some chaperones such as the members of the Hsp70 family also modulate polyglutamine (polyQ) aggregation and suppress its toxicity. These findings suggested that an imbalance between the neuronal chaperone capacity and the production of potentially dangerous polyQ proteins may trigger the onset of polyQ disease [61]. The formation of insoluble protein aggregates in neurons is a hallmark of neurodegenerative diseases caused by proteins with expanded polyQ repeats. In addition, the more frequent amyloid-related neurodegenerative diseases are caused by a gain of toxic function of misfolded proteins. Toxicity in these disorders may result from an imbalance between normal chaperone capacity and production of dangerous protein species. Increased chaperone expression can suppress the neurotoxicity of these molecules, suggesting possible therapeutic strategies [62]. Moreover, the effects of the Hsp70 were investigated in tau oligomers and tau toxicity linked to neurodegenerative disease. The authors illustrated that Hsp70 preferentially binds to tau oligomers rather than filaments and prevents the anterograde fast axonal transport inhibition observed with a mixture of both forms of aggregated tau [63]. All this evidence strengthens the idea that a reduced NO release linked to induced Hsp70 expression can mediate beneficial effects against oxidative stress injury,

inflammation, and apoptosis, during neurodegenerative and neurotoxicity diseases [64] (Fig. 12.1). In addition, abnormalities in NO signaling may constitute a trait-marker related to neuroinflammation, which could be explored for novel therapeutic targets [65].

Finally, the etiology of main neurodegenerative diseases is still unknown, but increasing evidence suggests that glutamate and mitochondria are two key players in the oxidative stress process that underlie these illnesses. Moreover, an emergent role of NO pathways linked to mitochondrial dysfunction has been proposed. It is of particular interest to current understanding. These findings were discussed in the section concerning neurotoxicity from glutamate-induced apoptosis. Taken together, evidence suggests that NO pathways modulation could prevent oxidative damage to neurons by apoptosis inhibition. The discussion remains open on emergent aspects of nitric oxide-mediated signaling in the brain, and how they can be related to neurotoxicity as well as to neurodegenerative diseases development.

Funding The author disclosed receipt of the following financial support for the research, authorship, and/or publication of this chapter: This work was supported by grants from the Research and Technology Council of Cuyo University (SECyT), Mendoza, Argentina, and from the National Agency of Scientific and Technical Research (APCyT), both of which were awarded to Walter Manucha. Grant PICT 2012-0234, BID 2777 OC/AR.

Conflict of Interest The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Tanaka K. Glutamate transporter dysfunction and major mental illnesses. Nihon Rinsho. 2016;74(1):163–73.
2. Brown GC, Vilalta A. How microglia kill neurons. Brain Res. 2015;1628(Pt B):288–97.
3. Takarada T, Fukumori R, Yoneda Y. Mitochondrial uncoupling protein-2 in glutamate neurotoxicity. Nihon Yakurigaku Zasshi. 2013;142(1):13–6.
4. Smaili SS, Ureshino RP, Rodrigues L, Rocha KK, Carvalho JT, Oseki KT, Bincoletto C, Lopes GS, Hirata H. The role of mitochondrial function in glutamate-dependent metabolism in neuronal cells. Curr Pharm Des. 2011;17(35):3865–77.
5. Pinazo-Durán MD, Zanón-Moreno V, Gallego-Pinazo R, García-Medina JJ. Oxidative stress and mitochondrial failure in the pathogenesis of glaucoma neurodegeneration. Prog Brain Res. 2015;220:127–53.
6. Repalli J. Translocator protein (TSPO) role in aging and Alzheimer's disease. Curr Aging Sci. 2014;7(3):168–75.
7. Pchelina SN, Emel'ianov AK, Usenko TS. Molecular basis of Parkinson's disease linked with mutations in the LRRK2 gene. Mol Biol (Mosk). 2014;48(1):3–14.
8. Son M, Elliott JL. Mitochondrial defects in transgenic mice expressing Cu, Zn superoxide dismutase mutations: the role of copper chaperone for SOD1. J Neurol Sci. 2014;336(1–2):1–7.
9. Goracci G, Ferrini M, Nardicchi V. Low molecular weight phospholipases A2 in mammalian brain and neural cells: roles in functions and dysfunctions. Mol Neurobiol. 2010;41(2–3):274–89.
10. Chabrier PE, Demerlé-Pallardy C, Auguet M. Nitric oxide synthases: targets for therapeutic strategies in neurological diseases. Cell Mol Life Sci. 1999;55(8–9):1029–35.
11. Urrutia PJ, Mena NP, Núñez MT. The interplay between iron accumulation, mitochondrial dysfunction, and inflammation during the execution step of neurodegenerative disorders. Front Pharmacol. 2014;5:38.
12. Vallés P, Manucha W. Nitric oxide in the kidney: Physiological roles and regulation. In: Advances in chemistry and biology of nitric oxide. Kerala/India: Research Signpost; 2008. p. 1–19.
13. Manucha W. Apoptosis y óxido nítrico, dos de los tópicos más estudiadosen investigación biomédica. Revista Médica Universitaria. 2008;4(1):1–12.
14. Sorokina EG, Reutov VP, Pinelis VG, Vinskaya NP, Vergun OV, Khodorov BI. The mechanism of potentiation of the glutamate-induced neurotoxicity by serum albumin.A possible role of nitric oxide. Membr Cell Biol. 2000;13(3):389–96.
15. Lee J. Mitochondrial drug targets in neurodegenerative diseases. Bioorg Med Chem Lett. 2016;26(3):714–20.
16. Marella M, Seo BB, Matsuno-Yagi A, Yagi T. Mechanism of cell death caused by complex I defects in a rat dopaminergic cell line. J Biol Chem. 2007;282(33):24146–56.
17. Cassano T, Pace L, Bedse G, Lavecchia AM, De Marco F, Gaetani S, Serviddio G. Glutamate and mitochondria: two prominent players in the oxidative stress-induced neurodegeneration. Curr Alzheimer Res. 2016;13(2):185–97.
18. Gruetter CA, Barry BK, McNamara DB, Gruetter DY, Kadowitz PJ, Ignarro L. Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosoamine. J Cyclic Nucleotide Res. 1979;5(3):211–24.

19. Molina MN, Ferder L, Manucha W. Emerging role of nitric oxide and heat shock proteins in insulin resistance. Curr Hypertens Rep. 2016;18(1):1. doi:10.1007/s11906-015-0615-4.
20. Manucha W. Mitochondria and oxidative stress participation in renal inflammatory process. Medicina (B Aires). 2014;74(3):254–8.
21. Pekarova M, Lojek A. The crucial role of l-arginine in macrophage activation: what you need to know about it. Life Sci. 2015;137:44–8.
22. Yuan S, Patel RP, Kevil CG. Working with nitric oxide and hydrogen sulfide in biological systems. Am J Physiol Lung Cell Mol Physiol. 2015;308(5):L403–15.
23. Szabo C. Gasotransmitters in cancer: from pathophysiology to experimental therapy. Nat Rev Drug Discov. 2015; doi:10.1038/nrd.2015.1.
24. Kim JI, Kim YS, Kang SK, Kim C, Park C, Lee MS, Huh Y. Electroacupuncture decreases nitric oxide synthesis in the hypothalamus of spontaneously hypertensive rats. Neurosci Lett. 2008;446(2–3):78–82.
25. Garthwaite J, Charles SL, Chess-Williams R. Endotheliumderivedrelaxing factor release on activation of NMDA receptorssuggests role as intercellular messenger in the brain. Nature. 1988;336:385–8.
26. Knowles RG, Palacios M, Palmer RMJ, et al. Formation of nitric oxide from L-arginine in the central nervous system:a transduction mechanism for stimulation of soluble guanylatecyclase. Proc Natl Acad Sci U S A. 1989;86:5159–62.
27. Hu Y, Zhu DY. Hippocampus and nitric oxide. Vitam Horm. 2014;96:127–60.
28. Bredt DS, Hwang PM, Snyder SH. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature. 1990;347(6295):768–70.
29. Gally JA, Montague PR, Reeke Jr GN, Edelman GM. The NO hypothesis: possible effects of a short-lived, rapidly diffusible signal in the development and function of the nervous system. Proc Natl Acad Sci U S A. 1990;87(9):3547–51.
30. Balez R, Ooi L. Getting to NO Alzheimer's disease: neuroprotection versus neurotoxicity mediated by nitric oxide. Oxid Med Cell Longev. 2016;2016:3806157. doi:10.1155/2016/3806157.
31. Pitsikas N. The role of nitric oxide donors in schizophrenia: Basic studies and clinical applications. Eur J Pharmacol. 2015;766:106–13.
32. Pitsikas N. The role of nitric oxide in the object recognition memory. Behav Brain Res. 2015;285:200–7.
33. Canteros MG. D-Arginine as a neuroprotective amino acid: promising outcomes for neurological diseases. Drug Discov Today. 2014;19(5):627–36.
34. Krishnamurthy K, Laskowitz DT. Chapter 5: Cellular and molecular mechanisms of secondary neuronal injury following traumatic brain injury. In: Laskowitz D, Grant G, editors. Translational research in traumatic brain injury. Boca Raton: CRC Press/Taylor and Francis Group; 2016.
35. Bruhwyler J, Chleide E, Liégeois JF, Carreer F. Nitric oxide: a new messenger in the brain. Neurosci Biobehav Rev. 1993;17(4):373–84.
36. Molina JA, Jiménez-Jiménez FJ, Ortí-Pareja M, Navarro JA. The role of nitric oxide in neurodegeneration. Potential for pharmacological intervention. Drugs Aging. 1998;12(4):251–9.
37. Dawson VL, Dawson TM, London ED, Bredt DS, Snyder SH. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. Proc Natl Acad Sci U S A. 1991;88(14):6368–71.
38. Wongprayoon P, Govitrapong P. Melatonin attenuates methamphetamine-induced neurotoxicity. Curr Pharm Des. 2015;22:1022–32.
39. Lazzara CA, Kim YH. Potential application of lithium in Parkinson's and other neurodegenerative diseases. Front Neurosci. 2015;9:403. doi:10.3389/fnins.2015.00403.
40. Wu H, Niu H, Shao A, Wu C, Dixon BJ, Zhang J, Yang S, Wang Y. Astaxanthin as a potential neuroprotective agent for neurological diseases. Mar Drugs. 2015;13(9):5750–566.
41. Fu W, Zhuang W, Zhou S, Wang X. Plant-derived neuroprotective agents in Parkinson's disease. Am J Transl Res. 2015;7(7):1189–202.
42. Leuner K, Schulz K, Schütt T, Pantel J, Prvulovic D, Rhein V, Savaskan E, Czech C, Eckert A, Müller WE. Peripheral mitochondrial dysfunction in Alzheimer's disease: focus on lymphocytes. Mol Neurobiol. 2012;46(1):194–204.
43. Kulic L, Wollmer MA, Rhein V, Pagani L, Kuehnle K, Cattepoel S, Tracy J, Eckert A, Nitsch RM. Combined expression of tau and the Harlequin mouse mutation leads to increased mitochondrial dysfunction, tau pathology and neurodegeneration. Neurobiol Aging. 2011;32(10):1827–38.
44. Chen H, Wang S, Ding JH, Hu G. Edaravone protects against MPP^+ - induced cytotoxicity in rat primary cultured astrocytes via inhibition of mitochondrial apoptotic pathway. J Neurochem. 2008;106(6):2345–52.
45. Kiedrowski L, Manev H, Costa E, Wroblewski JT. Inhibition of glutamate-induced cell death by sodium nitroprusside is not mediated by nitric oxide. Neuropharmacology. 1991;30(11):1241–3.
46. Lei SZ, Pan ZH, Aggarwal SK, Chen HS, Hartman J, Sucher NJ, Lipton SA. Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channelcomplex. Neuron. 1992;8(6):1087–99.
47. Risbano MG, Gladwin MT. Therapeutics targeting of dysregulated redox equilibrium and endothelial dysfunction. Handb Exp Pharmacol. 2013;218:315–49.
48. Lancaster Jr JR, Hibbs Jr JB. EPR demonstration of iron–nitrosyl complex formation by cytotoxic activated macrophages. Proc Natl Acad Sci U S A. 1990 Feb;87(3):1223–7.

49. Dai Z, Wu Z, Yang Y, Wang J, Satterfield MC, Meininger CJ, Bazer FW, Wu G. Nitric oxide and energy metabolism in mammals. Biofactors. 2013;39(4):383–91.
50. Hazell AS. Astrocytes and manganese neurotoxicity. Neurochem Int. 2002;41(4):271–7.
51. Wang H, Shimoji M, Yu SW, Dawson TM, Dawson VL. Apoptosis inducing factor and PARP-mediated injury in the MPTP mouse model of Parkinson's disease. Ann N Y Acad Sci. 2003;991:132–9.
52. Duchen MR. Roles of mitochondria in health and disease. Diabetes. 2004;53(Suppl 1):S96–102.
53. Zlatković J, Bernardi RE, Filipović D. Protective effect of Hsp70i against chronic social isolation stress in the rat hippocampus. J Neural Transm (Vienna). 2014;121(1):3–14.
54. Liu SG, Ren PY, Wang GY, Yao SX, He XJ. Allicin protects spinal cord neurons from glutamate-induced oxidative stress through regulating the heat shock protein 70/inducible nitric oxide synthase pathway. Food Funct. 2015;6(1):321–30.
55. Manucha W. HSP70 family in the renal inflammatory response. Inflamm Allergy Drug Targets. 2014;13(4):235–40.
56. Kim HK, Nunes PV, Oliveira KC, Young LT, Lafer B. Neuropathological relationship between major depression and dementia: A hypothetical model and review. Prog Neuropsychopharmacol Biol Psychiatry. 2016;67:51–7.
57. Chandramohan G, Bai Y, Norris K, Rodriguez-Iturbe B, Vaziri ND. Effects of dietary salt on intrarenal angiotensin system, NAD(P)H oxidase, COX-2, MCP-1 and PAI-1 expressions and NF-kappaB activity in salt-sensitive and -resistant rat kidneys. Am J Nephrol. 2008;28(1):158–67.
58. Martínez de Toda I, De la Fuente M. The role of Hsp70 in oxi-inflamm-aging and its use as a potential biomarker of lifespan. Biogerontology. 2015;16(6):709–21.
59. Savolainen KM, Loikkanen J, Eerikäinen S, Naarala J. Interactions of excitatory neurotransmitters and xenobiotics in excitotoxicity and oxidative stress: glutamate and lead. Toxicol Lett. 1998;102-103:363–7.
60. Rajdev S, Sharp FR. Stress proteins as molecular markers of neurotoxicity. Toxicol Pathol. 2000;28(1):105–12.
61. Sakahira H, Breuer P, Hayer-Hartl MK, Hartl FU. Molecular chaperones as modulators of polyglutamine protein aggregation and toxicity. Proc Natl Acad Sci U S A. 2002;99(Suppl 4):16412–8.
62. Barral JM, Broadley SA, Schaffar G, Hartl FU. Roles of molecular chaperones in protein misfolding diseases. Semin Cell Dev Biol. 2004;15(1):17–29.
63. Ward SM, Himmelstein DS, Lancia JK, Binder LI. Tau oligomers and tau toxicity in neurodegenerative disease. Biochem Soc Trans. 2012;40(4):667–71.
64. Giridharan VV, Thandavarayan RA, Arumugam S, Mizuno M, Nawa H, Suzuki K, Ko KM, Krishnamurthy P, Watanabe K, Konishi T. Schisandrin B ameliorates ICV-infused amyloid β induced oxidative stress and neuronal dysfunction through inhibiting RAGE/NF-κB/MAPK and up-regulating HSP/Beclin expression. PLoS One. 2015;10(11):e0142483. doi:10.1371/journal.pone.0142483.
65. Kudlow P, Cha DS, Carvalho AF, RS MI. Nitric oxide and major depressive disorder: pathophysiology and treatment implications. Curr Mol Med. 2016;16:206–15.

13 Psychoneuroendocrinological and Cognitive Interactions in the Interface Between Chronic Stress and Depression

Gustavo E. Tafet

Abstract

The role of chronic stress in the origin and development of depression may be conceived as the result of different factors, including the impact of current environmental stressors and the cumulative effects of stressful experiences during early periods of life. It has been shown that chronic stressful experiences, including current and early-life events, may lead to increased activation of the hypothalamic–pituitary–adrenal axis. These changes, including increased synthesis and release of Corticotrophin Releasing Hormone (CRH) and cortisol, have been also associated with functional changes in certain limbic structures, including increased reactivity of the amygdala and decreased activity of the hippocampus, and changes in different monoaminergic systems, including decreased serotonergic activity, therefore resulting in increased vulnerability to stress. Upon exposure to chronic stressful events, as well as stressful conditions in early life, other biological factors may also contribute to this process, including genetic polymorphisms and epigenetic mechanisms, altered immunological responses, and psychological factors, including negatively biased cognitive processing, with the resulting cognitive distortions and learned helplessness. This chapter aims to understand the role of these converging factors, the potential interactions between them, and the role they play in the interface between chronic stress and the development of depression.

Keywords

Stress • Depression • Neurobiology • Epigenetics • Cortisol • Serotonin

Introduction

The links between stress and depression have been widely observed, particularly at the clinical level, where chronic exposure to different stressful events has been associated with the origin and

G.E. Tafet, PhD
Department of Psychiatry and Neurosciences,
Maimonides University,
Hidalgo, 775 Buenos Aires, Argentina
e-mail: psychiatry@maimonides.edu

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_13

development of depressive symptoms [1–5]. In this regard, different environmental stressors may trigger an array of adaptive responses, depending on diverse aspects related to the characteristics of the stressors and the available resources of each individual to cope with them. Psychological aspects include all the cognitive processing of perceived environmental information, a subjective appraisal and assessment of potential resources, and the resulting coping strategies. Biological aspects include all the different components of the central nervous system (CNS) involved in emotional and cognitive processing, and the neural structures involved in the activation of adaptive responses, including the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal (HPA) axis [6, 7]. In this regard, adaptive responses should be rapidly activated to react effectively during stressful situations and efficiently concluded afterward. If these responses continue in a prolonged and excessive manner, as is observed in chronically stressful conditions, they may lead to maladaptive changes, which in turn are associated with the origin and development of various disorders [1, 6], including different anxiety disorders and depression.

Stress: From the Environment to the Brain

Bio-ecological and psycho-social factors are perceived as environmental stressors, and this information is transmitted through different sensory pathways to specific areas of the CNS, including the thalamus, limbic structures, and various cortices. Hence, sensory information may reach the amygdala through direct projections from the thalamus, or indirectly through cortical connections. Direct projections provide a primary representation of the input, which may be enhanced by noradrenergic stimulation from the locus coeruleus (LC) to provoke the primary stress response. Indirect projections include various cortical steps, including primary and higher-order sensory cortices, which create perceptual representations of environmental stimuli; association cortices, where unimodal stimuli are integrated into polymodal representations; and transitional cortices, including the parahippocampal, perirhinal, and entorhinal areas, which in turn project to the hippocampus, where more complex representations are integrated with contextual cues [8]. The hippocampus projects back to cortical areas and projects forward to the lateral nucleus of the amygdala, as well as the hypothalamic paraventricular nucleus (PVN), where it has been shown to play an inhibitory role [9, 10]. In addition, the hippocampus and transitional cortices participate in the formation and retrieval of explicit memories, which in turn may also reach the amygdala, therefore triggering stress responses, playing as internal stressors in the absence of any environmental stimuli. Hence, direct and indirect pathways converge in the lateral nucleus of the amygdala, which in turn projects to the basal, accessory basal, and central nuclei [11, 12]. This allows an integrated emotional processing, which in turn may be translated into different outputs to regulate the expression of an array of behavioral, autonomic, and neuroendocrine responses [13]. These pathways include projections to the lateral hypothalamus, which may activate the sympathetic component of the ANS [14]; projections to the dorsal motor nucleus of the vagus, which may activate the para-sympathetic component of the ANS; and projections to the hypothalamic PVN, which can provoke the activation of the HPA axis [13, 14].

Role of the HPA System

It has been shown that activation of the HPA axis is initiated in limbic structures, including direct projections from the central nucleus of the amygdala, or indirectly through the bed nucleus of the stria terminals (BNST), which project to the hypothalamic PVN [6]. Neurons of the PVN synthesize Corticotrophin Releasing Hormone (CRH), which is released to the hypophyseal portal blood to reach the anterior pituitary, where it up-regulates the transcription of the pro-opio-

melanocortin (POMC) gene, a common precursor for adrenocorticotropin (ACTH) and related peptides, therefore stimulating the release of ACTH into the bloodstream. In addition, arginine–vasopressine is also released to reinforce the effect of CRH. Hence, ACTH reaches the adrenal cortex, where it stimulates the biosynthesis and release of glucocorticoids, particularly cortisol, which participate in widespread metabolic effects, mostly involved in the mobilization of resources aimed at improving physiological conditions to successfully cope with stressful situations. These steroid hormones bind to both mineralocorticoid receptors (MRs or type I) and glucocorticoid receptors (GRs or type II), constituting a hormone-receptor complex. Upon cortisol binding, these receptors undergo conformational changes to facilitate their recognition by and subsequent binding to a glucocorticoid response element (GRE) located in the promoter region of target genes [15], therefore activating or deactivating the expression of various target genes. Up-regulation may be achieved through the constitution of homo- or hetero-dimers of the cortisol–GRs complex, which recognize and bind to GREs [16], bringing together other co-factors, constituting a pre-initiation complex at the promoter region. Down-regulation may be achieved through binding to a negative GRE, as has been described for the negative regulation of the POMC gene [17] and the CRH gene [18], thereby down-regulating the HPA axis. In addition, cortisol may also down-regulate the HPA axis through binding to GRs in the hippocampus, which stimulate inhibitory projections to the PVN.

During chronic stress, sustained and persistent activation of the HPA system may disrupt physiological mechanisms, including negative-feedback loops [15]. Physiological rhythms characterized by wide variations, with morning zeniths and evening nadirs, result in increased levels of cortisol and blunted circadian rhythm, reflected in increased levels during the evening and mild changes in the morning [1]. Therefore, alterations in the regulation of the HPA axis, such as those observed during chronic stress, may develop into different clinical conditions, such as anxiety disorders and depression. Moreover, a significant association between stress and depression is now well documented [1, 4, 19, 20], where hyperactivity of the HPA axis, with the consequent hypercortisolism, represents one of the most consistent findings in both conditions [21, 22].

The Role of CRH

It has been shown that CRH plays a critical role in the regulation of the HPA axis, which has been clearly associated with activation of the hypothalamic PVN in response to stress. More recently, CRH-containing neurons have been also described in different cortical and subcortical areas, such as the central nucleus of the amygdala, participating in neural pathways involved in cognitive and emotional responses [23, 24]. In this regard, CRH projections from the amygdala have been shown to exert stimulatory effect on cells of the PVN, therefore activating the HPA axis. Reciprocal connections have been described between these CRH neurons and aminergic nuclei, such as the LC and the raphe nuclei (RN) [3]. They represent pathways of reciprocal interaction between the noradrenergic and the serotonergic systems and the HPA axis. All of them are involved in the stress response [3, 25]. In this regard, it has been shown that CRH projections stimulate NA release in the LC [26], with the consequent noradrenergic activation of the ANS and the HPA axis. It also exerts an inhibitory effect on serotonergic neurons in the raphe nucleus (RN) [27]. In this manner other structures are affected, through serotonergic projections to the PVN, the amygdala, and the hippocampus [3]. Hence, CRH has been associated with the regulation of the serotonergic and the noradrenergic systems, which are critically involved in mood and anxiety disorders, producing anxiogenic and depressogenic effects [28]. In addition, CRH has been also associated with anxiety and encoding of emotional memories [21, 28], therefore demonstrating its critical role in the stress response, not only during adulthood, but also as an important factor in long-lasting effects of early stressful experiences.

The Role of Serotonin

It has been shown that serotonin (5-hydroxitriptamine, 5HT) plays a critical role in the pathophysiology of depression, developing and further supporting the serotonergic hypothesis of depression [29, 30], which associates a deficient or altered serotonergic neurotransmission in the CNS with the origin and development of depressive symptoms. The serotonergic system has its cell groups mainly located in the RN, which project to diverse cortical and limbic structures. The serotonergic projections to the forebrain originate mainly in the dorsal (DRN) and medial RN (MRN) [31]. The DRN–forebrain tract innervates diverse structures, including the amygdala and the nucleus accumbens (NAc) [32, 33]. They have also been associated with the state of anticipatory anxiety that plays an adaptive role in situations of alarm. It contributes to informing the amygdala about unpleasant experiences, and participates in the regulation of the resulting emotional reactions [10]. Dysfunction of this system has been associated with the development of phobic and generalized anxiety disorders [33]. The MRN–forebrain tract innervates complementary structures, most prominently the dorsal hippocampus and the hypothalamus [33, 34], It has been associated with tolerance to unpleasant, unavoidable, persistent aversive stimuli [35] such as those perceived during chronic stress. It is also associated with adaptive control on negative emotional experiences, generating relaxation, satisfaction, and inertia [10]. In consequence, dysfunction of this system, particularly involving MRN–hippocampal projections, may be associated with decreased tolerance to aversive stimuli, learned helplessness, and subsequent depression [33]. In addition, serotonergic neurons in the RN have been shown to be also interconnected and physiologically integrated with other monoaminergic systems, including the noradrenergic and dopaminergic [36].

At the molecular level, 5HT is released to the synaptic cleft to bind pre- and post-synaptic 5HT receptors. The control on serotonergic neurotransmission is exerted by the serotonin transporter (5HTT), which is responsible for the reuptake of 5HT, therefore regulating the concentrations of the neurotransmitter and its availability to bind and activate its receptors. The 5HTT represents the molecular target of various antidepressants, such as the selective serotonin reuptake inhibitors (SSRIs). Therefore, 5HTT blockade by SSRIs is expressed into increased concentrations of 5HT in the synaptic cleft, leading in turn to increased activation of 5HT receptors. The efficacy of these antidepressants has also been associated with adaptive changes produced by its continuous administration, including desensitization or down-regulation of somato-dendritic $5HT_{1A}$ autoreceptors in the RN, and up-regulation of post-synaptic $5HT_{1A}$ and desensitization of $5HT_{2A}$ receptors [37]. In addition, it has been shown that post-synaptic $5HT_{1A}$ receptors in different limbic structures may be down-regulated or desensitized by glucocorticoids or exposure to chronic stress [38–40]. Cortisol may inhibit 5HT neurotransmission tonically through binding to MRs, while increased levels of cortisol, such as those observed during chronic stressful conditions, bind predominantly to GRs, therefore interacting with GREs and inhibiting the expression of the 5HT1A gene [38]. In addition, it has been shown that cortisol may exert a stimulatory effect on 5HT uptake in vitro, and this has been attributed to an increased expression of the 5HTT gene by cortisol [41], further supporting the notion of a reciprocal regulation between the HPA and 5HT systems, and their potential interactions in the interface between stress and depression.

The Role of Dopamine

Dopamine (DA) has been also involved in the stress responses, including stress-related regulation of the HPA axis, as well as in the pathophysiology of depression [42, 43]. The main groups of dopaminergic neurons in the CNS comprise the retro–rubro field (A8), the substantia nigra pars compacta (A9), and the ventral–tegmental area (VTA, A10), which originates the mesolimbic (M-L) and mesocortical (M-C) pathways, which have been shown to participate in cognitive and emotional functions [44]. The M-L pathway

reaches the nucleus accumbens (NAc) and other limbic structures, such as the amygdala and the hippocampus, and participates in the processing and reinforcement of rewarding stimuli, the subjective experience of pleasure, and in motivation of behavioral responses [10]. The M-C pathway reaches the prefrontal cortex (PFC) and the anterior cingulate cortex (ACC), among other structures. It has been associated with cognitive functions such as concentration, working memory, judgment, planning and execution of behavioral responses [10, 44]. Increased activity of the amygdala has been associated with the impact of environmental stressors, which has also been associated with increased concentration of DA in the PFC, hence contributing to giving exaggerated salience to relatively mild negative stimuli, with the resulting anhedonia [45].

The M-L pathway has been shown to be sensitive to stressful experiences [46]; therefore, exposure to unavoidable or uncontrollable stressors may lead to decreased DA release in the NAc and impaired response to environmental stimuli, which may further lead to the expression and exacerbation of depressive symptoms induced by stress [42]. It has also been demonstrated that altered dopaminergic function is critically involved in altered reward processing underlying anhedonia [47]. In addition, reciprocal regulation has been also observed between the VTA and the RN [43], and dopaminergic pathways have been also involved in certain regulatory effect on the HPA axis.

The Role of Norepinephrine

The role of norepinephrine (NE) has been also recognized in the pathophysiology of affective disorders, providing the first aminergic hypothesis of depression [48]. The main group of NE-containing neurons in the CNS is located in the LC, which sends various projections to different cortical and subcortical structures [49], including the amygdala, the hippocampus, and the PVN [23, 50]. Among these, noradrenergic projections to the VTA have been described. Here, NE exerts stimulatory effect on DA release. Noradrenergic projections to the RN have been also described. In this site, NE exerts a regulatory effect on 5HT release [49]. Reciprocal regulations between NE and 5HT have been described, not only through connections between both aminergic systems, but also through limbic structures, such as the hippocampus [51]. In addition, reciprocal connections between NE and CRH containing neurons suggest a critical role of the LC in the regulation of neural and neuroendocrine responses to stress [23].

The LC is activated in response to acute stressors. It induces the resulting release of NE throughout different neural structures, thereby leading to enhanced arousal and vigilance, in the context of adaptive responses to stress [6]. Activation of the LC also stimulates the lateral hypothalamus, which in turn participates in the activation of the sympathetic branch of the ANS. This complements the adaptive response to stress [23]. During chronic stress a potential dysfunction of the LC has been observed, with the consequent decrease in NE release. This fact has been associated with some features of learned helplessness, as well as problems in cognitive functions frequently observed in depression [52]. Alteration of the NE system has been also associated with altered states of arousal [49], commonly observed in anxiety disorders and in depression.

The Role of Neurotrophic Factors

Various studies have focused on the role of neurotrophic factors in critical neural processes, such as neuroplasticity and neurogenesis, with particular attention on the neurotrophins (NT). This molecular group includes the nerve growth factor (NGF), the brain-derived neurotrophic factor (BDNF), NT3, and NT4. Among these, various studies have focused on the role of BDNF in the regulation of neuroplasticity and neurogenesis, strongly suggesting that decreased levels of BDNF may lead to depressive symptoms, whereas up-regulation of BDNF has been associated with clinical recovery [53]. In this regard, it has been shown that chronic stressful situations, with the resulting hyperactivity

of the HPA axis and hypercortisolism, may induce atrophy of neurons in the hippocampus, where high concentrations of GRs have been described [54], and these have been also associated with decreased levels of BDNF. Moreover, it has been also suggested that increased levels of glucocorticoids may be involved in down-regulation of BDNF and, on the other hand, it has been shown that various antidepressants up-regulated the expression of BDNF in the hippocampus [53], therefore supporting a potential role for BDNF in their mechanism of action [55, 56]. The association between the observed up-regulation of BDNF in the hippocampus and the successful effects of certain antidepressants suggested that the enduring effects of antidepressants could be associated with neuroplastic changes in the hippocampus, amygdala and PFC, and this could be associated with up-regulation of BDNF [53].

It has been shown that the sustained and prolonged impact of stressful conditions may lead to alteration of limbic structures, such as the amygdala and the hippocampus, therefore affecting their projections to the PFC and the ANS, which are critically involved in cognitive and emotional regulation [57]. Increased activation of the HPA axis, with the resulting hypercortisolism, neuroplastic, and neurogenetic processes, may result in a critical effect on the hippocampus . This may lead to altered formation of new cognitions, thereby contributing to impair depressogenic conditions. Hence, successful antidepressive strategies should lead to substantial recovery of hippocampal function, with the resulting up-regulation of neuroplasticity and increasing neurogenesis. This may be directly achieved through increasing levels of 5HT [58], or indirectly, through modulation of the HPA axis, and increasing levels of BDNF [53].

The Role of Cognitive Vulnerability

The link between stress and depression has been clearly observed at the clinical level, where cognitive processing plays a critical role. The potentially noxious impact of environmental stressors may depend on different characteristics related to the events, such as length, intensity, and strength of the impact, the availability of subjective resources to cope with them, and the resulting cognitive appraisal, particularly the potential balance between stressors and resources, and the resulting coping strategies [59]. In this regard, cognitive appraisal may lead to a realization that not every stressor is necessarily noxious or negative. Certain stimuli, perceived as desirable, predictable, and controllable challenges, could be perceived as pleasant or exciting, and therefore are known as eustress. In the opposite direction, the more intense, persistent, undesirable, unpredictable, and uncontrollable challenges could be perceived as threatening, may lead to maladaptive responses, and therefore are known as distress [60]. Accordingly, distressful situations may lead to a defense reaction, representing an active mode of response, characterized by effortful coping strategies produced in situations of perceived threat to control, or a defeat reaction, representing a passive mode of response, characterized by severe difficulty or inability to cope, associated with situations of subjective loss of controllability. Therefore, chronic exposure to undesirable, unpredictable, unavoidable, or uncontrollable situations may lead to decreasing resources, or the subjective assessment that resources are not enough, which in turn is associated with subjective feelings of helplessness [19]. This has been associated with chronic stressful situations and the development of depressive symptoms. Many cognitive resources are shaped during childhood, and according to the cognitive model [61] early-life experiences provide the raw material to develop cognitive schemas, which in turn represent the basis to transform environmental information into cognitions, and these are the result of every learned experience stored in long-term memory. Therefore, early adverse events contribute to the shaping of particular cognitive schemas, with the consequent negative biases. Dysfunctional schemas shaped during childhood may be retained in silence over long periods, to be later activated by additional experiences during adulthood, thereby leading to negative biases in the information processing, with the consequent dysfunctional effects, including negatively biased appraisals and limitations in further processing of the resulting cognitions, thereby leading to feelings of helplessness and subsequent depression [62].

The Role of Early Adverse Experiences

It has been demonstrated that, in addition to chronic stress during adulthood, the impact of adverse conditions and traumatic events experienced during childhood represents a strong factor of vulnerability in the origin and development of depression [3, 63]. The association between early adverse experiences, such as abuse, neglect, or loss, and the development of depression later in life has been shown to occur particularly in response to stressful conditions during adulthood [64]. A history of early adverse experiences has been associated with psychological and neurobiological consequences, which in turn have been associated with increased vulnerability to stress later in life. Various studies have focused on alterations in different limbic structures and the HPA axis. In this regard, it has been shown that early stressful experiences may lead to decreased availability and reduced efficacy of hippocampal GRs [63], therefore causing a predisposition to glucocorticoid resistance and increased reactivity of the HPA axis observed in response to additional stressful situations. Moreover, increased levels of cortisol and decreased GRs induced by early stressful situations have been associated with decreased hippocampal function and volume in adulthood [65]. Therefore, early adverse experiences may induce permanent changes, including hyper-reactivity of neural and neuroendocrine responses to stress, reflected in increased CRH activity, glucocorticoid resistance, and reduced volume of the hippocampus [63, 66], all of which may influence the potential response to additional stressful situations later in life.

The Role of Genetic Polymorphisms

The role of environmental stressors has been extensively studied; however, it has been observed that some individuals may exhibit stronger vulnerability to stress, while some others may be less sensitive, more resistant, or even resilient, therefore avoiding their potential depressogenic effects [67]. The potential influence of stressful events may depend not only on the attributes of environmental stressors, but on the interaction between these stressors and individual characteristics, including psychological subjective conditions, such as cognitive resources, and biological idiosyncratic conditions, such as certain genetic makeup [63]. The relationship between genetic variations and potential alterations in different neural structures and functions in the CNS provides an important link to understand the molecular mechanisms underlying potential gene-environment interactions. In this regard, various polymorphisms have been investigated in candidate genes, which are known to participate in important molecular pathways involved in the origin of depression. The presence of these genetic variations may be involved in the development of depression in response to stressful events, including adverse experiences during childhood and environmental stressors during adulthood, therefore constituting an important factor of risk and vulnerability [63, 67–69].

The serotonergic system, which is critically involved in the regulation of mood, has been shown to provide an important source of candidate genes [67]. The 5-HTT, which participates in 5-HT reuptake at brain synapses, was investigated, resulting in the identification of a polymorphism in the promoter region of the 5-HTT gene [70]. The promoter activity is regulated by sequence elements located in the upstream regulatory region, known as the 5-HTT gene-linked-polymorphic-region (5-HTTLPR), where a short (5-HTTLPR-S) and a long (5-HTTLPR-L) promoter variant have been identified [67]. The 5-HTTLPR-S allele was associated with decreased transcriptional efficiency in comparison with the 5-HTTLPR-L allele, resulting in reduced expression of the 5-HTT gene [70] and altered 5-HTT availability. Alterations in the regulation of the 5-HTT gene may be involved in the modulation of serotonergic activity in response to stress, and this has been further supported by clinical and preclinical studies [71]. They include the evidence, observed in functional brain imaging studies, that 5-HTTLPR-S carriers, homozygous or heterozygous for this allele, exhibited increased amygdala reactivity to fearful and threatening stressors, in comparison to 5-HTTLPR-L carriers [72]. This

strongly suggests that variations in the 5-HTT gene may be involved in psychological responses to stress [67].

It has been shown that the amygdala plays a critical role in the regulation of emotional reactions, and increased reactivity of the amygdala has been associated with anxiety and altered mood regulation [8, 12]. Hence, a potential association between this polymorphism and increased reactivity of the amygdala in response to negative stressors [73] may contribute to better understanding the potential impact of the mechanisms underlying this association. As has been described previously, the amygdala also plays a critical role in the activation of the HPA axis, and therefore hyper-activation of the amygdala may lead to hypercortisolism.

The potential association between the 5-HTTLPR-S variation and decreased expression of the 5-HTT gene, with the consequent alterations in 5-HTT availability, may appear paradoxical, considering the potential vulnerability attributed to 5-HTTLPR-S carriers. It has been shown that the therapeutic effect of SSRIs may depend, not only on 5-HTT blockade, with the resulting increase of 5-HT concentrations in the synaptic cleft, but also on down-regulation of pre-synaptic 5-HT_{1A} auto-receptors, with the consequent normalization of the serotonergic tone [37, 73]. Therefore, it is conceivable that alterations in 5-HTT gene regulation, with the consequent effect on synaptic 5-HT levels, may differ between the earlier expressed by congenital conditions, and the later triggered by environmental factors. Hence, it has been proposed that congenital alterations in the regulation of the serotonergic system, such as an essentially increased concentration of 5-HT, may result in down-regulation of 5-HT receptors, particularly post-synaptic, therefore leading to desensitization of the serotonergic system [73] providing a possible mechanism to understand the vulnerability suffered by 5-HTTLPR S allele carriers. The association of this polymorphism with altered serotonergic neurotransmission and hyper-reactivity of the amygdala, with the consequent activation of the HPA axis, provides a potential link to investigate the role of stressful events in the origin and development of depression in vulnerable individuals.

Some researchers have also investigated genetic variations in the BDNF gene. It has been shown that up-regulation of BDNF plays a critical role in the treatment of mood disorders through stimulation of neuroplasticity and neurogenesis in the hippocampus, as well as hippocampal connections with the amygdala and PFC [74]. By the opposite way, decreased levels of BDNF have been associated with depressive symptoms [75]. Various studies have focused on the BDNF gene regulation, and variations potentially involved in mood disorders, resulting in the identification of different single nucleotide polymorphisms (SNPs). Among these, a SNP has been identified at nucleotide position 196 in the coding region of the BDNF gene, where a guanine base is replaced by an adenine. In the mentioned variation, it is in turn translated into the substitution of valine (Val) by methionine (Met) at codon 66, therefore termed "Val66Met". Here, the presence of a "Met" allele has been associated with a functional alteration, which is translated into abnormal intracellular trafficking and decreased secretion of BDNF [54, 74, 76]. Studies of carriers of the Met-BDNF allele showed relatively smaller hippocampal volumes, compared with homozygous for the Val-BDNF allele [74]. This has also been associated with reduced hippocampal activation [76] and deficient cognitive performance [74], which have also been associated with lower emotional stability and increased vulnerability to develop depressive symptoms.

In addition, various studies have also focused on genes involved in the regulation of the HPA axis. In this regard, hyperactivity of CRH neurons in the CNS was associated with the origin of depression [21]. In consequence, transcriptional regulation of CRH and CRH type 1 receptor (CRHR1) genes were also studied. It resulted in the identification of various SNPs in the CRHR1, and haplotypes formed by certain SNPs, potentially involved in the moderation of the effects produced by early adverse experiences on adult depression [69]. Other genes involved in the regulation of the HPA system were also studied, such

as the corticosteroid receptors, including both the MR and GR genes, where various SNPs have been found in both of them. Among these, two different SNPs in the GR gene (BclI and Asp363Ser) have been associated with increased vulnerability for depression in the general population, probably through increased glucocorticoid sensitivity [77]. More recently, studies also focused on the FK-506-binding protein (FKBP5), a co-chaperone of hsp-90 involved in the regulation of GR sensitivity [78], and therefore also involved in HPA axis responsivity. Various SNPs have been identified in this gene, some of them associated with increased FKBP5 protein expression, which in turn may lead to changes in GR, with the resulting effect on HPA axis regulation [79]. These observations demonstrate the role of polymorphisms in gene–environment interactions, particularly in the origin and development of depression.

Conclusion

The role of chronic stress in the origin and development of depression may be conceived as the result of multiple factors, including converging psychological and neurobiological features. Recent research has focused on the persistent and long-lasting effects of stressful experiences during childhood, including alterations in the regulation of the HPA axis, with resulting vulnerability to acute and chronic stressors during adulthood. However, the observation that some individuals may exhibit stronger vulnerability to environmental stressors, while some others may be less sensitive to similar experiences, more resistant or even resilient, has highlighted the importance of further studying the nature of different risk factors, including genetic dispositions and the potential interactions between genetic and environmental factors. Future research should focus on further understanding the neurobiological background underlying these factors, and potential windows of intervention, including neural and molecular mechanisms involved in the interface between cognitive processing of environmental stressors and their potential impact in epigenetic processes. This may lead to the development of more successful treatments, aimed at restoring altered neural and neuroendocrine mechanisms, but also to prevent the development of mood disorders in vulnerable individuals. This would be possible either by identifying, and potentially neutralizing, different factors of vulnerability, or by increasing and promoting protective resources in individuals exposed to stressful conditions, particularly those victims of adverse conditions in early periods of life.

References

1. Gold PW, Goodwin FK, Chrousos GP. Clinical and biochemical manifestations of depression. Relation to neurobiology of stress (1). N Engl J Med. 1988;319(6):348–53.
2. Nemeroff CB. The preeminent role of early untoward experience on vulnerability to major psychiatric disorders: the nature–nurture controversy revisited and soon to be resolved. Mol Psychiatry. 1999;4:106–8.
3. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol Psychiatry. 2001;49(12):1023–39.
4. Tafet GE, Bernardini R. Psychoneuroendocrinological links between chronic stress and depression. Prog Neuropsychopharmacol Biol Psychiatry. 2003;27(6):893–903.
5. Tafet GE, Nemeroff CB. The links between stress and depression: psychoneuroendocrinological, genetic and environmental interactions. J Neuropsychiatry Clin Neurosci. 2015;9
6. Chrousos GP, Gold PW. The concept of stress and stress system disorders. JAMA. 1992;267:1244–52.
7. López JF, Akil H, Watson SJ. Neural circuits mediating stress. Biol Psychiatry. 1999;46(11):1461–71.
8. LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci. 2000;23:155–84.
9. McEwen BS, Brinton RE. Neuroendocrine aspects of adaptation. Prog Brain Res. 1987;72:11–26.
10. Smelik PG. Adaptation and brain function. Prog Brain Res. 1987;72:3–9.
11. LeDoux JE. Brain mechanisms of emotion and emotional leaming. Curr Opin Neurobiol. 1992;2:191–7.
12. LeDoux JE. The emotional brain: the misterious underpinnings of emotional life. New York: Simon & Schuster; 1996.
13. Davis M. The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. TIPS. 1992;13:35–41.
14. LeDoux JE, Iwata J, Cicchetti P, Reis DJ. Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. J Neurosci. 1988;8:2517–29.

15. De Kloet ER, Joëls M, Holsboer F. Stress and the brain: from adaptation to disease. Nat Rev Neurosci. 2005;6(6):463–75.
16. Reichel RR, Jacob ST. Control of gene expression by lipophilic hormones. FASEB J. 1993;7:427–36.
17. Drouin J, Sun Y, Chamberland M, Gauthier Y, De Lean A, Nemer M, Schmidt T. Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. EMBO J. 1993;1:145–56.
18. Malkoski S, Dorin R. Composite glucocorticoid regulation at a functionally defined negative glucocorticoid response element of the human corticotropin-releasing hormone gene. Mol Endocrinol. 1999;19:1629–44.
19. Abramson LY, Seligman M, Teasdale LD. Learned helplessness in humans: critique and reformulation. J Abnorm Psychol. 1978;87:49–78.
20. Holsboer F. Neuroendocrinology of mood disorders. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York: Raven Press Ltd.; 1995. p. 957–69.
21. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol. 1999;160(1):1–12.
22. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron. 2002;34:13–25.
23. Valentino RJ, Foote SL, Page ME. The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress response. Ann N Y Acad Sci. 1993;697:171–87.
24. LeDoux JE. The amygdala: contributions to fear and stress. Semin Neurol. 1994;6:231–7.
25. Owens MJ, Nemeroff CB. Physiology and pharmacology of corticotropin-releasing factor. Pharmacol Rev. 1991;43:425–73.
26. Valentino RJ, Foote SL, Aston-Jones G. Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. Brain Res. 1983;270:363–7.
27. Kirby LG, Rice KC, Valentino RJ. Effects of corticotropin-releasing factor on neuronal activity in the serotonergic dorsal raphe nucleus. Neuropsychopharmacology. 2000;22:148–62.
28. Nemeroff CB. Neurobiological consequences of childhood trauma. J Clin Psychiatry. 2004;65(Suppl 1):18–28.
29. Meltzer HY, Lowy MT. The serotonin hypothesis of depression. In: Meltzer H, editor. Psychopharmacology: the third Generation of Progress. New York: Raven Press; 1987. p. 513–26.
30. Owens MJ, Nemeroff CB. The role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin Chem. 1994;40:288–95.
31. Azmitia EC. The primate serotonergic system: progression towards a collaborative organization. In: Meltzer H, editor. Psychopharmacology: the third generation of progress. New York: Raven Press Ltd.; 1987. p. 61–74.
32. Azmitia EC, Whitaker-Azmitia PM. Anatomy, cell biology, and plasticity of the serotonergic system. Neuropsychopharmacological implications for the actions of psychotropic drugs. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York: Raven Press Ltd; 1995. p. 443–9.
33. Deakin JFW. Distinct roles of 5HT subsystems in panic, anxiety and depression. In: Racagai G, Brunello N, Fukuda T, editors. Biological psychiatry, vol. 1. Amsterdam: Elsevier; 1991. p. 305–7.
34. Hensler JG. Serotonergic modulation of the limbic system. Neurosci Biobehav Rev. 2006;30:203–14.
35. Kennett GA, Dickinson SL, Curzon G. Enhancement of some 5-HT-dependent behavioural responses following repeated immobilization in rats. Brain Res. 1985;330(2):253–63.
36. Ordway GA, Klimek V, Mann JJ. Neurocircuitry of mood disorders. In: Davis KL, Charney D, Coyle JT, Nemeroff CB, editors. Psychopharmacology: the fifth generation of progress. Philadelphia: Lippincott, Williams, & Wilkins; 2002. p. 1051–64.
37. Blier P, de Montigny C, Chaput Y. Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. J Clin Psychopharmacol. 1987;7:24S–35S.
38. Lopez JF, Chalmers DT, Little KY, Watson SJ. Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. Biol Psychiatry. 1998;43:547–73.
39. Karten YJ, Nair SM, van Essen L, Sibug R, Joels M. Long-term exposure to high corticosterone levels attenuates serotonin responses in rat hippocampal CA1 neurons. Proc Natl Acad Sci U S A. 1999;96:13456–61.
40. van Riel E, van Gemert NG, Meijer OC, Joels M. Effect of early life stress on serotonin responses in the hippocampus of young adult rats. Synapse. 2004;53:11–9.
41. Tafet GE, Toister-Achituv M, Shinitzky M. Enhancement of serotonin uptake by cortisol: a possible link between stress and depression. Cogn Affect Behav Neurosci. 2001;1(1):96–104.
42. Cabib S, Puglisi-Allegra S. Stress, depression and the mesolimbic dopamine system. Psychopharmacologie. 1996;128:331–42.
43. Dunlop BW, Nemeroff CB. The role of dopamine in the pathophysiology of depression. Arch Gen Psychiatry. 2007;64(3):327–37.
44. Le Moal M, Simon H. Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol Rev. 1991;71(1):155–234.
45. Nestler EJ, Carlezon Jr WA. The mesolimbic dopamine reward circuit in depression. Biol Psychiatry. 2006;59(12):1151–9.
46. Abercrombie ED, Keefe KA, Di Frischia DF, Zigmond MJ. Differential effects of stress on in vivo dopamine

release in striatum, nucleus accumbens and medial frontal cortex. J Neurochem. 1989;52:1655–8.
47. Stein DJ. Depression, anhedonia, and psychomotor symptoms: the role of dopaminergic neurocircuitry. CNS Spectr. 2008;13(7):561–5.
48. Schildkraut JJ. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry. 1965;122(5):509–22.
49. Ressler KJ, Nemeroff CB. Role of norepinephrine in the pathophysiology and treatment of mood disorders. Biol Psychiatry. 1999;46:1219–33.
50. Charney DS, Bremmer JD, Redmond DE. Noradrenergic neural substrates for anxiety and fear. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York: Raven Press Ltd.; 1995. p. 387–95.
51. Mongeau R, Blier P, de Montigny C. The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effects of antidepressant treatments. Brain Res Brain Res Rev. 1997;23(3):145–95.
52. Robbins TW, Everitt BJ. Central norepinephrine neurons and behavior. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York: Raven Press Ltd.; 1995. p. 363–72.
53. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry. 2006;59(12):1116–27.
54. Sapolsky RM. Glucocorticoids and atrophy of the human hippocampus. Science. 1996;273:749–50.
55. Malberg J, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult hippocampus. J Neurosci. 2000;20:9104–10.
56. Duman RS. Role of neurotrophic factors in the etiology and treatment of mood disorders. Neuromolecular Med. 2004;5:11–25.
57. Ota KT, Duman RS. Environmental and pharmacological modulations of cellular plasticity: role in the pathophysiology and treatment of depression. Neurobiol Dis. 2013;57:28–37.
58. Jacobs BL, van Praag H, Gage FH. Adult brain neurogenesis and psychiatry: a novel theory of depression. Mol Psychiatry. 2000;5(3):262–9.
59. Lazarus RS, Folkman S. Stress, appraisal and coping. New York: Springer; 1984.
60. Selye H. The stress of life. New York: McGraw-Hill; 1978.
61. Beck AT. Depression: clinical, experimental, and theoretical aspects. New York: Harper & Row; 1967.
62. Beck AT. The evolution of the cognitive model of depression and its neurobiological correlates. Am J Psychiatry. 2008 Aug;165(8):969–77.
63. Heim C, Newport DJ, Mletzko T, Miller AH, Nemeroff CB. The link between childhood trauma and depression: insights from HPA axis studies in humans. Psychoneuroendocrinology. 2008;33:693–710.
64. Kaufman J, Plotsky PM, Nemeroff CB, Charney DS. Effects of early adverse experiences on brain structure and function: clinical implications. Biol Psychiatry. 2000;48(8):778–90.
65. McEwen BS. Stress and hippocampal plasticity. Curr Opin Neurobiol. 1999;5:205–16.
66. Meaney MJ, Szyf M. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. Dialogues Clin Neurosci. 2005;7:103–23.
67. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, Mc Clay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science. 2003;301(5631):386–9.
68. Kaufman J, Yang BZ, Douglas-Palumberi H, Grasso D, Lipschitz D, Houshyar S, Krystal JH, Gelernter J. Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. Biol Psychiatry. 2006;59:673–80.
69. Bradley RG, Binder EB, Epstein MP, Tang Y, Nair HP, Liu W, Gillespie CF, Berg T, Evces M, Newport DJ, Stowe ZN, Heim CM, Nemeroff CB, Schwartz A, Cubells JF, Ressler KJ. Influence of child abuse on adult depression: moderation by the corticotropin-releasing hormone receptor gene. Arch Gen Psychiatry. 2008;65(2):190–200.
70. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science. 1996;274(5292):1527–31.
71. Murphy DL, Li Q, Engel S, Wichems C, Andrews A, Lesch KP, Uhl G. Genetic perspectives on the serotonin transporter. Brain Res Bull. 2001;56(5):487–94.
72. Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, Egan MF, Weinberger DR. Serotonin transporter genetic variation and the response of the human amygdala. Science. 2002;297:400–3.
73. Hariri AR, Drabant EM, Munoz KE, Kolachana BS, Mattay VS, Egan MF, Weinberger DR. A susceptibility gene for affective disorders and the response of the human amygdala. Arch Gen Psychiatry. 2005;62(2):146–52.
74. Gatt JM, Nemeroff CB, Dobson-Stone C, Paul RH, Bryant RA, Schofield PR, Gordon E, Kemp AH, Williams LM. Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. Mol Psychiatry. 2009;14(7):681–95.
75. Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakir C, Nakazato M, Watanabe H, Shinoda N, Okada S, Iyo M. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry. 2003;54:70–5.
76. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 2003;112(2):257–69.

77. De Rijk RH, Schaaf M, de Kloet ER. Glucocorticoid receptor variants: clinical implications. J Steroid Biochem Mol Biol. 2012;81:103–22.
78. Horstmann S, Lucae S, Menke A, Hennings JM, Ising M, Roeske D, Müller-Myhsok B, Holsboer F, Binder EB. Polymorphisms in GRIK4, HTR2A, and FKBP5 show interactive effects in predicting remission to antidepressant treatment. Neuropsychopharmacology. 2010;35(3):727–40.
79. Binder EB, Salyakin D, Lichtner P, Wochnik GM, Ising M, Pütz B, Papiol S, Seaman S, Lucae S, Kohli MA, Nickel T, Künzel HE, Fuchs B, Majer M, Pfennig A, Kern N, Brunner J, Model S, Baghai T, Deiml T, Zill P, Bondy B, Rupprecht R, Messer T, Köhnlein O, Dabitz H, Brückl T, Müller N, Pfister H, Lieb R, Mueller JC, Lõhmussaar E, Strom TM, Bettecken T, Meitinger T, Uhr M, Rein T, Holsboer F, Muller-Myhsok B. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. Nat Genet. 2004;36(12):1319–25.

14 Mechanisms Involved in Memory Processes: Alterations Induced by Psychostimulants—Targeting the Central AT_1 Receptors

Natalia Andrea Marchese, Osvaldo Martin Basmadjian, Victoria Belén Occhieppo, Gustavo Baiardi, and Claudia Bregonzio

Abstract

Learned experiences are indispensable for adaptation and survival of every living organism. The generation of a memory trace is an active physiological process which implies association and organization of the new impressions with already stored ones. Therefore, memory is explained as activity-dependent synaptic plasticity, involving electrophysiological, biochemical and morphological changes in functional synapse.

Throughout the different stages of information processing, emotion and memories interact to shape data encoding and retrieval. Dopamine plays a key role in the control of mood, fear and anxiety, and modulates the biochemical processes of memory. Hence, more plastic and appropriate responses can be achieved to variable external demands.

Among drugs of abuse worldwide consumed, psychostimulants are known for their stimulant properties within the central nervous system. Monoaminergic neurotransmission elicited by amphetamine alters neuronal connectivity in several brain areas; thus, its pharmacological actions can be extended to learning and memory processes. Evidence indicates a complex scenario after psychostimulant administration where long-term memory can be either impaired or enhanced, according to the experimental conditions.

N.A. Marchese (✉) • O.M. Basmadjian
V.B. Occhieppo • C. Bregonzio
Departamento de Farmacología, Facultad de Ciencias Químicas, Instituto de Farmacología Experimental Córdoba (IFEC-CONICET), Universidad Nacional de Córdoba (UNC), Córdoba 5000, Argentina
e-mail: nmarchese@fcq.unc.edu.ar

G. Baiardi
Laboratorio de Neurofarmacología, Instituto de Investigaciones Biológicas y Tecnológicas (IIBYT-CONICET), Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Universidad Católica de Córdoba, Córdoba, Argentina

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_14

Brain angiotensin II, by activation of its brain AT_1 receptors, plays an active role as neuromodulator in noradrenergic and dopaminergic transmission. AT_1 receptors activation positively regulates catecholamine synthesis and release, and their expression in brain areas is linked to noradrenaline and dopamine receptors. Angiotensin II AT_1 receptors are known to play a key role in acute responses as well as in the development of neuroadaptation induced by amphetamine. This evidence leads towards new modulatory pharmacotherapy approaches concerning psychostimulant-related alterations over cognitive processes.

Keywords
Memory • Synaptic plasticity • Emotions • Dopamine • Psychostimulants • Angiotensin II

Introduction

The ability to remember past experiences in order to modify the ongoing behavior outreaches the irreversible straightforward direction of time [1]. Memory implies the faculty of acquiring, processing, storing and retrieving information. These processes are indispensable for learning in regard to adaptation and survival of every living organism [2, 3]. Thus, the marine gastropod mollusk from genus *Aplysia* shows its siphon-withdrawal response to noxious stimuli, and rodents can learn to map environments according to food access or threatening places. Moreover, in humans memory is critical, not only for survival, but for the rich array of memories associated with emotion, acquired skills and habits, facts and experiences with personal tags, that make possible to constantly develop personal identity and individuality [2, 4, 5].

Memory gathers broad types of categories in response to different interpretations. According to the temporal duration of the stored information, *working memory* accounts for the small amount of information held regarding the ongoing conscious present as it is used to plan and carry out behavior; *short-term memory* reflects the faculty to temporarily hold a limited amount of information in a very accessible state; whereas *long-term memory* indicates the vast body of knowledge stored over a lifetime [6]. The distinction between these categories is widely discussed: the differences between working and short-term memories, mainly rely on definitions; meanwhile, between short-term and long-term memories, the main proposed differences are duration and capacity of the stored information. On these last issues, items in short-term memory decay as a function of time, and there is a limit in how many items this type of memory can hold [6]. In humans, we can identify as well *implicit* and *explicit* memories, concerning the consciousness of remembering. It is proposed that implicit memory may be the only type of memory observed in lower invertebrates, whereas explicit forms of memory can be most effectively studied in vertebrates [7]. Moreover, memories can be related to *declarative* and *non-declarative* learning. The first one is used to form stimulus–stimulus associations, such as semantic and episodic memories, which enables the conscious recall of facts and events. Meanwhile, non-declarative learning refers to a collection of several different mnemonic abilities that can be unconsciously expressed through performance. To this regard, procedural memories can be mentioned, which make it possible to perform basic motor skills and daily life tasks [3, 5, 7–9].

Stored knowledge, upon which changes in behavior over time are dependent, reflects time-sequential processes. Long-term memory implies acquisition, consolidation, retention, retrieval, and performance. In experimental conditions,

memory acquisition occurs as the animal learns an association between two stimuli. Consolidation comes from the Latin for "to make firm," and involves progressive post-acquisition, so this association moves from a labile to a more fixed state. One of the hallmarks during long-term memory storage is that newly learned information is sensitive to disruption after acquisition. Memories can be impaired or enhanced during the consolidation period, suggesting that this labile state may have evolved as a way to allow memory to be integrated along with the new experience. Within a short time after training, new memories become resistant to interferences or agents. The time window of susceptibility depends on the task and type of interference or blocker, and ranges from seconds to minutes to hours [10, 11]. Consolidation implies two physiological processes: one is needed for the formation and storage of a memory trace, and the second one is required to assimilate the newly acquired memory into an already existing body of knowledge, which in turn also influences what could be learned subsequently [8]. Finally, retrieval gives access to the activation and use of stored memory in order to guide behavior [7]. Analyses of retrieval are faced with the challenge of disentangling the effects of retrieval manipulation on the performance of a behavioral task [11].

The construction of a memory trace depends on an active physiological process; it is not merely the impression of the new experience upon the nerve cells, but it is also the association and organization of the new impressions with the old ones [8]. Thus, memory-encoding implies dynamic spatio-temporal patterns of synchronized cellular activity within widespread neural networks. This dynamic and reverberating activity progressively results in altered patterns of connectivity among co-activated neurons. Within this framework, any memory representation would correspond with specific sets of patterns of activity in overlapping networks [2, 12]. Hence, each memory is influenced by different variables and mediated by different neural structures and mechanisms that form distinguishable, and dissociable, systems. Memory systems are organized structures of more elementary operating components, and according to the situations involve different concatenations of components from one or more systems. Intervention with the operation of a system—even with a single component of it—affects all those learning and memory performances that depend on that system [8].

Changes in brain activity after learning provide insights into the time course of consolidation processes. The study of functional brain activity reveals shifts in activity among different brain regions occurring over a period of several hours after learning, supporting time-dependent reorganization of brain representations along with consolidation [13]. According to the standard model, memory consolidation begins when information, registered initially in the neocortex, is integrated by the hippocampal complex/medial temporal lobes and related structures in the diencephalon. The new memory trace consists of an ensemble of bound hippocampal complex–neocortical neurons [8, 10, 12]. However, it should be noted that brain areas which are involved in consolidation are not necessarily sites of long-term memory storage [7].

The *Synaptic Plasticity* Hypothesis

Long-lasting modified brain function in response to experience is presumed to underlie an organism's ability to learn and memorize new facts and events [14, 15]. So, *how does experience modify brain function in order to guide behavior?* The initial and currently accepted proposal was presented by Donald Hebb in his book "The Organization of Behavior"(1943), where he first propossed that objects or ideas are represented by patterns of activity generated in groups of neurons. In this sense, "neural ensembles" formed by specific groups of neurons possess recurrent connections, and have the ability to maintain newly acquired patterns of neural activity through

reverberation. This reverberator activity leads to changes in the efficacy of the working synapses, a phenomenon that is nowadays known as "synaptic plasticity" [16]. This phenomenon is thought to allow associations to be encoded as the synapse between cell A (pre-synaptic) and cell B (post-synaptic) is strengthened by the co-occurrence of: a) an active synapse from cell A onto cell B, and b) a strong depolarization that triggers action potentials in cell B [17, 18]. Moreover, associative plasticity can be implemented if a strong presynaptic input depolarizes the postsynaptic neuron at the same time that another presynaptic input weakly stimulates the neuron. In this circumstances, the ultimate result would be a weak input strengthened by its temporal relationship with the strong input [18].

The synaptic plasticity phenomenon would explain the ability of cell assemblies to reproduce patterns of neural activity long after they were acquired through experience [16]. Therefore, experience-dependent internal representations (memories) could be explained as activity-dependent synaptic plasticity, induced at appropriate synapses during memory formation, which would be necessary and sufficient for the information storage [12]. A process of stabilization or consolidation would lead to a "structural trace," a memory trace that is maintained in some form of a dormant state. Thus, neural ensembles formed in this way will subsequently serve as the elements responsible for reproducing the patterns of neural activity acquired during experience, as these correspond to the neural representation of the stimulus [2, 16]. What persists throughout the lifetime of a memory is the capacity to reactivate, or reconstruct, the original, or a similar, pattern representation by the process of retrieval whenever a subset of the original information, or related information, is available [2, 12]. Currently, it is well accepted that activity-dependent synaptic plasticity is a principal mechanism for cell-assembly formation, and consequently for memory consolidation. Consequently, synaptic plasticity determines the future dynamics of the neural network associated with memory retrieval and behavioral performance [16].

Molecular Mechanisms in Synaptic Plasticity

Long-Term Potentiation

Experimental support for the Hebbian theory came about 20 years later with the finding of long-term electrophysiological changes in the efficacy of synapses as a consequence of high-frequency stimulation. This phenomenon was later defined as long-term potentiation (LTP), and has been proved to occur as a result of the activity history of a particular pathway and to be required for memory formation [16, 17].

The main cellular mechanisms that trigger LTP have been assessed in invertebrates as well as in vertebrates, including mammals; wherein the hippocampus plays a critical role in the formation of long-term memories. The main excitatory neurotransmitter involved in this phenomenon is glutamate. Synaptic release of glutamate triggers an initial activation of AMPA, metabotropic and particularly NMDA glutamate receptors. These activations lead to Ca2+ influx through NMDA receptors and the consequent increase of the its intracellular concentration. This early event is followed by an enhancement of Ca2+/calmodulin-dependent kinase II (CaMKII) activity, together with the downstream stimulation of post-synaptic adenylyl cyclase and cAMP production. These second messengers mediate the transient reinforcement of synaptic connections; which includes enhancement of neurotransmitter release by pre-synaptic terminals, through retrograde messengers, and post-synaptic modifications. These last comprise an increased number of AMPARs in the plasma membrane, the phosphorylation of specific subunits, and the consequent modification of the biophysical properties of this channel. All together, these events are

responsible for the modified electrophysiological efficacy of synapses [19, 20].

Kinases and Immediate Early Genes

Kinases have been implicated in the consolidation of long-term memories, as they are critical mediators at the early events of synaptic strengthening, maintenance and/or stabilization. Models of plasticity consider protein kinases in two contexts: as a storage device through modification of the properties of synaptic receptors, and as a switch that triggers other plasticity's mechanisms by activation of specific genomic programs [2, 12].

The transient elevation of intracellular Ca2+ activates numerous signaling proteins including CaMKII, protein kinase C (PKC), and small GTPase proteins such as Ras and Rho [12, 16]. Moreover, the local increase in cAMP leads to the activation of the cAMP-dependent protein kinase A (PKA); this takes place by the dissociation of the catalytic subunits of this enzyme from their regulatory subunits [19]. Together, all of these proteins actively regulate long-term plasticity through actin polymerization and depolymerization, protein trafficking, exocytosis and endocytosis of glutamate receptors, and phosphorylation of different substrates in the synaptic terminals (such as channels and proteins involved in exocytosis), leading to enhanced transmitter availability and release [16, 19].

The genomic response generally occurs within minutes after neuronal activation, and is mediated via kinase-dependent activation of constitutively expressed transcriptional regulators [2]. These events are of great importance, considering that use-dependent modulation of gene expression might confer long-term plastic changes with immunity to the brief life span imposed on single copies of protein molecules by molecular turnover [10, 12]. Modulation of cAMP-response element (CRE)-regulated gene expression by CRE-binding protein (CREB) appears to be a universal and fundamental element in this process. Genetic and pharmacologic manipulations of CREB validate how its inducible and transient expression is required for LTP and memory consolidation [10, 19, 21]. Moreover, the catalytic subunit of PKA is known to recruit p42-MAPK, and then they move to the nucleus where they phosphorylate nuclear targets including other kinases, which later on will later on phosphorylate transcription factors and activate gene expression [19].

CREB modulates the expression of CRE-regulated genes, including a number of immediate–early genes, such as transcription factors that, in turn, regulate the expression of late response genes. Other transcription factors, such as serum response factor, c-fos, early growth response gene-1EGR-1, or NF-jb, are also likely to contribute to the transcriptional regulation that accompanies long-lasting forms of synaptic plasticity [19]. Processes of neuronal protein synthesis that correlate with, and are required for, consolidation are now known to be multiphasic, involving the concerted recruitment of synaptic and cell-wide mechanisms [10].

Interestingly, the new approaches regarding epigenetic modifications point out that critical chromatin changes occur during the formation of long-term memory and that these changes are required for the stable maintenance of memories. Histone modification, chromatin remodeling, and the activity of retrotransposons may have long-term consequences in the transcriptional regulation of specific loci involved for long-term synaptic changes [22, 23].

Synaptic Modifications

The ultimate speculation is, how do newly synthesized gene products lead to the persistence of memories? In addition to electrophysiological modifications and transcriptional regulation, synaptic plasticity also involves protein synthesis from *de novo* and preexisting mRNAs, which are trafficked to the dendrites. These new products permit rapid and localized changes at specific

synapses, which appear to culminate in the remodeling of existing synapses or the emergence of new ones [2, 12]. This persistent type of synaptic plasticity may be possible under particular behavioral and environmental conditions. Structural synaptic changes, observed after robust behavioral experience, may be related to long-term memory, but also possibly to functional improvement of the neural network [16].

Following transcriptional activation, mRNAs and proteins have to be delivered specifically to the synapses whose activation originally triggered the wave of gene expression. The *synaptic capture hypothesis*, sometimes referred to as synaptic tagging, proposes that the gene expression products are delivered throughout the cell but are only functionally incorporated in those synapses that have been tagged by previous synaptic activity [24]. The molecular details underlying these processes are still under debate, but they include the local and transient activity of PKA, BDNF and protein synthesis. Moreover, the complex control of translation at dendrites involves mRNA transport and docking, cytoplasmic polyadenylation, and the phosphorylation of different translation factors [10, 19]. Overall, synaptic plasticity involves coordination between the activated synapse and the nucleus to optimize the metabolic resources of the neuron and the specificity of the long-term change. Furthermore, pre-synaptic terminals show two types of alterations: focal changes for membrane specialization of the synapse that mediate transmitter release, and a widespread effect involving modulation of the total number of presynaptic varicosities [19].

In addition to the already described changes in synapse (trafficking of new receptor molecules into the synaptic membrane, and altered association of receptors with cellular cytoskeleton and signal transduction cascades), there is evidence that long-term synaptic plasticity and long-term memory are correlated with morphological changes in synapses [10]. Morphological changes at existing synapses, have been observed after LTP and after learning. They include changes in synapse curvature, size of the active zone and spine volume, increases in synaptic length, generation and enlargement of dendritic spines, and appearance of perforations of the synapses and multiple-synapse boutons [2, 19].

Although structural rearrangements and remodeling of neural networks could be a prime candidate mechanism underlying the persistence of memories, a long-standing issue is whether the cellular mechanisms of memory lead to the growth of new synapses, or merely to morphological remodeling of existing ones [2].

Emotion and Memories/Fear Memories

Emotion interacts with cognitive processes such as perception, attention and memory. Emotion–memory interaction occurs at different stages of information processing, from the initial encoding and consolidation of memory traces to their long-term retrieval [25]. Emotions have been hypothesized to be a biological strategy for rapidly integrating previously recorded data (weighted for significance), thus assigning a motivational value to the stimulus and orchestrating an appropriate behavioral response [26]. From this evolutionary perspective, the memory imprinting of experiences which are related to strong emotions, either positive or negative seems useful [27]. The affective space is parsed according to two orthogonal dimensions: arousal (emotion that varies from calm to excitement) and valence (emotion that varies from unpleasant—negative to pleasant—positive, with neutral as an intermediate value) [28]. An emotionally-arousing experience is more likely to be remembered than a neutral one and to be faithfully transferred to long-term stores (consolidation). Furthermore, these stored emotional experiences show increased resistance to future perturbations [27].

Whenever emotion and memory are discussed together, it is usually referred to emotion somehow influencing—either enhancing or inhibiting—memory. Memory and emotion are two distinguishable phenomena; however, emotion can itself be a memory [29]. The term fear refers to a subjective feeling state and to the behavioral and physiological responses that occur in response to threatening environmental situations.

The fear system can be viewed as a set of processing circuits that detect and respond to danger, rather than a mechanism by which subjective states are experienced. Considering this approach, fear is operationalized, and is thus experimentally tractable [18, 30].

Accumulating evidence demonstrates that the processing of fear memories involves a spatially distributed network and a multitude of parallel circuits. These are proposed to modulate the efficacy of existing pathways whenever the stimuli or the context have an emotional tag. In this scenario, the same pathways are used to encode and store neutral and emotional memories; however, when there is an emotional context or when the stimulus itself has valence, they are modulated in order to act more efficiently [27]. Regions involved in emotional valence processing are comprised of many different cell-types with heterogeneous functional roles. Indeed, many regions such as the amygdala, ventral tegmental area and hypothalamus have been implicated in both, positive and negative valence processing [26]. The amygdala is part of the brain's limbic system, which regulates emotions. It is one of the most primitive parts of the brain and plays a critical role in our capacity to avoid threats and survive by its modulatory activity over hippocampal function, and thereby over the consolidation process [5, 25, 28].

Although molecular changes are known to occur in other areas of the amygdala, the molecular contributions to plastic changes with regard to fear learning are understood in most detail in the basolateral subdivision of the amygdala (BLA). Studies have focused on the BLA because molecular changes in this area have been shown to make essential contributions to the formation, storage and expression of the emotional experiences' memories [18]. The hypothesis that the amygdala modulates hippocampal memory storage is rooted in several lines of research, at anatomical, electrophysiological, molecular and functional levels. BLA projects substantially to the entorhinal cortex and the hippocampal formation, including the CA1 and CA3 fields, and the parasubiculum [14]. Furthermore, evidence from several studies indicates that amygdalae activity influences the induction of hippocampal LTP. BLA has been proved to either attenuate this phenomenon (by disruption of its activity) or promote it (after BLA stimulation).However, it should be noted that these effects are not necessarily indicative of a direct, monosynaptic BLA-hippocampus projection [14]. Moreover, synaptic plasticity has been shown to occur locally in the BLA during the consolidation of fear memories, with recruitment of the same molecular mediators as in hippocampus. These include overexpression and phosphorylation of glutamate receptors, as well as transcription and protein translation through CaMKII, PKA, MAPK, PKC, and phosporylated CREB pathways. Structural modifications at synapses have also been reported [18].

Interestingly, it has been proposed that the amygdala has a bi-phasic effect on hippocampal plasticity: a fast excitatory phase that may serve as a marker for emotional events (emotional tagging), and a slower inhibitory phase that may be beneficial in reducing the masking effects of subsequent, less-significant, events during the initial consolidation stage [14]. The cross-talk between the hippocampus and BLA plays an important role in regulating the strength of memory for emotionally based experiences. This dynamic connection defines under what circumstances emotional memories endure, whereas in others, emotion interferes with consolidation [14].

Inhibitory Avoidance Considering experimental approaches, one-trial avoidance has been widely used for the study of fear-memory consolidation in laboratory animals. This paradigm corresponds with many important examples of learning in humans, e.g., not to stick your fingertips into electric plug sockets. One-trial step-through inhibitory (or passive) avoidance in rodents has long been a selected model for biochemical and pharmacological studies of memory [20]. The term "inhibitory" is usually preferred to "passive" because this behavioral test involves the specific repression of the natural tendency of rats to avoid lightened places, without affecting the performance of exploratory behavior [31]. The animals learn that stepping through a door

from a lightened compartment towards a dark one is followed by a foot-shock. Thus, on a subsequent exposure to the task, they will stay much longer on the safe side of the door before eventually stepping through again; even if it means staying in the non-preferred lightened compartment. Usually, one trial is enough to establish a long-lasting memory of these tasks [20, 32]. The most remarkable characteristic of this test is the brief nature of the training procedure: a single fast association is made between a movement and an aversive stimulus. The association takes place in seconds, provides LTP induction, and makes it possible to precisely determine the onset of memory consolidation [20]. At the time of training and for a short period afterward, the amygdala contributes to memory processing by bringing in emotional information and modulating the chain of biochemical events that take place in the hippocampus. Two waves of concatenated events have been identified in this chain, one right after training and the other one 3–6 h later. There are both related to cAMP-sensitive gene transcription and to changes in glycoprotein synthesis and cell adhesion. It is likely that the second wave is triggered by the reflex activation of noradrenergic and dopaminergic afferents to the hippocampus, and controlled by serotoninergic fibers [31].

With regard to learning procedures with multiple-trial tasks, each successive trial involves not only short-term and long-term memory consolidation but also retrieval, extinction, relearning and reconsolidation. Pharmacological effects or biochemical measures observed within this task could be linked to any of those memory stages. There have been many successful inferences from inhibitory avoidance to other forms of memory, especially for fear-motivated learning and spatial tasks [20].

Dopamine Modulation

An extensive body of research has focused on how other systems, modulatory systems, interact with the basic fear network. Indeed, once the amygdala recognizes a dangerous situation, multiple parallel and redundant circuits are activated. These pathways might mediate slightly different aspects of fear, allowing fine-tuning of the ultimate behavioral response under a variety of external and internal conditions [30]. Thus, this should allow the fear system to be more plastic and to respond in a more appropriate way to variable external demands.

Dopamine (DA) is one of the most recognized neurotransmitters involved in the processing of emotional experiences [30]. The varied roles for DA in emotional-associated learning are not surprising, given the distribution of its receptors in the central nervous system. Its presence throughout the regions for aversive and reward memory sets a modulatory regional activity pattern that will have different outcomes depending on the behavioral experience. DA neurons in the midbrain have long been thought to serve a central role in reward prediction.The initial studies regarding a functional role for DA in learning described mechanisms through which DA increases stimulus–response associations, whenever the responses lead to a rewarding experience [33]. However, behavioral theories for the role of dopamine in aversive learning remain poorly defined. There are multiple mechanisms through which dopamine may alter the establishment, maintenance, expression and extinction of fear [34].

The mesolimbic dopaminergic system consists on the ventral tegmental area (VTA) and the regions it provides innervation to, including the nucleus accumbens, amygdala, hippocampus and prefrontal cortex. The VTA is a heterogeneous structure; it comprises 65% of dopamine neurons, 30% of inhibitory GABAergic neurons and about 5% of glutamate neurons. Neuron activation by both, rewarding and aversive stimuli, may reflect the salience of the stimulus. More importantly, DA neurons inhibited and excited by aversive stimuli are anatomically distinct and project to different target regions [26]. DA neurons in the dorsal VTA are excited by reward or reward-associated stimuli, whereas in the ventral VTA show high electrical activity in response to foot-shock [30, 34]. In addition, afferent projections to the VTA may also target distinct neuron subpopulations within this area

of [26]. Moreover, the interactions of the nucleus accumbens with the hippocampus and the amygdala are critical for allowing the generation of appropriate behaviors in fear and reward experiences. Concurrent activation of DA signals in the nucleus accumbens and basolateral amygdala contributes to long-term memory formation. These regions are supposed to integrate DA signaling to generate appropriate behavioral responses to new contingencies. Moreover, the role of hippocampal DA receptors on fear comes from studies of acquisition and long-term consolidation mechanism [34]. In this sense, DA in the nucleus accumbens would act to optimize or adapt emotional responses based on the varying significance of a situation or a cue [30].

Along with different regional activation patterns, DA function in learning is also determined by the different intra-cellular signaling cascades that are triggered by receptor binding of DA. The same receptor subtype can have very different molecular effects, which may result in different behavioral endpoints. These effects may be excitatory or inhibitory, and may occur through different second messengers. DA receptors can be divided into two main subfamilies: D1-like receptors (D1 and D5), that activate the stimulatory G proteins Gαs and Gαolf, and D2-like receptors (D2, D3, and D4) which activate the inhibitory G proteins Gαi and Gαo [18, 34]. The classical view of D1-like receptor activity has focused on intracellular signaling through adenylate cyclase and cAMP activity; in addition, they can operate through activation of (PLC) and IP3 production, particularly in the amygdala [18]. In the same way, D2-like receptors also activate signaling pathways distinct from Gαi/o-mediated inhibition of adenylate cyclase [34].

Monoaminergic neurotransmitters, released in emotional situations, regulate glutamatergic transmission and Hebbian plasticity. This modulatory activity has brought into light a new concept termed *heterosynaptic* or *neoHebbian* plasticity (as an alternative to the classical homosynaptic or Hebbian plasticity). This proposal maintains that stable synaptic modification requires a third signal [17, 18]. DA receptors may modulate Hebbian processes directly by reducing feed-forward inhibition or in a parallel fashion with Hebbian mechanisms to implement BLA plasticity and fear learning through their respective signaling pathways [18]. The proposal that DA in the amygdala is involved in negative emotions is supported on the basis that fear-arousing environmental stimuli can activate DA neurons and enhance its neurotransmission in the amygdala. Anatomical studies have shown that D1 and D2 receptors are highly expressed in the amygdala and are proposed to modulate consolidation of fear conditioning and extinction [30]. This idea is supported by findings that demonstrate that LTP in the amygdala depends on the coordinated activation of D1 receptors with TrkB receptors. Moreover, the PLC-dependent pathway may be critical for D1 receptor-mediated changes, including the persistence of late LTP. Thereby, D1 receptor stimulation may support synaptic plasticity necessary to consolidate associations in fear memories [30, 34]. The role of amygdalae D2 receptors in fear conditioning is less characterized. However, it is well accepted that D1 and D2 receptor activation in the BLA is necessary for the initial acquisition of fear [34].

The biochemical cascades in a given set of synapses in the hippocampus may change depending on direct or indirect influences of the various neuromodulators and hormones on this structure. The dopaminergic pathways to D1 receptors in the hippocampus and neocortex are well known to mediate alertness, emotion and anxiety levels. D1 receptors, through the already described second messengers, can regulate the PKA-dependent CREB phosphorylation processes that underlie memory consolidation [20]. Moreover, there is one kind of LTP whose late protein-dependent phase can be modulated by D1 receptors, and it appears to occur physiologically around 3 h after training. This leads to the idea that there must be hippocampal pathways that inform the dopaminergic nucleus that a learning situation took place 3 h before. The message back from these areas to the hippocampus may instruct it to produce more cAMP and so activate the PKA/CREB-P pathway, induce protein synthesis and save the learned information [31]. From a biologic perspective, in changing environments,

it would be of vital relevance to form long-lasting memories of novel events after a single experience. Hippocampal DA has been proved to participate in consolidating novel associations after only one trial in rats. Moreover, the modulatory effect of monoamines on plasticity underlying memory formation has been shown in a broad variety of model systems [18].

Finally, it is important to outstand that a large body of evidence demonstrates that monoamine releasers might facilitate memory at low doses, but impair memory at high doses. It is known that low to moderate levels of arousal, or mild anxiety, facilitate learning and retrieval, whereas too high levels of arousal or anxiety have an impairing effect [31]. Just as DA can alter learning through a variety of actions on different behavioral circuits, it also can alter learning through a variety of actions on different molecular processes. The existence of multiple signaling pathways for one receptor creates the possibility for another way in which efficacy can differ among agonists, often referred to as functional selectivity. A ligand may be a full agonist at one signaling pathway and a partial agonist or even an antagonist at another signaling pathway regulated by the same receptor [34]. It is important to keep in mind all of these modulatory possibilities when thinking about DA and fear memories. They illustrate the complexities that should be taken into account when evaluating the function of DA in learning, concerning not just behavioral processes, but also cellular and intra-cellular mechanisms [34]. DA, by directly affecting the biochemical processes of memory, introduces information coming from their own circuits, adding emotional or affective components to memories [31]. In spite of some mixed effects, it is clear that DA plays a modulatory role in aspects of fear acquisition [34].

Amphetamine and Memory

Psychostimulants are classified as indirectly acting sympathomimetic amines. Amphetamine (Amph) is a drug of abuse, worldwide consumed, with stimulant properties across the nervous system. The classical recognized effects of this drug are increased heart rate and blood pressure, together with behavioral alterations resembling wakefulness, better achievement in executive tasks and euphoria. Amph promotes mainly noradrenergic and dopaminergic neurotransmission and induces long-term changes in multiple neuronal circuits, modifying their future responses to pharmacologic or non-pharmacological challenges [35, 36]. The altered neuronal connectivity observed by Amph is shown not only in brain areas involved in reward processing, but also in those related to learning and memory processes. As described in the previous section, DA is involved in cognitive processes, and provides connectivity between the different brain areas associated with the consolidation of long-term memories. Amph, by its mimetic activity over DA neurotransmission, elicits differential responses in different cognitive processes, although there is no conclusive experience with regarding to the relationship between Amph and memory consolidation (Table 14.1). On one hand, some studies have reported that medium doses of Amph impaired memory acquisition, while at lower and higher doses it enhanced the learning of a passive avoidance task [38, 39]. On the other hand, an inverted U shaped response for the effects of Amph on memory retention has been described in DBA/2 mice and Wistar rats (Fig. 14.1) [32, 42]. In the same way, when working under low foot-shock training conditions, Haycock et al. demonstrated that a single drug administration enhances retention in a passive avoidance test [43]; meanwhile, Sahgal and Wright [49] reported that the dose–response curve for drug-enhancement of memory processes showed an inverted-U form. However, no differences in memory consolidation in rats pre-treated with Amph have been reported [50]. For Pavlovian conditioning, post-training Amph enhances the occurrence of memory consolidation. In this particular test, it is proposed that the stimulant-induced enhancement of memory consolidation may contribute to Pavlovian-like influences acting over drug addiction [44–46]. All of these events are further supported by different studies which report that post-training

Table 14.1 Amph-induced alteration over memory stages

Species (*strains*)	Test	Drugs (doses)	Memory stage			Reference
			Acquisition	Consolidation	Retrieval	
Rats *(Wistar)*	LI	*d*-AMP (1.5 mg/kg i.p.)	=			[86]
Rats *(Sprague–Dawley)*	PA	*d*-AMP	–/+			[37, 38]
Human	APST	*d*-AMP (20 mg v.o.)	=			[48]
Human	PAL	d-AMP (0.2 mg/kg v.o.)	+			[49]
Mice *(DBA/2)*	PA	*d*-AMP (2–3 mg/kg i.p.)		–		[39]
Mice *(ARS HA/ICR (Sprague–Dawley)*	PA	*d*-AMP (0.03, 0.1, 0.3, 1.0 and 3.0 mg/kg i.p.)		+		[40]
Rats *(Sprague–Dawley)*	PA/ NOR/PCA	*d*-AMP		+		[43-45, 87, 88]
Rats *(Long–Evans /Wistar)*	FC/ PA	*d*-AMP (i.p.)		=		[41, 42]
Human	FR	d-AMP (10 mg v.o.)		+		[47]

(continued)

Table 14.1 (continued)

Mice *(DUB-ICR and Swiss Webster)*	PA/ CDS	*d*-AMP (1–2 mg/kg)			+	[50, 89]
Rats *(Wistar)*	LI	*d*-AMP (1.5 mg/kg i.p.)			–	[86]
Rats *(Sprague–Dawley/ Wistar)*	MT/ PA/ AA	*d*-AMP (s.c.)			+	[46, 51]
Human	RS/ APST/PAL	*d*-AMP			=	[48, 49, 52]
Mice *(Swiss Webster)*	PA	Meth (10–20 mg/kg i.p.)		–		[90]
Rats *(Wistar)*	PA	MDMA (10 mg/kg i.p.)	–			[91]
Mice *(C57BL/6, 6D2F1, C57)*	PA	DA Agonist (SKF 38393 and LY 171555)		–		[92]
Mice *(C57BL/6, 6D2F1, C57)*	PA	DA Antagonist (SCH 23390 and (–)sulpiride)		+		

The psychostimulant administration was observed to either improve (+), interfere (−) or have no effect (=) in the performance of rodents and humans in several learning trials: *PA* passive avoidance, *AA* active avoidance, *PCA* Pavlovian conditioned approach, *FC* fear conditioning, *RS* retrieval of emotional and unemotional stimuli, *APST* affective picture system test, *PAL* paired-associate learning, *LI* latent inhibition, *FR* free recall, *CDS* conditioned drink suppression, *MT* maze task

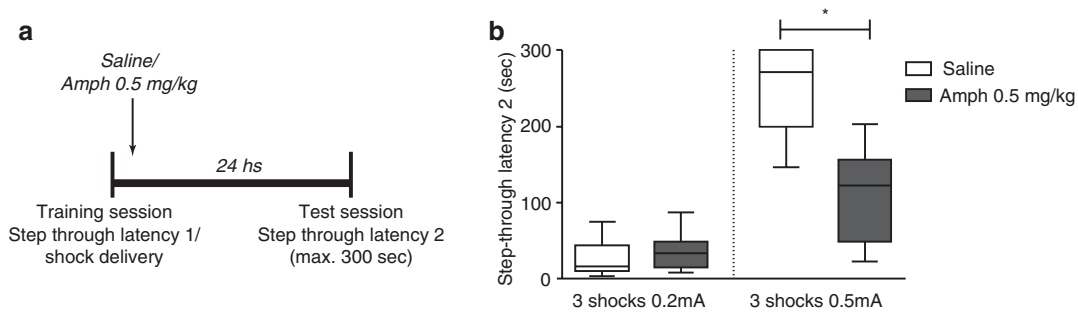


Fig. 14.1 Interfering effect of Amph over long-term memory consolidation. (**a**) Schematic representation of experimental protocol performed to evaluate Amph effect over consolidation in the one-trial step-through inhibitory avoidance test. (**b**) Amph effects on consolidation of a fear memory were observed as interference when administered after training with a medium shock-intensity protocol (0.5 mA). * different from saline injected-animals, $p < 0.05$

administration of the selective D1 or D2 agonists impairs retention of an inhibitory avoidance response in mice, while the selective D1 or D2 antagonists improve retention [54]. Finally, a positive influence of Amph on human-memory consolidation has been reported, with a significant increase of word recall after 24 hs [51]. Clinical reports demonstrated that Amph shows a facilitative trend at all stages of paired--associate learning in humans [40, 41].

From what it is reported, Amph appears to have a facilitatory role on retrieval stage in various animal models of memory [52, 54, 55]. In passive avoidance test, Kovacs et al. noted an improvement in performance in rats which had been treated 1 h previously with Amph [54]. Similar results were reported by Stone et al. in mice. However, these results have not been reproduced in humans, given that no significant differences were found upon administration of Amph in various memory tests [40, 41, 56].

The Brain Renin–Angiotensin System

The classical effects regarded for the Renin–Angiotensin System (RAS) were related to its endocrine role in electrolytic homeostasis and control of blood pressure. Angiotensin II (AngII) control implies a rapid increase in vascular resistance, and long-term effects acting on vasculature, heart, kidney, sympathetic output, and the central nervous system (CNS), by promoting vasopressin and aldosterone release and regulating thirst and water intake [60–63].

Among the receptors that have been identified to be activated by AngII, the AT_1 receptor (AT_1-R) is the one mediating most of the peptide's physiological and pathological functions. This surface receptor belongs to the G-protein-coupled receptor family, and has a conformation of seven transmembrane domains [63–65]. AT_1-R activates multiple intracellular signaling pathways; mainly, it promotes IP3 formation and Ca^{2+} release from intracellular compartments, adenylate cyclase inhibition, modulation of voltage-dependent Ca^{2+} channels and activation of PLC. Secondary pathways involving MAPK, ERK or JNK activation have also been described for its participation in trophy events. Independent G-protein activation pathways involve later desensitization and even internalization by endocytosis mediated by β-arrestins [63].

Over the years, it has come to be appreciated that a local autocrine or paracrine RAS exist in a number of tissues, which subsequently implies new roles for this system [66]. Moreover, tissues and systemic activities show significant differences. Even though circulating AngII levels may not be intensively high, AT_1-R expression in different organs can be abundant enough to promote intracellular signaling. Furthermore,

locally produced AngII concentration may be higher than plasma levels, and elicit a response in tissues with relative low AT_1-R expression.

Numerous pieces of evidence support that AngII acts as a neuromodulator in different brain pathways. The activation of the AT_1-R subtype by AngII elicits neuronal depolarization through facilitatory activity over different channels. The AT_1-R facilitator effects would be possible through the inhibition of potassium channel or opening of a non-selective sodium–calcium channel. The resulting depolarization triggered by the neuropeptide over different signaling pathways would reflect a fine regulation in overall cellular activation [67–69].

Across the CNS, AngII plays an important role in central dopaminergic neurotransmission modulation, and there is evidence that a cross-regulation exists between these two systems. It has been demonstrated that AT_1-R modifies tyrosine–hydroxylase activity and hence alters monoamine production. DA release is elevated in a concentration-dependent manner when AngII is applied either to striatal slices or locally in the rat striatum. Furthermore, microdialisys of AngII in the same brain structure increases the level of DA and its metabolites (DOPAC and HVA). Similar effects have been obtained for DA concentration in NAc after AngII administration [70–72]. AT_1-R blockers did not only reverse these changes in the neurotransmitter levels but it also decreased its concentration without AngII stimulus, suggesting a regulatory role on basal DA release [72, 73]. All together, these results point towards the possible action of AngII pre-synaptically modulating the synthesis and release of DA through activation of AT_1-R, as they are located in the soma and terminals of dopaminergic neurons [74]. Moreover, recently it has been shown that an alteration in the dopaminergic system, such as decreased levels of DA, can induce an increase in local RAS components, as an attempt to compensate for the deficit in dopaminergic activity [74]. Therefore, AT_1-R expression is closely linked to DA levels, given that decreased levels of DA promotes an increase in AT_1-R expression, which can be reversed when the DA activity is reestablished. Similarly, the knockout mice for D_1 and D_2 receptors, have increased levels of AT_1-R, while transgenic mice overexpressing D_2 receptors show reduced levels [75, 76]. Moreover, aged rats show decreased levels of DA receptors and increased expression of AT_1-R simultaneously, when compared to young animals [77]. In the same way, acute or chronic manipulation of brain RAS with AT_1-R antagonists decreased D_2 and increased D_1 receptor expression without affecting striatal DA release or motor behavior [78]. Recent evidence highlights functional and physical interactions between AT_1-R and β- adrenergic, D_1 and D_2 receptors [79–81]. Blockade of the D_2/AT_1-R heterodimer by an AT_1-R antagonist, leads to enhanced dopaminergic transmission and has direct impact on the basal ganglia system involved in motor control. Thus, AngII levels would control striatal dopaminergic neurotransmission, and would serve to immediately regulate DA function [79]. Moreover, AngII has a modulatory neurochemical effect over DA-mediated behavior, such as the control of movement and reward processing, which might be susceptible to be controlled by pharmacological manipulation of the RAS [70, 72].

Renin–Angiotensin System and Memory

AngII is actively involved in learning and memory processes. There is a broad spectrum of effects described in this peptide over cognitive tasks. This is probably the result of different methodological procedures, with regard to time and route of administration and type of memory evaluated. It has been observed that local administration of AngII on the hippocampus generates disruptive effects on the acquisition and consolidation of memories [82, 83]. Moreover, it has been found that LTP induction is inhibited by the injection of AngII in CA1 region and BLA [84, 85].

On the other hand, in contrast to the previous mentioned results, numerous authors have reported an improvement in the three stages of memory formation after intra-cerebroventricular administration of AngII (Table 14.2) [86–89].

Table 14.2 Ang II-induced alteration over memory stages

Species *(strains)*	Test	Drugs (doses)	Memory stage			Reference
			Acquisition	Consolidation	Retrieval	
Rats *(Sprague-Dawley)*	PA	Ang II (5–50 ngintra-DG)	–			[75]
Rats *(Wistar)*	PA	Ang II (1–3 nmoli.c.v.)	=			[82]
Rats *(Wistar)*	CARs/ PA	Ang II (1 nmoli.c.v.)	+			[79-81, 93]
Mice *(Laka)*	TL	Ang II (10–20 ngi.c.v.)		+		[94]
Rats *(Proton and Wistar)*	TL/NOR/CARs/PA	Ang II (i.c.v.)		+		80, 81, [94, 95]
Rats *(Wistar)*	PA	Ang II (intra-CA1 and i.c.v.)		–		[76, 82]
Rats *(Wistar)*	PA/ CARs	Ang II (1 nmoli.c.v.)			+	[79, 81, 82, 93]

(continued)

Table 14.2 (continued)

Rats *(Sprague-Dawley and Wistar)*	PA	Renin (i.c.v.)	–			[96, 97]
Rats *(Wistar)*	PA	Renin (0.01–0.1 U i.c.v.)			=	[97]
Mice *(Laka)*	AA	Captopril (5–10 mg/kg i.p.) Losartan (5–10 mg/kg i.p.)	+	+		[98]

The different RAS components administration was observed to either improve (+), interfere (−) or have no effect (=) in the performance of rodents in several learning trials: *PA* passive avoidance, *AA* active avoidance, *CARs* conditioned avoidance responses, *TL* transfer latency

AngII facilitates acquisition in active conditioning trials and retention in passive avoidance tests. This facilitator effect of AngII on memory appears to be mediated by central monoamine systems. In addition, it has been found that dopaminergic projection to the nucleus accumbens is involved in the improving effect of angiotensin peptides on recognition memory in rats [96].

Final Considerations

The functional relation between Amph and AngII activity has been studied over the last decade by this working group. Overall, it has been shown that Amph-induced long-term changes at the reward circuit involve AT_1-R activation with regard to responses of the reward circuit. We have observed that AT_1-R are involved in the behavioral and neurochemical adaptive responses induced by Amph exposure [5, 97]. Moreover, Amph exposure has been shown to induce long-term changes in AT_1-R density and in angiotensinogen mRNA in CPu, a rich DA area strongly related to drugs of abuse responses [98].

Recently, we have shown that AT_1-R play a functional role in Amph-induced alterations over neurocognitive processes. In our experimental model, acute Amph impaired memory retention in male rats in the one-trial inhibitory avoidance test when administered immediately post-training. This effect involved central AT_1-R activation, since central AT_1-R antagonist administration before the psychostimulant partially prevented drug-induced memory impairment [32].

Moreover, a previous experience of repeated Amph followed by 7 days of withdrawal modified the animals' performance in the inhibitory avoidance test, observed as a resistance to the acute Amph interference effect in the behavioral response. Moreover, these animals showed an altered neuronal activation pattern in BLA after the test session. Long-term Amph-induced alterations were shown in hippocampus synaptic transmission, measured as a lower threshold necessary to generate LTP. It is noteworthy that AT_1-R blockade prevented the behavioral, neurochemical, and electrophysiological alterations observed in the repeated Amph group, pointing out a functional role for AT_1-R in the psychostimulant-induced neuroadaptations [32].

Brain RAS is a neuromodulatory system of superior brain activities and by its AT_1-R can actively modulate Amph-induced alterations. Because AT_1-R blockers are currently and safely used in clinics for different pathologies, our results suggest that they would be prominent candidates for pharmacological treatment in pathologies related to altered DA and cognitive alterations.

References

1. Tulving E. Episodic memory: from mind to brain. Annu Rev Psychol. 2002;53:1–25.
2. Bruel-Jungerman E, Davis S, Laroche S. Brain plasticity mechanisms and memory: a party of four. Neuroscientiste. 2007;13(5):492–505.
3. Fietta P, Fietta P. The neurobiology of the human memory. Theor Biol Forum. 2011;104(1):69–87.
4. Kandel ER, Squire LR. Neuroscience: breaking down scientific barriers to the study of brain and mind. Science. 2000;290(5494):1113–20.
5. Glannon W. Psychopharmacology and memory. J Med Ethics. 2006;32(2):74–8.
6. Cowan N. What are the differences between long-term, short-term, and working memory? Prog Brain Res. 2008;169:323–38.
7. Setlow B. The nucleus accumbens and learning and memory. J Neurosci Res. 1997;49(5):515–21.
8. Moscovitch M, Rosenbaum RS, Gilboa A, Addis DR, Westmacott R, Grady C, et al. Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. J Anat. 2005;207(1):35–66.
9. Squire LR. Declarative and nondeclarative memory: multiple brain systems supporting learning and memory. J Cogn Neurosci. 1992;4(3):232–43.
10. Dudai Y. The neurobiology of consolidations, or, how stable is the engram? Annu Rev Psychol. 2004;55:51–86.
11. Abel T, Lattal KM. Molecular mechanisms of memory acquisition, consolidation and retrieval. Curr Opin Neurobiol. 2001;11(2):180–7.
12. Dudai Y. Molecular bases of long-term memories: a question of persistence. Curr Opin Neurobiol. 2002;12(2):211–6.
13. McGaugh JL. Memory — a century of consolidation. Science. 2000;287(5451):248–51.
14. Richter-Levin G, Akirav I. Amygdala–hippocampus dynamic interaction in relation to memory. Mol Neurobiol. 2000;22(1–3):11–20.
15. Arshavsky YI. "The seven sins" of the Hebbian synapse: can the hypothesis of synaptic plasticity explain long-term memory consolidation? Prog Neurobiol. 2006;80(3):99–113.
16. Carasatorre M, Ramirez-Amaya V. Network, cellular, and molecular mechanisms underlying long-term memory formation. Curr Top Behav Neurosci. 2013;15:73–115.
17. Lisman J, Grace AA, Duzel E. A neoHebbian framework for episodic memory; role of dopamine-dependent late LTP. Trends Neurosci. 2011;34(10):536–47.
18. Johansen JP, Cain CK, Ostroff LE, LeDoux JE. Molecular mechanisms of fear learning and memory. Cell. 2011;147(3):509–24.
19. Barco A, Bailey CH, Kandel ER. Common molecular mechanisms in explicit and implicit memory. J Neurochem. 2006;97(6):1520–33.
20. Izquierdo I, Bevilaqua LR, Rossato JI, Bonini JS, Medina JH, Cammarota M. Different molecular cascades in different sites of the brain control memory consolidation. Trends Neurosci. 2006;29(9):496–505.
21. Kida S, Josselyn SA, Pena de Ortiz S, Kogan JH, Chevere I, Masushige S, et al. CREB required for the stability of new and reactivated fear memories. Nat Neurosci. 2002;5(4):348–55.
22. Guan Z, Giustetto M, Lomvardas S, Kim JH, Miniaci MC, Schwartz JH, et al. Integration of long-term-memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. Cell. 2002;111(4):483–93.
23. Hsieh J, Gage FH. Chromatin remodeling in neural development and plasticity. Curr Opin Cell Biol. 2005;17(6):664–71.
24. Frey U, Morris RG. Synaptic tagging and long-term potentiation. Nature. 1997;385(6616):533–6.
25. Srinivasan RGaN. Cognitive neuroscience of emotional memory. Advances in Developmental Neuroscience and Imaging; 2009.
26. Nieh EH, Kim SY, Namburi P, Tye KM. Optogenetic dissection of neural circuits underlying emotional valence and motivated behaviors. Brain Res. 2013;1511:73–92.
27. Paz R, Pare D. Physiological basis for emotional modulation of memory circuits by the amygdala. Curr Opin Neurobiol. 2013;23(3):381–6.

28. LaBar KS, Cabeza R. Cognitive neuroscience of emotional memory. Nat Rev Neurosci. 2006;7(1):54–64.
29. LeDoux JE. Emotional memory: in search of systems and synapses. Ann N Y Acad Sci. 1993;702:149–57.
30. Pezze MA, Feldon J. Mesolimbic dopaminergic pathways in fear conditioning. Prog Neurobiol. 2004;74(5):301–20.
31. Izquierdo I, Medina JH. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol Learn Mem. 1997;68(3):285–316.
32. Marchese NA, Artur de laVillarmois E, Basmadjian OM, Perez MF, Baiardi G, Bregonzio C. Brain Angiotensin II AT receptors are involved in the acute and long-term amphetamine-induced neurocognitive alterations. Psychopharmacology (Berl). 2016;233:795–807.
33. Wise RA. Dopamine, learning and motivation. Nat Rev Neurosci. 2004;5(6):483–94.
34. Abraham AD, Neve KA, Lattal KM. Dopamine and extinction: a convergence of theory with fear and reward circuitry. Neurobiol Learn Mem. 2014;108:65–77.
35. Pierce RC, Kalivas PW. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Brain Res Rev. 1997;25(2):192–216.
36. Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl). 2000;151(2–3):99–120.
37. Weiner I, Lubow RE, Feldon J. Abolition of the expression but not the acquisition of latent inhibition by chronic amphetamine in rats. Psychopharmacology (Berl). 1984;83(2):194–9.
38. Seliger DL. Dose–response effects of d-amphetamine on passive avoidance learning in the rat. Psychopharmacologia. 1975;44(2):191–3.
39. Seliger DL. Passive avoidance learning in the rat as functions of d-amphetamine dosage and shock intensity. Psychopharmacology (Berl). 1977;54(3):241–2.
40. Weafer J, Gallo DA, de Wit H. Amphetamine fails to alter cued recollection of emotional images: study of encoding, retrieval, and state-dependency. PLoS One. 2014;9(2):e90423.
41. Hurst PM, Radlow R, Chubb NC, Bagley SK. Effects of D-amphetamine on acquisition, persistence, and recall. Am J Psychol. 1969;82(3):307–19.
42. Cabib S, Castellano C. Impairments produced by amphetamine and stress on memory storage are reduced following a chronic stressful experience. Psychopharmacology (Berl). 1997;129(2):161–7.
43. Haycock JW, Van Buskirk R, Gold PE. Effects on retention of posttraining amphetamine injections in mice: interaction with pretraining experience. Psychopharmacology (Berl). 1977;54(1):21–4.
44. Simon NW, Setlow B. Post-training amphetamine administration enhances memory consolidation in appetitive Pavlovian conditioning: Implications for drug addiction. Neurobiol Learn Mem. 2006;86(3):305–10.
45. Blaiss CA, Janak PH. Post-training, but not post-reactivation, administration of amphetamine and anisomycin modulates Pavlovian conditioned approach. Neurobiol Learn Mem. 2007;87(4):644–58.
46. Hall DA, Gulley JM. Disruptive effect of amphetamines on Pavlovian to instrumental transfer. Behav Brain Res. 2011;216(1):440–5.
47. Lee EH, Ma YL. Amphetamine enhances memory retention and facilitates norepinephrine release from the hippocampus in rats. Brain Res Bull. 1995;37(4):411–6.
48. Wiig KA, Whitlock JR, Epstein MH, Carpenter RL, Bear MF. The levo enantiomer of amphetamine increases memory consolidation and gene expression in the hippocampus without producing locomotor stimulation. Neurobiol Learn Mem. 2009;92(1):106–13.
49. Sahgal A, Wright C. A comparison of the effects of vasopressin and oxytocin with amphetamine and chlordiazepoxide on passive avoidance behaviour in rats. Psychopharmacology (Berl). 1983;80(1):88–92.
50. Lee HJ, Berger SY, Stiedl O, Spiess J, Kim JJ. Post-training injections of catecholaminergic drugs do not modulate fear conditioning in rats and mice. Neurosci Lett. 2001;303(2):123–6.
51. Soetens E, D'Hooge R, Hueting JE. Amphetamine enhances human-memory consolidation. Neurosci Lett. 1993;161(1):9–12.
52. Quartermain D, Judge ME, Jung H. Amphetamine enhances retrieval following diverse sources of forgetting. Physiol Behav. 1988;43(2):239–41.
53. Stone WSR, Rudd J, Gold PE. Amphetamine, epinephrine, and glucose enhancement of memory retrieval. Psychobiology. 1990;18(2):227–30.
54. Kovacs GL, de Wied D. Effects of amphetamine and haloperidol on avoidance behavior and exploratory activity. Eur J Pharmacol. 1978;53(1):103–7.
55. Sara SJ, Deweer B. Memory retrieval enhanced by amphetamine after a long retention interval. Behav Neural Biol. 1982;36(2):146–60.
56. Ballard ME, Gallo DA, de Wit H. Amphetamine increases errors during episodic memory retrieval. J Clin Psychopharmacol. 2014;34(1):85–92.
57. Murnane KS, Perrine SA, Finton BJ, Galloway MP, Howell LL, Fantegrossi WE. Effects of exposure to amphetamine derivatives on passive avoidance performance and the central levels of monoamines and their metabolites in mice: correlations between behavior and neurochemistry. Psychopharmacology (Berl). 2012;220(3):495–508.
58. Moyano S, Del Rio J, Frechilla D. Acute and chronic effects of MDMA on molecular mechanisms implicated in memory formation in rat hippocampus: surface expression of CaMKII and NMDA receptor subunits. Pharmacol Biochem Behav. 2005;82(1):190–9.

59. Cestari V, Castellano C, Cabib S, Puglisi-Allegra S. Strain-dependent effects of post-training dopamine receptor agonists and antagonists on memory storage in mice. Behav Neural Biol. 1992;58(1):58–63.
60. Braun-Menendez E, Fasciolo JC, Leloir LF, Munoz JM. The substance causing renal hypertension. J Physiol. 1940;98(3):283–98.
61. Antunes-Rodrigues J, de Castro M, Elias LL, Valenca MM, McCann SM. Neuroendocrine control of body fluid metabolism. Physiol Rev. 2004;84(1):169–208.
62. Saavedra JM. Brain and pituitary angiotensin. Endocr Rev. 1992;13(2):329–80.
63. Hunyady L, Catt KJ. Pleiotropic AT1 receptor signaling pathways mediating physiological and pathogenic actions of angiotensin II. Mol Endocrinol. 2006;20(5):953–70.
64. Sasaki K, Yamano Y, Bardhan S, Iwai N, Murray JJ, Hasegawa M, et al. Cloning and expression of a complementary DNA encoding a bovine adrenal angiotensin II type-1 receptor. Nature. 1991;351(6323):230–3.
65. Murphy TJ, Alexander RW, Griendling KK, Runge MS, Bernstein KE. Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. Nature. 1991;351(6323):233–6.
66. Lavoie JL, Sigmund CD. Minireview: overview of the renin–angiotensin system — an endocrine and paracrine system. Endocrinology. 2003;144(6):2179–83.
67. Allen AM, Moeller I, Jenkins TA, Zhuo J, Aldred GP, Chai SY, et al. Angiotensin receptors in the nervous system. Brain Res Bull. 1998;47(1):17–28.
68. Bai D, Renaud LP. ANG II AT1 receptors induce depolarization and inward current in rat median preoptic neurons in vitro. Am J Physiol. 1998;275(2 Pt 2):R632–9.
69. Yang CR, Phillips MI, Renaud LP. Angiotensin II receptor activation depolarizes rat supraoptic neurons in vitro. Am J Physiol. 1992;263(6 Pt 2):R1333–8.
70. Mendelsohn FA, Jenkins TA, Berkovic SF. Effects of angiotensin II on dopamine and serotonin turnover in the striatum of conscious rats. Brain Res. 1993;613(2):221–9.
71. Hoebel BG, Rada P, Mark GP, Hernandez L. The power of integrative peptides to reinforce behavior by releasing dopamine. Ann N Y Acad Sci. 1994;739:36–41.
72. Brown DC, Steward LJ, Ge J, Barnes NM. Ability of angiotensin II to modulate striatal dopamine release via the AT1 receptor in vitro and in vivo. Br J Pharmacol. 1996;118(2):414–20.
73. Dwoskin LP, Jewell AL, Cassis LA. DuP 753, a nonpeptide angiotensin II-1 receptor antagonist, alters dopaminergic function in rat striatum. Naunyn Schmiedebergs Arch Pharmacol. 1992;345(2):153–9.
74. Labandeira-Garcia JL, Garrido-Gil P, Rodriguez-Pallares J, Valenzuela R, Borrajo A, Rodriguez-Perez AI. Brain renin–angiotensin system and dopaminergic cell vulnerability. Front Neuroendocrinol. 2014;8:67.
75. Villar-Cheda B, Rodriguez-Pallares J, Valenzuela R, Munoz A, Guerra MJ, Baltatu OC, et al. Nigral and striatal regulation of angiotensin receptor expression by dopamine and angiotensin in rodents: implications for progression of Parkinson's disease. Eur J Neurosci. 2010;32(10):1695–706.
76. Labandeira-Garcia JL, Rodriguez-Pallares J, Villar-Cheda B, Rodriguez-Perez AI, Garrido-Gil P, Guerra MJ. Aging, Angiotensin system and dopaminergic degeneration in the substantia nigra. Aging Disease. 2011;2(3):257–74.
77. Villar-Cheda B, Dominguez-Meijide A, Valenzuela R, Granado N, Moratalla R, Labandeira-Garcia JL. Aging-related dysregulation of dopamine and angiotensin receptor interaction. Neurobiol Aging. 2014;35(7):1726–38.
78. Dominguez-Meijide A, Villar-Cheda B, Garrido-Gil P, Sierrra-Paredes G, Guerra MJ, Labandeira-Garcia JL. Effect of chronic treatment with angiotensin type 1 receptor antagonists on striatal dopamine levels in normal rats and in a rat model of Parkinson's disease treated with L-DOPA. Neuropharmacology. 2014;(76 Pt A):156–68.
79. Martinez-Pinilla E, Rodriguez-Perez AI, Navarro G, Aguinaga D, Moreno E, Lanciego JL, et al. Dopamine D2 and angiotensin II type 1 receptors form functional heteromers in rat striatum. Biochem Pharmacol. 2015;96(2):131–42.
80. Barki-Harrington L. Oligomerisation of angiotensin receptors: novel aspects in disease and drug therapy. J Renin Angiotensin Aldosterone Syst. 2004;5(2):49–52.
81. Khan F, Spicarova Z, Zelenin S, Holtback U, Scott L, Aperia A. Negative reciprocity between angiotensin II type 1 and dopamine D1 receptors in rat renal proximal tubule cells. Am J Physiol Renal Physiol. 2008;295(4):F1110–6.
82. Lee EH, Ma YL, Wayner MJ, Armstrong DL. Impaired retention by angiotensin II mediated by the AT1 receptor. Peptides. 1995;16(6):1069–71.
83. Kerr DS, Bevilaqua LR, Bonini JS, Rossato JI, Kohler CA, Medina JH, et al. Angiotensin II blocks memory consolidation through an AT2 receptor-dependent mechanism. Psychopharmacology (Berl). 2005;179(3):529–35.
84. Denny JB, Polan-Curtain J, Wayner MJ, Armstrong DL. Angiotensin II blocks hippocampal long-term potentiation. Brain Res. 1991;567(2):321–4.
85. von Bohlen und Halbach O, Albrecht D. Angiotensin II inhibits long-term potentiation within the lateral nucleus of the amygdala through AT1 receptors. Peptides. 1998;19(6):1031–6.
86. de Souza FA, Sanchis-Segura C, Fukada SY, de Bortoli VC, Zangrossi Jr H, de Oliveira AM. Intracerebroventricular effects of angiotensin II on a step-through passive avoidance task in rats. Neurobiol Learn Mem. 2004;81(1):100–3.
87. Braszko JJ, Kupryszewski G, Witczuk B, Wisniewski K. Angiotensin II-(3-8)-hexapeptide affects motor activity, performance of passive avoidance and a conditioned avoidance response in rats. Neuroscience. 1988;27(3):777–83.

88. Braszko JJ, Wisniewski K. Alpha 1 and alpha 2-adrenergic receptor blockade influences angiotensin II facilitation of avoidance behavior and stereotypy in rats. Psychoneuroendocrinology. 1990;15(4):239–52.
89. Kulakowska A, Karwowska W, Wisniewski K, Braszko JJ. Losartan influences behavioural effects of angiotensin II in rats. Pharmacol Res. 1996;34(3–4):109–15.
90. Braszko JJ. AT(2) but not AT(1) receptor antagonism abolishes angiotensin II increase of the acquisition of conditioned avoidance responses in rats. Behav Brain Res. 2002;131(1–2):79–86.
91. Raghavendra V, Chopra K, Kulkarni SK. Brain renin–angiotensin system (RAS) in stress-induced analgesia and impaired retention. Peptides. 1999;20(3):335–42.
92. Tchekalarova J, Kambourova T, Georgiev V. Interaction of angiotensin II and III with adenosine A(1) receptor-related drugs in passive avoidance conditioning in rats. Behav Brain Res. 2002;129(1–2):61–4.
93. DeNoble VJ, DeNoble KF, Spencer KR, Chiu AT, Wong PC, Timmermans PB. Non-peptide angiotensin II receptor antagonist and angiotensin-converting enzyme inhibitor: effect on a renin-induced deficit of a passive avoidance response in rats. Brain Res. 1991;561(2):230–5.
94. Koller M, Krause HP, Hoffmeister F, Ganten D. Endogenous brain angiotensin II disrupts passive avoidance behavior in rats. Neurosci Lett. 1979;14(1):71–5.
95. Raghavendra V, Chopra K, Kulkarni SK. Comparative studies on the memory-enhancing actions of captopril and losartan in mice using inhibitory shock avoidance paradigm. Neuropeptides. 2001;35(1):65–9.
96. Winnicka MM. Dopaminergic projection to the nucleus accumbens mediates the memory-enhancing effect of angiotensins in rats. Pharmacol Biochem Behav. 1999;62(4):625–30.
97. Paz MC, Marchese NA, Cancela LM, Bregonzio C. Angiotensin II AT(1) receptors are involved in neuronal activation induced by amphetamine in a two-injection protocol. Biomed Res Int. 2013;2013:534817.
98. Paz MC, Marchese NA, Stroppa MM, Gerez de Burgos NM, Imboden H, Baiardi G, et al. Involvement of the brain renin–angiotensin system (RAS) in the neuroadaptive responses induced by amphetamine in a two-injection protocol. Behav Brain Res. 2014;272:314–23.

The Extent of Neuroadaptive Responses to Psychostimulants: Focus on Brain Angiotensin System

15

Claudia Bregonzio, Natalia Andrea Marchese, Maria Constanza Paz, Emilce Artur de la Villarmois, Gustavo Baiardi, and Mariela Fernanda Pérez

Abstract

Amphetamine and cocaine are drugs of abuse worldwide consumed for their stimulant properties in the central nervous system. They mainly potentiate noradrenergic and dopaminergic neurotransmission and induce long-term changes in multiple neuronal circuits, modifying the future responses to pharmacological or non-pharmacological challenges. The altered neuronal connectivity induced by psychostimulants has long been studied in reward processing brain areas and in behavioral responses. Different neurotransmitter systems are involved in these responses, including the neuropeptide angiotensin II. Locally produced brain angiotensin II, acting through AT_1 receptors, plays an important role in the modulation of central dopaminergic neurotransmission. Dopamine-innervated areas such as caudate putamen, nucleus accumbens, substantia nigra, hypothalamus, and ventral pallidum express high AT_1 receptor density. Our recent studies show the role of angiotensin II AT_1 receptors in the development of neuroadaptative behavioral and neurochemical changes induced by amphetamine. Moreover, we found alterations in the components of the renin angiotensin system (RAS) and in the functionality of AT_1 receptors after amphetamine exposure. The evidence presented in this chapter highlight the RAS as a neuromodulatory system of superior brain activities, and further validate Angiotensin II involvement in amphetamine-induced alterations through AT_1 receptor activation. The AT_1 receptor blockers are currently and safely used in clinic for different pathologies, so they would

C. Bregonzio, PhD (✉) • N.A. Marchese • M.C. Paz
E.A. de la Villarmois • M.F. Pérez
Departamento de Farmacología, Facultad de Ciencias Químicas, Instituto de Farmacología Experimental Córdoba (IFEC-CONICET), Universidad Nacional de Córdoba (UNC), Haya de la Torre y Medina Allende s/n, Cordoba 5000, Argentina
e-mail: bregonzio@fcq.unc.edu.ar

G. Baiardi
Laboratorio de Neurofarmacología, Instituto de Investigaciones, Biológicas y Tecnológicas (IIBYT-CONICET), Universidad Nacional de Córdoba, Córdoba, Cordoba, Argentina

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_15

be prominent candidates for pharmacological treatment in pathologies related to altered dopamine neurotransmission, such as drug addiction, schizophrenia, or even depression.

Keywords
Angiotensin II • Neuroadaptation • Dopamine • AT_1 receptors • Psychostimulants • Amphetamine

Psychostimulant Pharmacology

D-amphetamine and cocaine are drugs known as psychostimulants. These drugs have a common pharmacological target as indirect catecholaminergic agonists. They produce their stimulant and psychotic effects by increasing synaptic concentrations of dopamine (DA) through inhibition of reuptake and potentiation of presynaptic release. The potency of the psychotogenic effects induced by psychostimulants is associated with the dopaminergic agonist action, although no consistent correlation has been found between symptoms and measures of DA neuronal activity [1–3]. However, the physiological actions induced by drug administration extend beyond the transient changes in synaptic concentrations of DA, since a temporal dissociation exists between behavioral response and plasma drug concentrations [1–3].

Neuroadaptative Changes Associated with Psychostimulants

Repeated administration of stimulants may produce a supersensitive condition in the central nervous system by upward regulation of postsynaptic DA receptors [4, 5]. This is a consequence of presynaptic DA depletion and lowered tonic basal DA levels after the increased dopaminergic synaptic activation induced by psychostimulants. Furthermore, sustained dopaminergic activation could develop autoreceptor subsensitivity associated with a reduced inhibition of presynaptic DA synthesis and release [6]. These events could lead to increased DA neurotransmission due to dysregulation of presynaptic and postsynaptic mechanisms.

At behavioral level, the supersensitivity induced by psychostimulants is a phenomenon termed *behavioral sensitization*, characterized by an enhanced response to psychostimulants that relies on neuroplastic changes. These changes are time-dependent and involve the motivational behavior circuitry of the brain [7, 8]. The behavioral sensitization involves changes associated with long-lasting hyperactivity of the mesolimbic dopaminergic pathway [8, 9]. Moreover, considerable evidence indicates that exposure to a drug of abuse does not need to be repeated to induce locomotor sensitization. In this regard, it has been described in mouse and rat studies that a single exposure to psychostimulants (cocaine or amphetamine) induces behavioral sensitization [10, 11]. The sensitization process presents two temporally distinct phases: induction and expression [8, 12]. The mesocorticolimbic dopaminergic projections play a key role in both described phases of amphetamine-induced behavioral sensitization. In this respect, it is known that sensitization can be induced by microinjection of amphetamine into the ventral tegmental area; meanwhile, its expression is associated with time-dependent adaptations in forebrain DA-innervated areas, such as the nucleus accumbens (NAc) and caudate putamen (CPu) [13].

In contrast with observations of enhanced DA neurotransmission within the NAc and CPu in response to the repeated administration of psychostimulants, the DA response in the medial prefrontal cortex (mPFC) has been reported to be decreased in animals sensitized to cocaine [14–16]. Moreover, the evidence suggests that the dorsal mPFC, which provides glutamatergic afferents specifically to NAc core, enhances the

expression of behavioral sensitization to cocaine by increasing glutamate transmission [17]. Oppositely, lesions in the prelimbic area of the mPFC were shown to affect the development of sensitization to the locomotor activating effects of cocaine [18]. Furthermore, a negative relationship has been described between DA levels in the ventral and dorsal mPFC and locomotor activity in response to the acute systemic administration of cocaine. Meanwhile, in the shell of the NAc a positive relationship between DA levels and locomotor response to cocaine has been described [19]. Based on the available evidence, it seems that DA tone in the mPFC is involved in the balance between hyper- or hypo-stimulation states.

Schizophrenia and Interactions with Psychostimulants

The neurobiological bases of schizophrenia and chronic stimulant drug effects suggest the potential for their interaction. Schizophrenic patients who abuse psychostimulant drugs are susceptible to incur several types of risk because they can present symptom exacerbations that otherwise might not have occurred. Beyond this, given the occurrence of behavioral sensitization and neurotoxic effects, chronic stimulant use could conceivably exacerbate the pathophysiology of the disease, resulting in an acceleration of disease progress and/or an increase in the severity of symptoms. In addition, the antipsychotic treatment response might be altered in an unfavorable direction. However, schizophrenic patients use smaller quantities of drugs of abuse than healthy drug abusers. Even though the dose–response parameters could be different for schizophrenic patients, the concern whether chronic stimulant abuse in healthy persons can induce a schizophrenia-like condition persisting without stimulant abuse is currently unsettled. In this regard, the evidence suggests that the onset of schizophrenia may be precipitated in pre-psychotic or latent schizophrenic patients, but not directly caused by psychostimulant abuse [3, 20].

Role of Angiotensin in Brain Excitability

Currently, it is well established that the brain possesses its own and distinct angiotensin system [21]. The pharmacological manipulation of this system may modulate a number of events coordinated by the central nervous system such as drinking behavior [22], hormone release [23], anxiety [24–26], cognition [27–30], locomotor activity [31, 32], and stereotypy [32, 33]. The pharmacological and molecular evidence indicates the presence of at least two receptors for the octapeptides angiotensin II AT_1 and AT_2 [34, 35]. These receptor subtypes mediate responses evoked by angiotensin II, although to date the majority of the known physiological responses evoked by angiotensin II appear to be mediated via the AT_1 receptor [36, 37].

The immunohistochemical and neuropharmacological evidence suggests that angiotensin II and its derived peptides angiotensin III and/or IV are neurotransmitters or neuromodulators in specific neuronal pathways in the brainstem, forebrain, and hypothalamus. AT_1 receptor activation by angiotensin II elicits neuronal depolarization by affecting the permeability of different ion channels. In this regard, AT_1 receptor mediates an inhibition of potassium channel [38] or opening of a non-selective sodium-calcium channel [39]. The activation of these different signaling pathways inducing depolarization would reflect the fine tuning level of the overall cellular activation. The electrophysiological evidences show that angiotensin II applied in vitro induces a firing increase in the median preoptic neurons [40], paraventricular nucleus of the hypothalamus [41], basolateral nucleus of amygdala [42], central nucleus of amygdala [43] and the hippocampus [44, 45]. Similarly to Angiotensin II, angiotensin IV exerts stimulating effects on the firing rate and burst discharges in the hippocampus [45]. The different distribution of the co-localized AT_1 and AT_4 receptors in the same hippocampal neuron could be a reason for the observed difference in excitation produced by either angiotensin II or angiotensin IV respectively. The excitatory effects induced by angiotensin II are due to presynaptic AT_1 receptor activation and modulatory effects on

classical neurotransmitter release [38], or activation of postsynaptic AT_1 receptors that induces membrane depolarization and an inward current [40]. It has been found that angiotensin II and angiotensin IV exert dual effects on the dorsal lateral geniculate nucleus [46], amygdala [42] and hippocampus [44, 45], but the inhibitory role predominates in the locus coeruleus [47], superior colliculus [48], and septum [49].

In many brain areas, there is an interaction between angiotensinergic and glutamatergic systems, and this interaction could explain in part the inhibitory effects induced by angiotensin II. In this regard, angiotensin II modulates the response to glutamate in the superior colliculus acting through AT_1 and AT_2 receptors, while visual potentials evoked by glutamate receptor agonist have been shown to be attenuated by postsynaptic AT_1 receptor activation only [50]. In the locus coeruleus, angiotensin II blocked the excitation evoked by glutamate [47]. Moreover, it has been described that angiotensin inhibited the NMDA- and/or kainate-evoked increase in the firing rate of dorsal lateral geniculate nucleus, and the AT_1 receptors may be involved [46]. In-vivo studies show that angiotensin II modulates the baroreceptor reflex response, acting on the area postrema, and in an electrophysiological study, an inhibitory effect was found in this area, induced by angiotensin II. Neurons from the area postrema are in reciprocal connection with the nucleus tractus solitarius, a region involved in integration of baroreceptor reflex response [51]. Moreover, angiotensin II and III inhibit the neurons of nucleus reticularis ventrolateralis, and subsequently the spontaneous baroreceptor reflex response. This action could be reversed by AT_2 receptor antagonists [52]. The presented evidence strongly suggests angiotensin II and derived peptides as modulators of neuronal activity.

Relationship Between Dopamine and Angiotensin II

There is a large body of evidence supporting the relationship between brain angiotensin II and catecholamine systems [53, 54]. This interaction could be involved in some central actions of angiotensin II such as cardiovascular control, dipsogenesis, and complex behaviors, supporting the idea that drugs able to modulate brain angiotensin II may be useful in regulating central DA activity. In this respect, high AT_1 receptor density has been described in DA-rich regions, in CPu, hypothalamus, NAc, and ventral pallidum [55, 56]. The evidence shows that brain angiotensin II increases the DA release in CPu and NAc [57]. Moreover, it has been found that in CPu the DA release induced by angiotensin II is mediated by AT_1 receptors [55], and the stereotype behavior induced by apomorphine (DA receptor agonist) could also be blocked by AT_1 receptor antagonists [58].

Given the established role of the nigro-striatal dopamine system in the control of movement, and the fact that angiotensin II enhances the release of dopamine in the rat striatum (that provides a neurochemical mechanism underlying the modulation of locomotor activity and other DA mediated behaviors) we may hypothesize that the angiotensin system could be a useful tool for pharmacological manipulation of the DA system (see above).

The available evidence suggests that DA and angiotensin II systems directly regulate against each other in the striatum and substantia nigra of rodents [59, 60]. In this regard, reserpine-induced DA depletion produced a significant increase in the expression of AT_1 and AT_2 receptors, which decreased when the dopaminergic function was restored. The same phenomenon was observed after dopaminergic denervation with 6-hydroxydopamine. In this case, the administration of L-Dopa decreased AT_1 and increased AT_2 receptor density [59]. Moreover, changes in angiotensin II levels may affect angiotensin II receptor density**.** In this regard, transgenic rats with very low levels of brain angiotensin II showed increased AT_1 receptors [61]. The Labandeira-Garcia group found evidence suggesting that AT_1 receptors expression is closely related to DA levels through direct (DA and AT_1 receptors) and indirect (changes in angiotensin II levels) mechanisms [60].

Brain Angiotensin II and Cocaine

Although it is generally recognized that cocaine has a potentially toxic effect upon the cardiovascular system, the process by which this occurs is extremely complex and is not, at present, fully understood. Acutely, cocaine administration has been associated with a wide range of effects, including increases in heart rate and blood pressure, coronary vasoconstriction, increases in myocardial contractility, and decreases in ejection fraction [62]. Based on the available evidence, it seems that these effects are associated with cocaine-induced increases in sympathetic output subsequent to potentiation of monoamines. Consistent with its local anesthetic properties, mediated by voltage-gated sodium channel blockade, cocaine also may depress heart function. These 'opposing' effects may create significant imbalances in oxygen demand and supply, which, particularly in a cocaine-induced prothrombotic context, have the potential to significantly degrade the electrical and mechanical functioning of the heart [63]. Findings in chronic cocaine abusers include hypertension, left ventricular hypertrophy, malignant arrhythmias, myocardial ischemia, and cardiomyopathy [64]. The clinical etiologic context is complex, as it is necessary to disentangle the effects of cocaine from cardiovascular risk factors associated with a drug-abusing lifestyle, including different factors such as poor diet and intravenous drug use [65].

Angiotensin I converting enzyme (ACE), the main angiotensin II generating enzyme, is an essential part of the renin–angiotensin system (RAS) present in the brain. Interestingly, research points to central nervous system effects of ACE-inhibitors that may bear upon their potential utility for the treatment of cocaine addiction. A number of studies in animals point to activation of the hypothalamic–pituitary–adrenal (HPA) axis by drugs of abuse, which may affect the drug's positive reinforcement properties, as well as mediate anxiogenic-like behavior associated with drug withdrawal [66]. There is evidence suggesting that cocaine withdrawal is associated with activation of corticotropin-releasing factor (CRF) [67]. Extrapolated to a clinical population, CRF release may mediate the abstinence-associated dysphoria, as well as the stress-related relapse to cocaine [68]. It has been found that ACE-inhibitors in cocaine-abusing patients decrease CRF release [69]**.** Conversely, angiotensin II increases CRF release [70]. Decrease in CRF by ACE-inhibitors could potentially play a role in reducing stress-related relapse to cocaine [65].

As was described above, DA release in the striatum of the rat can be directly affected by angiotensin II [71, 72] or through a metabolite-like angiotensin IV [55, 73, 74]. Angiotensin II increases CRF release [75], an effect also induced by cocaine withdrawal [76]. The available evidence supports the importance and possible implications of the complete RAS in the brain, where it acts to regulate a number of physiological processes (e.g., cardiovascular maintenance, memory, fluid intake, energy balance). ACE-inhibitors are ligands that form a complex with Zn2+ at the active site of ACE. ACE-inhibitors are effective in reducing blood pressure in hypertensive individuals, as they block the conversion of angiotensin I to angiotensin II and reduce the degradation of bradykinin. It has been described that ACE-inhibitors are able to increase dopamine release in the striatum, an effect probably mediated by the opioid system [65]. As they could indirectly block CRF release and directly block angiotensin II production, ACE-inhibitors have been suggested to be used in the treatment of cocaine abuse [65]. Moreover, it has been observed that chronic administration of ACE-inhibitors increased the turnover of dopamine in the striatum of rats [77]. Similarly, it has been found that sodium depletion, known as a treatment that induces RAS activation, is able to induce behavioral cross-sensitization with cocaine, showing the involvement of angiotensin II in the neuroplastic events induced by this psychostimulant [78].

The central nucleus of the amygdala (CeA) plays a critical role in integrating sympathetic and behavioral responses to stress and the stimulation of the CeA produces increases in blood pressure and heart rate [79]. The CeA also contains angiotensin II, ACE, and angiotensin

receptors [80]. In addition, CRF-like immunoreactivity exists in the CeA [81, 82]. Moreover, it has been described that microinjection of angiotensin II in the CeA elicits a pressor response, whereas CRF evokes both an increase in plasma catecholamines and arterial pressure [83, 84]. Cocaine and acute stress increase CRF and/or its mRNA in the amygdala [68, 85, 86]. Therefore, multiple studies suggest that angiotensin II and CRF are key neurotransmitters in the CeA involved in regulation of sympathetic and hemodynamic responses to stress [79].

Brain Angiotensin II and Amphetamine

Long-Lasting Changes in Brain Angiotensin II Involved in the Neuroadaptive Responses to Amphetamine

A direct relationship between angiotensin II and behavioral sensitization induced by amphetamine was found in our laboratory. In this regard, it was shown that angiotensin II AT_1 receptors are involved in the development of behavioral and neurochemical sensitization induced by a single exposure to amphetamine [87, 88]. Moreover, it was recently reported by our group that amphetamine exposure induces persistent alterations in brain angiotensin II components within CPu and NAc [89]. In this respect, both studied regions, CPu and NAc, presented long-lasting increase in AT_1 receptor density after amphetamine exposure but in CPu, a significant decrease in angiotensinogen (angiotensin II precursor) was found. The available evidence suggests a relationship between the AT_1 receptors and angiotensinogen in the brain. In this regard, it has been described that the administration of AT_1 receptor antagonists induced widespread up-regulation of angiotensinogen mRNA levels with low doses and down-regulation with higher doses [90] (Fig. 15.1). Meanwhile, other authors found a decrease in angiotensinogen, angiotensin II, and angiotensin-converting enzyme mRNA levels in basal ganglia after systemic administration of candesartan, an AT_1 receptor antagonist [91]. This evidence supports the view that manipulations of AT_1 receptors could induce changes in brain angiotensinogen levels. The results obtained for our group showing amphetamine-induced decrease in angiotensinogen in CPu could be related to an overstimulation of AT_1 receptors. In this regard, it was also found that the expression of behavioral sensitization was attenuated by AT_1 receptor blockade in CPu [89]. In NAc, no changes were observed in angiotensinogen after amphetamine exposure, and the AT_1 receptor

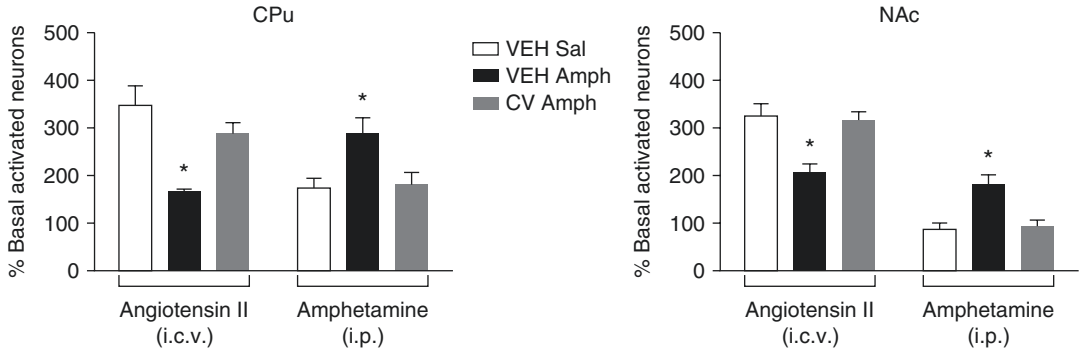


Fig. 15.1 AT_1 receptors are involved in the altered neuronal activation induced by amphetamine exposure in response to different challenges. Angiotensin II (400 pmol) was administered intracerebrally and amphetamine (0.5 mg/kg) intraperitoneally. The neuronal activation was measured as Fos expression in the two brain areas from animals exposed to amphetamine 21 days before, pretreated with AT_1 receptor antagonist (CV) or vehicle. The values were calculated as percentage respect control group (vehicle-saline animals exposed to saline challenge) and expressed as mean ± SEM. Two-way ANOVA analysis, $*p < 0.05$, $n = 7–10$

antagonists were ineffective in preventing the expression of behavioral sensitization induced by the psychostimulant [89]. Even though the two brain areas evaluated are rich in dopaminergic terminals and are strongly related to psychostimulants neuroadaptive responses, they show differential DA release in response to electrical stimulation. Moreover, the DA release in the CPu is not regulated by dopamine autoreceptor activation, in contrast to NAc [92]. Two mechanisms have been suggested for regulating DA release in the projections of dopaminergic systems: a phasic release regulated by depolarization of dopaminergic nerve cell bodies, and a tonic regulation of DA release independent of electrical activity of these neurons [93]. The tonic influences are more significant in the CPu than in the NAc [92]. This last fact is in agreement with the evidence showing the tonic influence of angiotensin II on DA synthesis and release in CPu through AT_1 receptors [55, 94]. However, CPu and NAc seem to have different roles in the neuroadaptive responses to drugs of abuse [95, 96].

Repeated Amphetamine Exposure Modifies Brain Angiotensin II AT_1 Receptor Functionality

Sodium depletion, which activates RAS, develops cross-sensitization effects leading to enhanced locomotor activity responses to amphetamine [97]. These experiments indicate that treatments implying RAS activation show reciprocal behavioral cross-sensitization with psychostimulants. In relation to these findings, our group, using a protocol of repeated amphetamine, found long-lasting changes affecting brain response to angiotensin II [98]. These alterations were revealed by exogenously intracerebrally injected angiotensin II in conscious rats, known to produce a marked increase in water and sodium intake, as well as an increased natriuresis [98]. All these effects have been previously and exhaustively described [99–103]; however, the results obtained in our study showed that previous exposure to repeated amphetamine administration modified the described effects of angiotensin II i.c.v. on these parameters in a long-term manner (1 week after amphetamine withdrawal) [104]. In this respect, it was found that repeated amphetamine exposure markedly decreased the sodium intake induced by angiotensin II; meanwhile, water intake was unaffected. Sodium intake behavior is likely to reflect the differential regulation of intracellular signaling pathways. In this regard, it has been hypothesized that differential AT_1 receptors signaling pathways play separable roles in water and saline intake stimulated by angiotensin II [105, 106]. There are results that support this hypothesis, demonstrating that G protein-dependent pathways appear to be more important for water intake stimulated by angiotensin II, whereas G protein-independent pathways may be more relevant for angiotensin II-stimulated sodium intake [107]. In accordance with this last fact are the results showing that repeated i.c.v. angiotensin II administration reduced the dipsogenic effect without affecting sodium intake [108]. Therefore, a possible explanation for amphetamine exposure effects is the alteration of intracellular signaling pathway involved in the effects of angiotensin II on sodium intake. This altered response obtained in amphetamine-exposed animals may involve the desensitization of AT_1 receptors through internalization of these receptors [109]. This last is supported by the evidence showing that angiotensin II i.c.v. induces internalization of AT_1 receptors [110]. Accordingly, a decrease in the response to angiotensin II after a persistent or repetitive stimulation of AT_1 receptors has been described [111]. Moreover, it has been shown that the early inducible genes, *c-fos*, *c-jun*, and *delta-fos* are involved in the control of transcription factors expression that ultimately mediate the desensitization to the angiotensin II signal [112]. In our laboratory, we found that angiotensin II i.c.v. induced a threefold increase in NAc and CPu neuronal activation; this effect was blunted by repeated amphetamine exposure. This decreased response could demonstrate an AT_1 receptor desensitization induced by repeated psychostimulant administration. In this regard, AT_1 receptor desensitization-reduced Fos expression has been described as a consequence of repetitive angiotensin II i.c.v. administration in

different brain areas that co-expressed AT_1 receptors [112]. Interestingly, these results are in agreement with those obtained in regard to the decreased response in sodium intake to angiotensin II i.c.v.

It is known that exogenous i.c.v. angiotensin II administration stimulates oxytocin release from the pituitary gland [113, 114]. It has been found that the increase of sodium intake through sodium deprivation or adrenalectomy decreases basal oxytocin levels; meanwhile, treatments that stimulate oxytocin secretion (e.g., hypertonic saline, lithium chloride, and copper sulfate) inhibit sodium intake in sodium-deprived rats [115–117]. Moreover, blockade of central oxytocin receptors before i.c.v. angiotensin II administration resulted in a potentiation of angiotensin II-induced sodium intake, although in the absence of exogenously administered angiotensin II, blockade of oxytocin receptors does not interfere with the dipsogenic properties of angiotensin II, nor does it stimulate sodium intake [115]. In rats, the oxytocin receptor antagonist administration-induced sodium intake is blunted by AT_1 receptor antagonist administration [118]. This evidence supports the idea of an inhibitory oxytocinergic tone involved in the activation or disinhibition of AT_1 receptors [118].

The results obtained in our study using repeated amphetamine administration reveal a long-lasting effect of amphetamine exposure.

Moreover, it is possible to suggest that the decreased response in sodium intake induced by angiotensin II i.c.v. in amphetamine-exposed animals could be attributed to an increased oxytocin response to angiotensin II as a consequence of AT_1 receptors altered functionality. This explanation is supported by our results, showing that amphetamine exposure increased the number of Fos-oxytocin positive neurons in response to angiotensin II. The mechanisms by which angiotensin II i.c.v. induces natriuretic effects could involve brain oxytocin release [119]. It has been shown that angiotensin II i.c.v. activates oxytocin neurons in paraventricular and supraoptic nucleus [113–115]. In our study, the repeated amphetamine administration potentiated the activation of oxytocin neurons induced by angiotensin II i.c.v. in different oxytocinergic subnuclei of paraventricular and supraoptic nucleus, possibly showing an increased oxytocin response to angiotensin II because of the reduced AT_1 receptor functionality mentioned above. Therefore, the repeated amphetamine exposure could reduce AT_1 receptor functionality (desensitization-like) shown as a potentiated oxytocinergic response to i.c.v. angiotensin II that elicits a decrease in sodium intake, an increase in natriuresis, and decreases in plasma renin activity. These results are also supported by the increased number of Fos-oxytocin positive neurons in paraventricular and supraoptic nucleus in response to i.c.v. angiotensin II found in the amphetamine-exposed animals [104].

In conclusion, the results presented here support the view that long-lasting changes in brain RAS could be considered among the psychostimulant-induced neuroadaptations.

References

1. Angrist B, Corwin J, Bartlik B, Cooper T. Early pharmacokinetics and clinical effects of oral D-amphetamine in normal subjects. Biol Psychiatry. 1987;22(11):1357–68.
2. Sherer MA. Intravenous cocaine: psychiatric effects, biological mechanisms. Biol Psychiatry. 1988;24(8):865–85.
3. Lieberman JA, Kinon BJ, Loebel AD. Dopaminergic mechanisms in idiopathic and drug-induced psychoses. Schizophr Bull. 1990;16(1):97–110.
4. Klawans HL, Margolin DI. Amphetamine-induced dopaminergic hypersensitivity in guinea pigs. Implications in psychosis and human movement disorders. Arch Gen Psychiatry. 1975;32(6):725–32.
5. Dackis C, Gold MS. Neurotransmitter and neuroendocrine abnormalities associated with cocaine use. Psychiatr Med. 1985;3(4):461–83.
6. Antelman SM, Chiodo LA. Dopamine autoreceptor subsensitivity: a mechanism common to the treatment of depression and the induction of amphetamine psychosis. Biol Psychiatry. 1981;16(8):717–27.
7. Kalivas PW, Pierce RC, Cornish J, Sorg BA. A role for sensitization in craving and relapse in cocaine addiction. J Psychopharmacol. 1998;12(1):49–53.
8. Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol. 1993;4(4):289–312.
9. Pierce RC, Kalivas PW. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Brain Res Rev. 1997;25(2):192–216.

10. Vanderschuren LJ, Schmidt ED, De Vries TJ, Van Moorsel CA, Tilders FJ, Schoffelmeer AN. A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. J Neurosci. 1999;19(21):9579–86.
11. Valjent E, Bertran-Gonzalez J, Aubier B, Greengard P, Herve D, Girault JA. Mechanisms of locomotor sensitization to drugs of abuse in a two-injection protocol. Neuropsychopharmacology Off Publ Am Col Neuropsychopharmacology. 2010;35(2):401–15.
12. Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl). 2000;151(2–3):99–120.
13. Vezina P. D1 dopamine receptor activation is necessary for the induction of sensitization by amphetamine in the ventral tegmental area. J Neurosci. 1996;16(7):2411–20.
14. Prasad BM, Hochstatter T, Sorg BA. Expression of cocaine sensitization: regulation by the medial prefrontal cortex. Neuroscience. 1999;88(3):765–74.
15. Sorg BA, Kalivas PW. Effects of cocaine and footshock stress on extracellular dopamine levels in the medial prefrontal cortex. Neuroscience. 1993;53(3):695–703.
16. Sorg BA, Davidson DL, Kalivas PW, Prasad BM. Repeated daily cocaine alters subsequent cocaine-induced increase of extracellular dopamine in the medial prefrontal cortex. J Pharmacol Exp Ther. 1997;281(1):54–61.
17. Pierce RC, Reeder DC, Hicks J, Morgan ZR, Kalivas PW. Ibotenic acid lesions of the dorsal prefrontal cortex disrupt the expression of behavioral sensitization to cocaine. Neuroscience. 1998;82(4):1103–14.
18. Tzschentke TM, Schmidt WJ. The development of cocaine-induced behavioral sensitization is affected by discrete quinolinic acid lesions of the prelimbic medial prefrontal cortex. Brain Res. 1998;795(1–2):71–6.
19. Hedou G, Feldon J, Heidbreder CA. Effects of cocaine on dopamine in subregions of the rat prefrontal cortex and their efferents to subterritories of the nucleus accumbens. Eur J Pharmacol. 1999;372(2):143–55.
20. Richard ML, Liskow BI, Perry PJ. Recent psychostimulant use in hospitalized schizophrenics. J Clin Psychiatry. 1985;46(3):79–83.
21. Lippoldt A, Paul M, Fuxe K, Ganten D. The brain renin–angiotensin system: molecular mechanisms of cell to cell interactions. Clin Exp Hypertens. 1995;17(1–2):251–66.
22. Steckelings UM, Bottari SP, Unger T. Angiotensin receptor subtypes in the brain. Trends Pharmacol Sci. 1992;13(9):365–8.
23. Steele MK, Stephenson KN, Meredith JM, Levine JE. Effects of angiotensin II on LHRH release, as measured by in vivo microdialysis of the anterior pituitary gland of conscious female rats. Neuroendocrinology. 1992;55(3):276–81.
24. Barnes NM, Costall B, Kelly ME, Murphy DA, Naylor RJ. Anxiolytic-like action of DuP753, a non-peptide angiotensin II receptor antagonist. Neuroreport. 1990;1(1):20–1.
25. Kaiser FC, Palmer GC, Wallace AV, Carr RD, Fraser-Rae L, Hallam C. Antianxiety properties of the angiotensin II antagonist, DUP 753, in the rat using the elevated plus-maze. Neuroreport. 1992;3(10):922–4.
26. Georgiev V, Tanaka M, Tsuda A, Koga C, Takeda S, Yokoo H, et al. Effects of angiotensin II on regional brain noradrenaline metabolism in non-stressed and stressed rats. Kurume Med J. 1992;39(4):235–44.
27. Barnes NM, Costall B, Kelly ME, Murphy DA, Naylor RJ. Cognitive enhancing actions of PD123177 detected in a mouse habituation paradigm. Neuroreport. 1991;2(6):351–3.
28. Barnes JM, Barnes NM, Costall B, Coughlan J, Kelly ME, Naylor RJ, et al. Angiotensin-converting enzyme inhibition, angiotensin, and cognition. J Cardiovasc Pharmacol. 1992;19(Suppl 6):S63–71.
29. Dennes RP, Barnes JC. Attenuation of scopolamine-induced spatial memory deficits in the rat by cholinomimetic and non-cholinomimetic drugs using a novel task in the 12-arm radial maze. Psychopharmacology (Berl). 1993;111(4):435–41.
30. Wayner MJ, Armstrong DL, Polan-Curtain JL, Denny JB. Role of angiotensin II and AT1 receptors in hippocampal LTP. Pharmacol Biochem Behav. 1993;45(2):455–64.
31. Georgiev V, Kambourova T. Behavioural effects of angiotensin II in the mouse following MPTP administration. Gen Pharmacol. 1991;22(4):625–30.
32. Banks RJ, Mozley L, Dourish CT. The angiotensin converting enzyme inhibitors captopril and enalapril inhibit apomorphine-induced oral stereotypy in the rat. Neuroscience. 1994;58(4):799–805.
33. Georgiev V, Getova D, Opitz M. Mechanisms of the angiotensin II effects on exploratory behavior of rats in open field. III. Modulatory role of GABA. Methods Find Exp Clin Pharmacol. 1991;13(1):5–9.
34. Wong PC, Duncia JV, Santella 3rd JB, Smith RD, Wexler RR, Timmermans PB, et al. EXP597, a non-peptide angiotensin II receptor antagonist with high affinities for the angiotensin AT1 and AT2 receptor subtypes. Eur J Pharmacol. 1994;260(2–3):261–4.
35. Inagami T, Iwai N, Sasaki K, Yamano Y, Bardhan S, Chaki S, et al. Cloning, expression and regulation of angiotensin II receptors. Eur Heart J. 1994;15(Suppl D):104–7.
36. Brown L, Sernia C. Angiotensin receptors in cardiovascular diseases. Clin Exp Pharmacol Physiol. 1994;21(10):811–8.
37. Dzau VJ, Pratt R, Gibbons GH. Angiotensin as local modulating factor in ventricular dysfunction and failure due to coronary artery disease. Drugs. 1994;47(Suppl 4):1–13.
38. Allen AM, Moeller I, Jenkins TA, Zhuo J, Aldred GP, Chai SY, et al. Angiotensin receptors in the nervous system. Brain Res Bull. 1998;47(1):17–28.

39. Yang CR, Phillips MI, Renaud LP. Angiotensin II receptor activation depolarizes rat supraoptic neurons in vitro. Am J Physiol. 1992;263(6 Pt 2):R1333–8.
40. Bai D, Renaud LP. ANG II AT1 receptors induce depolarization and inward current in rat median preoptic neurons in vitro. Am J Physiol. 1998;275(2 Pt 2):R632–9.
41. Latchford KJ, Ferguson AV. ANG II-induced excitation of paraventricular nucleus magnocellular neurons: a role for glutamate interneurons. Am J Physiol Regul Integr Comp Physiol. 2004;286(5):R894–902.
42. Albrecht D, Nitschke T, Von Bohlen Und Halbach O. Various effects of angiotensin II on amygdaloid neuronal activity in normotensive control and hypertensive transgenic [TGR(mREN-2)27] rats. FASEB J Off Publ Federation Am Soc Exp Biol. 2000;14(7):925–31.
43. Martial FP, Thornton SN, Lienard F, Mousseau MC, Nicolaidis S. Tonic neuronal inhibition by AII revealed by iontophoretic application of Losartan, a specific antagonist of AII type-1 receptors. Brain Res Bull. 1994;34(6):533–9.
44. Palovcik RA, Phillips MI. Saralasin increases activity of hippocampal neurons inhibited by angiotensin II. Brain Res. 1984;323(2):345–8.
45. Albrecht D, Broser M, Kruger H. Excitatory action of angiotensins II and IV on hippocampal neuronal activity in urethane anesthetized rats. Regul Pept. 1997;70(2–3):105–9.
46. Albrecht D, Broser M, Kruger H, Bader M. Effects of angiotensin II and IV on geniculate activity in nontransgenic and transgenic rats. Eur J Pharmacol. 1997;332(1):53–63.
47. Xiong HG, Marshall KC. Angiotensin II modulation of glutamate excitation of locus coeruleus neurons. Neurosci Lett. 1990;118(2):261–4.
48. Merabet L, de Gasparo M, Casanova C. Dose-dependent inhibitory effects of angiotensin II on visual responses of the rat superior colliculus: AT1 and AT2 receptor contributions. Neuropeptides. 1997;31(5):469–81.
49. Simonnet G, Bioulac B, Rodriguez F, Vincent JD. Evidence of a direct action of angiotensin II on neurones in the septum and in the medial preoptic area. Pharmacol Biochem Behav. 1980;13(3):359–63.
50. Mooney RD, Zhang Y, Rhoades RW. Effects of angiotensin II on visual neurons in the superficial laminae of the hamster's superior colliculus. Vis Neurosci. 1994;11(6):1163–73.
51. Cai Y, Bishop VS. Effects of arginine vasopressin and angiotensin II on area postrema neurons in rabbit brain slice preparation. Neurosci Lett. 1995;190(2):125–8.
52. Lin KS, Chan JY, Chan SH. Involvement of AT2 receptors at NRVL in tonic baroreflex suppression by endogenous angiotensins. Am J Physiol. 1997;272(5 Pt 2):H2204–10.
53. Georgiev V, Gyorgy L, Yonkov D, Getova D, Markovska V, Petkova B. Dopaminergic influence on the effects of angiotensin II in behavioural reactions. Physiol Bohemoslov. 1985;34(Suppl):45–8.
54. Huang BS, Malvin RL. Dopaminergic modulation of some central actions of angiotensin II in vivo. Proc Soc Exp Biol Med Soc Exp Biol Med. 1988;188(4):405–9.
55. Brown DC, Steward LJ, Ge J, Barnes NM. Ability of angiotensin II to modulate striatal dopamine release via the AT1 receptor in vitro and in vivo. Br J Pharmacol. 1996;118(2):414–20.
56. Daubert DL, Meadows GG, Wang JH, Sanchez PJ, Speth RC. Changes in angiotensin II receptors in dopamine-rich regions of the mouse brain with age and ethanol consumption. Brain Res. 1999;816(1):8–16.
57. Hoebel BG, Rada P, Mark GP, Hernandez L. The power of integrative peptides to reinforce behavior by releasing dopamine. Ann N Y Acad Sci. 1994;739:36–41.
58. Zhuo J, Moeller I, Jenkins T, Chai SY, Allen AM, Ohishi M, et al. Mapping tissue angiotensin-converting enzyme and angiotensin AT1, AT2 and AT4 receptors. J Hypertens. 1998;16(12 Pt 2):2027–37.
59. Labandeira-Garcia JL, Rodriguez-Pallares J, Dominguez-Meijide A, Valenzuela R, Villar-Cheda B, Rodriguez-Perez AI. Dopamine–angiotensin interactions in the basal ganglia and their relevance for Parkinson's disease. Mov Disord. 2013;28(10):1337–42.
60. Labandeira-Garcia JL, Rodriguez-Pallares J, Villar-Cheda B, Rodriguez-Perez AI, Garrido-Gil P, Guerra MJ. Aging, angiotensin system and dopaminergic degeneration in the substantia nigra. Aging Dis. 2011;2(3):257–74.
61. Villar-Cheda B, Rodriguez-Pallares J, Valenzuela R, Munoz A, Guerra MJ, Baltatu OC, et al. Nigral and striatal regulation of angiotensin receptor expression by dopamine and angiotensin in rodents: implications for progression of Parkinson's disease. Eur J Neurosci. 2010;32(10):1695–706.
62. Kloner RA, Hale S, Alker K, Rezkalla S. The effects of acute and chronic cocaine use on the heart. Circulation. 1992;85(2):407–19.
63. Billman GE. Cocaine: a review of its toxic actions on cardiac function. Crit Rev Toxicol. 1995;25(2):113–32.
64. Mouhaffel AH, Madu EC, Satmary WA, Fraker Jr TD. Cardiovascular complications of cocaine. Chest. 1995;107(5):1426–34.
65. Margolin A, Avants SK, Setaro JF, Rinder HM, Grupp L. Cocaine, HIV, and their cardiovascular effects: is there a role for ACE-inhibitor therapy? Drug Alcohol Depend. 2000;61(1):35–45.
66. Goeders NE. A neuroendocrine role in cocaine reinforcement. Psychoneuroendocrinology. 1997;22(4):237–59.
67. DeVries AC, Taymans SE, Sundstrom JM, Pert A. Conditioned release of corticosterone by contextual stimuli associated with cocaine is mediated by corticotropin-releasing factor. Brain Res. 1998;786(1–2):39–46.

68. Sarnyai Z. Neurobiology of stress and cocaine addiction. Studies on corticotropin-releasing factor in rats, monkeys, and humans. Ann N Y Acad Sci. 1998;851:371–87.
69. Zacharieva S, Matrozov P, Stoeva I, Andonova K. The effect of angiotensin-converting enzyme inhibition on ACTH response to corticotropin-releasing hormone (CRH) in normal men. Horm Metab Res. 1991;23(5):245–6.
70. Aguilera G, Kiss A, Luo X. Increased expression of type 1 angiotensin II receptors in the hypothalamic paraventricular nucleus following stress and glucocorticoid administration. J Neuroendocrinol. 1995;7(10):775–83.
71. Mendelsohn FA, Jenkins TA, Berkovic SF. Effects of angiotensin II on dopamine and serotonin turnover in the striatum of conscious rats. Brain Res. 1993;613(2):221–9.
72. Dwoskin LP, Jewell AL, Cassis LA. DuP 753, a non-peptide angiotensin II-1 receptor antagonist, alters dopaminergic function in rat striatum. Naunyn Schmiedebergs Arch Pharmacol. 1992;345(2):153–9.
73. Braszko JJ. Participation of D 1-4 dopamine receptors in the pro-cognitive effects of angiotensin IV and des-Phe 6 angiotensin IV. Neurosci Biobehav Rev. 2010;34(3):343–50.
74. Stragier B, Sarre S, Vanderheyden P, Vauquelin G, Fournie-Zaluski MC, Ebinger G, et al. Metabolism of angiotensin II is required for its in vivo effect on dopamine release in the striatum of the rat. J Neurochem. 2004;90(5):1251–7.
75. Aguilera G, Young WS, Kiss A, Bathia A. Direct regulation of hypothalamic corticotropin-releasing-hormone neurons by angiotensin II. Neuroendocrinology. 1995;61(4):437–44.
76. DeVries AC, Pert A. Conditioned increases in anxiogenic-like behavior following exposure to contextual stimuli associated with cocaine are mediated by corticotropin-releasing factor. Psychopharmacology (Berl). 1998;137(4):333–40.
77. Jenkins TA, Mendelsohn FA, Chai SY. Angiotensin-converting enzyme modulates dopamine turnover in the striatum. J Neurochem. 1997;68(3):1304–11.
78. Acerbo MJ, Johnson AK. Behavioral cross-sensitization between DOCA-induced sodium appetite and cocaine-induced locomotor behavior. Pharmacol Biochem Behav. 2011;98(3):440–8.
79. Watanabe MA, Kucenas S, Bowman TA, Ruhlman M, Knuepfer MM. Angiotensin II and CRF receptors in the central nucleus of the amygdala mediate hemodynamic response variability to cocaine in conscious rats. Brain Res. 2010;1309:53–65.
80. von Bohlen und Halbach O, Albrecht D. Visualization of specific angiotensin II binding sites in the rat limbic system. Neuropeptides. 1998;32(3):241–5.
81. Sakanaka M, Shibasaki T, Lederis K. Distribution and efferent projections of corticotropin-releasing factor-like immunoreactivity in the rat amygdaloid complex. Brain Res. 1986;382(2):213–38.
82. Uryu K, Okumura T, Shibasaki T, Sakanaka M. Fine structure and possible origins of nerve fibers with corticotropin-releasing factor-like immunoreactivity in the rat central amygdaloid nucleus. Brain Res. 1992;577(1):175–9.
83. Brown MR, Gray TS. Peptide injections into the amygdala of conscious rats: effects on blood pressure, heart rate and plasma catecholamines. Regul Pept. 1988;21(1–2):95–106.
84. Ku YH, Tan L, Li LS, Ding X. Role of corticotropin-releasing factor and substance P in pressor responses of nuclei controlling emotion and stress. Peptides. 1998;19(4):677–82.
85. Hsu DT, Chen FL, Takahashi LK, Kalin NH. Rapid stress-induced elevations in corticotropin-releasing hormone mRNA in rat central amygdala nucleus and hypothalamic paraventricular nucleus: an in situ hybridization analysis. Brain Res. 1998;788(1–2):305–10.
86. Gardi J, Biro E, Sarnyai Z, Vecsernyes M, Julesz J, Telegdy G. Time-dependent alterations in corticotropin-releasing factor-like immunoreactivity in different brain regions after acute cocaine administration to rats. Neuropeptides. 1997;31(1):15–8.
87. Paz MC, Assis MA, Cabrera RJ, Cancela LM, Bregonzio C. The AT(1) angiotensin II receptor blockade attenuates the development of amphetamine-induced behavioral sensitization in a two-injection protocol. Synapse. 2011;65(6):505–12.
88. Paz MC, Marchese NA, Cancela LM, Bregonzio C. Angiotensin II AT(1) receptors are involved in neuronal activation induced by amphetamine in a two-injection protocol. Biomed Res Int. 2013;2013:534817.
89. Paz MC, Marchese NA, Stroppa MM, Gerez de Burgos NM, Imboden H, Baiardi G, et al. Involvement of the brain renin–angiotensin system (RAS) in the neuroadaptive responses induced by amphetamine in a two-injection protocol. Behav Brain Res. 2014;272(314):23.
90. Pediconi D, Martarelli D, Fontanazza A, Pompei P. Effects of losartan and irbesartan administration on brain angiotensinogen mRNA levels. Eur J Pharmacol. 2005;528(1–3):79–87.
91. Pelisch N, Hosomi N, Ueno M, Masugata H, Murao K, Hitomi H, et al. Systemic candesartan reduces brain angiotensin II via downregulation of brain renin–angiotensin system. Hypertens Res Off J Jpn Soc Hypertens. 2010;33(2):161–4.
92. Trout SJ, Kruk ZL. Differences in evoked dopamine efflux in rat caudate putamen, nucleus accumbens and tuberculum olfactorium in the absence of uptake inhibition: influence of autoreceptors. Br J Pharmacol. 1992;106(2):452–8.
93. Grace AA. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience. 1991;41(1):1–24.
94. Mertens B, Vanderheyden P, Michotte Y, Sarre S. Direct angiotensin II type 2 receptor stimulation

decreases dopamine synthesis in the rat striatum. Neuropharmacology. 2010;58(7):1038–44.
95. Vanderschuren LJ, Everitt BJ. Behavioral and neural mechanisms of compulsive drug seeking. Eur J Pharmacol. 2005;526(1–3):77–88.
96. Wise RA. Drug-activation of brain reward pathways. Drug Alcohol Depend. 1998;51(1–2):13–22.
97. Clark JJ, Bernstein IL. Reciprocal cross-sensitization between amphetamine and salt appetite. Pharmacol Biochem Behav. 2004;78(4):691–8.
98. Marchese NA, Artur de laVillarmois E, Basmadjian OM, Perez MF, Baiardi G, Bregonzio C. Brain angiotensin II AT receptors are involved in the acute and long-term amphetamine-induced neurocognitive alterations. Psychopharmacology. 2016;233(5):795–807.
99. Ferguson AV, Washburn DL, Latchford KJ. Hormonal and neurotransmitter roles for angiotensin in the regulation of central autonomic function. Exp Biol Med (Maywood). 2001;226(2):85–96.
100. Fitzsimons JT. Angiotensin, thirst, and sodium appetite. Physiol Rev. 1998;78(3):583–686.
101. Fluharty SJ, Manaker S. Sodium appetite elicited by intracerebroventricular infusion of angiotensin II in the rat: I. Relation to urinary sodium excretion. Behav Neurosci. 1983;97(5):738–45.
102. Geerling JC, Loewy AD. Central regulation of sodium appetite. Exp Physiol. 2008;93(2):177–209.
103. Unger T, Horst PJ, Bauer M, Demmert G, Rettig R, Rohmeiss P. Natriuretic action of central angiotensin II in conscious rats. Brain Res. 1989;486(1):33–8.
104. Casarsa BS, Marinzalda MA, Marchese NA, Paz MC, Vivas L, Baiardi G, et al. A previous history of repeated amphetamine exposure modifies brain angiotensin II AT1 receptor functionality. Neuroscience. 2015;307:1–13.
105. Daniels D, Yee DK, Faulconbridge LF, Fluharty SJ. Divergent behavioral roles of angiotensin receptor intracellular signaling cascades. Endocrinology. 2005;146(12):5552–60.
106. Daniels D, Yee DK, Fluharty SJ. Angiotensin II receptor signalling. Exp Physiol. 2007;92(3):523–7.
107. Daniels D, Mietlicki EG, Nowak EL, Fluharty SJ. Angiotensin II stimulates water and NaCl intake through separate cell signalling pathways in rats. Exp Physiol. 2009;94(1):130–7.
108. Vento PJ, Daniels D. Repeated administration of angiotensin II reduces its dipsogenic effect without affecting saline intake. Exp Physiol. 2010;95(6):736–45.
109. Hunyady L, Catt KJ, Clark AJ, Gaborik Z. Mechanisms and functions of AT(1) angiotensin receptor internalization. Regul Pept. 2000;91(1–3):29–44.
110. Sasamura H, Dzau VJ, Pratt RE. Desensitization of angiotensin receptor function. Kidney Int. 1994;46(6):1499–501.
111. Tang H, Shirai H, Inagami T. Inhibition of protein kinase C prevents rapid desensitization of type 1B angiotensin II receptor. Circ Res. 1995;77(2):239–48.
112. Moellenhoff E, Blume A, Culman J, Chatterjee B, Herdegen T, Lebrun CJ, et al. Effect of repetitive icv injections of ANG II on c-Fos and AT(1)-receptor expression in the rat brain. Am J Physiol Regul Integr Comp Physiol. 2001;280(4):R1095–104.
113. Lang RE, Rascher W, Heil J, Unger T, Wiedemann G, Ganten D. Angiotensin stimulates oxytocin release. Life Sci. 1981;29(14):1425–8.
114. Ferguson AV, Kasting NW. Angiotensin acts at the subfornical organ to increase plasma oxytocin concentrations in the rat. Regul Pept. 1988;23(3):343–52.
115. Blackburn RE, Demko AD, Hoffman GE, Stricker EM, Verbalis JG. Central oxytocin inhibition of angiotensin-induced salt appetite in rats. Am J Physiol. 1992;263(6 Pt 2):R1347–53.
116. Stricker EM, Verbalis JG. Central inhibition of salt appetite by oxytocin in rats. Regul Pept. 1996;66(1–2):83–5.
117. Stricker EM, Verbalis JG. Central inhibitory control of sodium appetite in rats: correlation with pituitary oxytocin secretion. Behav Neurosci. 1987;101(4):560–7.
118. Fitts DA, Zierath DK, Wilkins EE, Bassett JE. Losartan blocks drinking and cFos expression induced by central ornithine vasotocin in rats. Physiol Behav. 2005;86(4):573–7.
119. Verbalis JG, Mangione MP, Stricker EM. Oxytocin produces natriuresis in rats at physiological plasma concentrations. Endocrinology. 1991;128(3):1317–22.

Habit Learning and Addiction

16

Enrico Patrono, Hisao Nishijo, Antonella Gasbarri, Assunta Pompili, and Carlos Tomaz

Abstract

Drug addiction is a chronic compulsion and relapsing disorder defined as a "pathological pattern of use of a substance", and characterized by the loss of control in drug-taking-related behaviors, the pursuance of those behaviors even in the presence of negative consequences, and a strongly motivated desire to consume substances. Several brain areas and circuits are involved, encoding cognitive functions such as reward, motivation, and memory. Addiction research has moved the focus to those psycho-neurobiological mechanisms that have a crucial role on the transition from an occasional use to the abuse of drugs. It has been hypothesized that drug addiction may start as a "goal-directed behavior"; later, with the maintenance of the "instrumental behavior", it can turn into a "habitual behavior", inducing a form of habit-based learning. At a brain level, it has been suggested that DA-ergic/GLU-ergic/NE-ergic meso-cortico-limbic transmission may have a crucial role in the pathological habit-based learning of a drug-seeking behavior.

The present chapter reviews the more recent studies on drug addiction, investigating the psycho-neurobiological hypotheses concerning what drives the transition from an occasional use to abuse of drugs. Then, a "habit learning" theory of drug addiction is described. Further,

E. Patrono (✉) • H. Nishijo
System Emotional Science, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama 930-0194, Japan
e-mail: e.patrono@gmail.com

A. Gasbarri • A. Pompili
Department of Applied Clinical Science and Biotechnology, University of L'Aquila, Coppito, L'Aquila 67100, Italy

C. Tomaz
Neuroscience Research Group, CEUMA University, UNICEUMA, São Luis, MA, Brazil

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_16

the possibility of an engagement of different memory systems in a "learned drug-seeking" behavior is discussed. The next section describes the role of prefrontal NE-ergic neurotransmission in drug addiction. Finally, the chapter raises some questions about a conceptual framework linking pathological learning with memory and drug addiction.

Keywords
Drug addiction • Habit-learning • Habit-memory • Mesocorticolimbic reward system

Introduction

Addiction in Latin (*addictus*) means "slave to debt" or "subjugate", and it is very closely associated with the concept of psychological dependence from pharmacological substances. Drug addiction is a chronically compulsive and recidivist disorder that affects individuals more psychologically than physically. The life of an addict is a progressive top-down circle of searching for, obtaining, using, and recovering from drug effects, in spite of related illness, disrupted relationships, and work/life failures. The social burden created by addiction can be quantified and measured in social and health contexts, in order to express the overall severity of this psychological disease. The extent of problematic drug use—by regular drug users—remains stable at between 16 million and 39 million people [1]; globally, these rates of drug abuse are relatively stable despite the fact that it is on the rise in developing countries. Substances of abuse reduce socio-economic development and boost organized crime, instability, and national insecurity [1].

Addiction has recently been defined by the 5th edition of *Diagnostic and Statistical Manual of Mental Disorders* (DSM-V) [2] as a "pathological pattern of use of the substance", where the loss of control over drug-seeking/drug-taking behavior, the persistence of drug-taking behavior despite negative consequences, and a high motivation to take drugs at the expense of other activities are the main features. The loss of control, persistence, and high motivation to take drugs can be analyzed and conceptualized at several levels, from psychological to molecular. In particular, a major hypothesis guiding experimental research considers the level of exposure to the substance as a key factor that leads to addiction [3–6]. Primarily, addiction is a chronic disease that involves brain systems related to reward, motivation, and memory, forming circuitry between each other. Secondarily, dysfunction in these circuits leads to bio-psycho-social manifestations of pathological behavior. Addiction researchers have recently hypothesized that there is a crucial role of the prefrontal cortex (pfC) in the limbic circuit of reward, and the pfC is considered to be one of the major components of the neurobiology of addiction [7–11].

The major aim of this chapter is to review recent studies highlighting the key features of drug addiction, and the nature of a transition from occasional to compulsive use of pharmacological substances. First, this chapter overviews two major hypotheses currently driving drug addiction research, all of which indicates that the level of exposure to the substance is a key factor leading to addiction [3–6]. Second, this chapter highlights a recent hypothesis related to "habit learning" that can explain the transition from occasional to compulsive drug use. The third part of this chapter discusses the possibility of the engagement of different memory systems in a "learned drug-seeking" behavior. The fourth part deals with a neurobiological conceptualization of addiction in relation to the "habit learning" and "habit memory" hypotheses. The fifth part of this chapter focuses on the role of catecholaminergic transmission in the pfC in addiction. In conclusion,

this chapter highlights several questions about a conceptual framework linking pathological learning and memory with drug addiction.

An Aberrant Motivation and a Hedonic Dysregulation Driving Drug-Seeking Behavior

In this section we review two major psychological theories explaining the passage from occasional use to pharmacological substance abuse: the "incentive-sensitization" theory and the "hedonic dysregulation" theory.

The "Incentive-Sensitization" Theory

Following the "drive-reduction theories", psychobiology of addiction has pointed out to a link between reward brain system and motivation. Motivational concepts can help us to understand how and why limbic brain systems are evolved to mediate psychological processes that guide drug seeking/taking behavior. Hedonic reward is a key concept in motivated behavior [4, 5], and cognitive expectations together with physiological internal states can modulate hedonic incentives [5]. It can be argued that learned Pavlovian incentive stimuli become both consummatory phase ("liking", hedonic value) and appetitive phase ("wanting", incentive salience), as a consequence of reward learning. It has been found that unconditioned affective reaction patterns elicited by sucrose and quinine solutions are essentially normal in rats after 6-hydroxydopamine (6-OHDA) lesions of the "striatal–accumbal" dopamine (DA) system [12]. Moreover, rats with extensive dopamine depletions can change their hedonic evaluation of a stimulus based on predictive relations with another event, meaning that reward learning models have posited dopamine systems to play a similar role in learned increments and learned decrements in prediction of hedonic rewards [12]. Pre-clinical research on drug addiction has found concepts to explain the compulsive use of drugs as well as the phenomenon of relapse in an interesting "motivation-based theory". Different drugs of abuse with different pharmacological actions cause *sensitization* via the alteration of the mesolimbic DA system. Sensitization happens when repeated drug administration leads to an enhancement of outcomes related to that drug or to another addictive substance (cross-sensitization, [5, 13, 14]). Compulsive drug seeking/taking behavior and the relapse (through the exposition to stimuli associated with the substance or due to stress) are attributable to modifications in the motivational system related to the appetitive phase (wanting). This phenomenon was explained by Berridge and Robinson with the "incentive-sensitization" theory [15]. They consider that long-term drug use leads to mounting neuroadaptations at the "brain reward system" level, enhancing the sensitization to the substances of abuse and to associated stimuli. Repeated use of a drug induces specific associations between stimuli and, consequently, induces specific actions tagging a specific behavior such as the rewarded outcome. Increasing of drug-stimuli pairings increases the incentive value of the stimuli, leading addicts to *want* to take drugs, even they do not particularly *like* them [5]. However, even if this theory explains many aspects of human addiction, such as excessive preoccupation with the drug and with seeking it out, the intense craving, and relapse, it fails to explain a central feature of drug addiction: the inability of addicts to regulate or stop the use of a drug despite negative consequences and the self-destructive nature of its prolonged use. This theory doesn't deny the pleasure obtained from the drug, the withdrawal, or habits such as reasons why people become addicted. However, it suggests that a sensitized *wanting* could better explain long-term compulsive drug seeking/taking behavior.

The "Hedonic Dysregulation" Theory

This theory describes a vicious "top-down" circle from occasional and controlled drug use into addiction passing through at least three stages: "preoccupation/anticipation", "binge/intoxication", and "withdrawal/negative effect" [16].

The first theories on drug addiction considered that drugs prevented or relieved psycho-physical

negative states resulting from abstinence (i.e., withdrawal) or from adverse environmental circumstances (i.e., stress). While initial drug use is motivated by the hedonic rewarding properties of the drug itself, it has been hypothesized that drug use becomes motivated more by a "negative reinforcement" (abstinence symptoms avoidance) than by a positive reinforcement (euphoric high state, [17]). Negative reinforcement can be defined as the process by which removal of an aversive stimulus (i.e., negative emotional state of drug withdrawal) increases the probability of a response (i.e., dependence-induced drug intake, [18]). Drug users progress from occasional use to addiction, and the factors motivating drug use are hypothesized to shift in importance, in which impulsivity often dominates early stages, and compulsivity dominates terminal stages. A shift occurs from impulsivity to compulsivity, and a similar shift occurs from positive reinforcement to negative reinforcement, driving the motivated behavior.

On the other hand, the role of sensitization in addiction has been explained as a shift in an incentive-salience state, described as "wanting", attributed to a pathological "over-activity" of mesolimbic dopamine function. Other factors such as an increased secretion of glucocorticoids may function in the long-term maintenance of this sensitized state [19]. Drug-taking follows the pattern of intoxication, tolerance, escalation in intake, profound dysphoria, physical discomfort, and somatic withdrawal signs during abstinence. The "craving" is an intense preoccupation/desire to obtain substances that often precedes the somatic signs of withdrawal, having a crucial role in compulsive seeking-behavior and in relapse. Moreover, craving has a role in the associated stimuli related to drug-taking behavior and to withdrawal. Finally, craving is a key part in the vicious circle of addiction, and it has been considered important in the three stages driving to drug addiction: "preoccupation/anticipation", "binge/intoxication", and "withdrawal/negative affect" [16]. These three stages are conceptualized as interacting with each other, becoming more intense, dysregulating the hedonic homeostasis of the reward system, and ultimately leading to the pathological state known as addiction [4]. The transition from occasional drug use to addiction involves neuroplasticity in all of these elements, and may begin with initial drug use in vulnerable individuals or individuals at particularly vulnerable developmental periods (i.e., in adolescence).

The preoccupation/anticipation (craving) stage of the addiction cycle has long been hypothesized to be a key element of relapse in humans, and it defines addiction as a chronic relapsing disorder. The binge/intoxication stage has a pattern of intake characterized by high intake of the drug except during periods of sleep and negative emotional states during abstinence, including dysphoria, irritability, and intense craving. Such "binges" can last hours or days, and are often followed by a withdrawal characterized by extreme dysphoria and inactivity. When craving is driven by environmental cues, intense substance-craving can anticipate withdrawal, signifying the availability of the substance and internal states linked to negative emotional states and stress (withdrawal/negative effect stage).

The hedonic dysregulation theory elucidates the passage from use to abuse of drugs as a "top-down" vicious circle, considering the key role of a sort of imbalance in the hedonic status of drug users. However, the theory fails to explain the role of other main features of drug addiction such as an abnormal sensitization to the substance and the instrumental behaviors to obtain the substance.

The Neural Basis of a Drug-Motivated Behavior

In addition to the behavioral criteria described above, different studies in the neurobiology of addiction also support the idea that DA plays a crucial role in drug-motivated behavior. The clearest mechanism in drug-seeking/taking behavior is the activation of the DA-ergic transmission in the brain reward circuitry [20–22]; and DA-ergic mesolimbic/nigrostriatal pathways are thought to be mainly involved in drug-induced neuroplastic changes. Furthermore, it has been widely shown that increased DA-ergic transmission in the nucleus accumbens (NA)

plays a mediating role in the rewarding/reinforcing effects of addictive drugs [6, 23–26].

One of the two NA subnuclei (the "shell") receives DA-ergic innervations from the ventral tegmental area (VTA), and is crucial in the modulation of "motivational salience", also contributing to the establishment of "Pavlovian" learned associations between motivational events and concurrent environmental perceptions [27, 28]. The NA shell also projects to subcortical structures, such as the lateral hypothalamus (LHyp, which mediates autonomic responses), permitting regulatory activity in hunger/satiety modulation of food motivation and reward [28]. Neurochemical lesions of the NA DA-ergic pathways or receptor-blocking drugs reduce the "wanting" to eat, but do not reduce facial expressions of "liking" for the same reward [12, 29, 30]. Furthermore, opiates increase extracellular DA in the NA [31], and drug priming reinstates drug-seeking behavior by activating the mesolimbic DA-ergic incentive motivation system [12, 28]. Adaptive behavioral responses to the motivational situation occur under DA release, inducing cellular changes that establish learned associations with the event [32]. By contrast, in a repeated drug administration, DA release is no longer induced by a particular event, as a motivational event becomes familiar by repeated exposure [33]. In this case, the behavioral response remains goal-directed and well learned, and further DA-induced neuroplastic changes are not necessary.

In contrast, the other sub-nucleus of NA (the "core") appears to be a primary site mediating the expression of learned behaviors in response to stimuli predicting motivationally relevant events [34, 35]; and DA is released into the core in response to stimuli predicting a rewarding event, which probably modulates the expression of adaptive behaviors [34]. Therefore, in learned associations induced by repeated motivational situations, DA will likely be released as part of the overall experience. In sum, DA might have two functions. The first is to alert the organism to the appearance of novel salient stimuli, and thereby promote neuroplasticity (learning). The second is to alert the organism to the pending appearance of a familiar, motivationally relevant event, on the basis of learned associations which were previously made with environmental stimuli predicting the event [36]. Finally, a series of parallel cortico-striato-pallido-cortical loops have been defined whereby the ventral striatum, including NA, relates to emotional learning and the dorsal striatum relates to cognitive and motor functions [37, 38].

In parallel with neurobiological studies, electrophysiological studies have revealed highly heterogeneous changes in striatal neuron firing during a motivated behavior [39–41].

Interestingly, two major neuronal types have been identified in the NA [42, 43]: fast spiking interneurons (FSIs) and medium spiny projection neurons (MSNs). FSIs strongly inhibit MSNs and control their spike timing [43, 44], and have been shown to respond differently than MSNs to rewards [45], suggesting that FSIs and MSNs play different roles in those behaviors related to motivation and habit learning. Finally, the NA plays an important role in both appetitive and consummatory behavior. A common finding in electrophysiological studies of the NA or ventral striatum (VS) in animal models of behavior is that subpopulations of neurons respond phasically to each identifiable component of both appetitive and consummatory phases of the task [41, 46–48]. However, because many more NA neurons are inhibited during consumption than are excited, manipulations that inhibit the NA may enhance food consumption, not because the NA is generally inactivated, but because the specific population of neurons whose firing inhibits consumption is silenced by such manipulations. Many of the same neurons whose inhibition may drive consumption are also excited by cues, and during operant responding are excited by behaviors that are incompatible with consumption.

A "Habit-Based Learning" Hypothesis for Drug Addiction

The "Habit Learning" Theory

Recently, addiction research has placed a special focus on what happens in the real world where drug abusers have to stoke up drugs because of

their not free availability [49]. In line with this idea, an animal model of drug-seeking/taking behavior has been created. In this model, rats no longer respond to stimuli in order to obtain drug infusions. Thus, it has been defined that the drug sensitivity is due to an instrumental behavior–drug administration relationship. In fact, drug-associated stimuli have a considerable effect on behaviors, and play an important role in addiction development [50, 51]. However, it has been shown that drug-seeking behavior is not affected by pharmacological effects of the drug, because the maladaptive behavior occurs prior to drug infusion [52]. If the drug-seeking behavior is still present even if the drug is not delivered, it is arguable that the drug-seeking behavior depends on the contingency in the presentation of drug-associated cues. This animal model provides an opportunity to study the neural basis of cue-associated drug-seeking behavior. Moreover, it is useful in order to address new potential treatments that would decrease cue-associated drug-seeking behavior. The main characteristics of drug addiction are the compulsive drug-seeking/taking drug behavior in spite of adverse consequences and the relapse to the substances of abuse. When desire becomes a need, the subject acts out a different kind of behavior. It leads him or her to take substances. Goal-directed behavior and habit learning perform two forms of instrumental learning: the first one is quickly acquired and tuned by its outcome, the second one is more willful, and elicited by previous stimuli rather than their consequences [53]. The psychobiology of drug addiction identifies the first of these behaviors as simply aberrant, and the second as pathological.

Different behavioral procedures have been developed, each of which focuses more directly on component processes. The critical procedure for demonstrating this motivational influence is the Pavlovian–instrumental transfer (PIT) design, in which the role of a separately trained conditioned stimulus (CS) on instrumental responses is assessed [6, 49, 52]. Pavlovian CSs can modulate instrumental performance. For example, a stimulus that predicts the arrival of a tasty solution will enhance lever pressing for that solution (*specific* PIT) or another reward (*general* PIT). The approach takes into account two conditions: (1) the Pavlovian processes that define sensitivity to the contingency between stimuli (S) and reinforcers (R), and (2) the instrumental processes sensitive to the contingencies between actions, or responses (R), and outcomes (O, [54, 55]). This R–O process can be contrasted with the first, S–R instrumental process in which seeking behavior is a simple habitual response triggered by the environment and drug-associated stimuli. It has been argued that drug seeking is initiated under the control of the goal-directed R–O process, but the onset of addiction becomes a compulsive habit under the control of the S–R process [52]. For example, an action such as lever-pressing works as a contingency between action and its outcomes. Combination of this contingency, along with an unconditioned stimuli (US)-induced instrumental incentive value, regulates goal-directed responses, defining a motivational incentive salience. On the other hand, the S–R process can induce an incentive learning process. Additionally, PIT provides a "motivational boost" and enhances R-O process.

Everitt considers drug addiction the final stage of several steps from the initial and controlled use of a substance [6, 49, 52, 56]. When the substance is taken voluntarily for its incentive effect, seeking behavior progressively becomes a "habit", through a gradual loss of control. Thus, the stimulus–response mechanism plays an important role in the maintenance of an instrumental behavior. Finally, the capacity of the stimulus (substance) to act as reinforcement (conditioned reinforcer) exerts a kind of control over the seeking/taking behavior. Thus, drug addiction may start as a "goal-directed behavior"; later, with the maintenance of the "instrumental behavior", it could turn into a "habitual behavior", inducing a form of learning based on the habit (habit learning, [6, 49, 52, 56]).

Three major theories guide the experimental research in the field of drug addiction. The *incentive-sensitization theory* states that "aberrant motivation" to seek and take drugs could characterize addiction, and considers that "wanting" plays a major role in addiction development.

The *hedonic dysregulation theory* defines a top-down spiraling, from use to abuse of drugs, and focuses on the role of dysregulation in hedonic homeostasis, taking into account a crucial role of a "liking" dysregulation. Finally, the *habit learning theory* highlights the role of an instrumental learning behavior that becomes habit, in order to explain the complex use/abuse transition in the drug seeking/taking behavior, and places equal weight on both the "liking" and "wanting" roles.

The Neural Basis of a Drug-Habit-Learned Behavior

Accumulating evidence suggests a critical role for dissociable neurochemical mechanisms in the basolateral amygdala (BLA) and the NA core that underlie drug-seeking behavior maintained by conditioned reinforcers [55, 57–60]. The BLA complex performs fundamental roles in memory formation and storage linked with emotional events [61, 62]. Moreover, it is involved in appetitive (positive) conditioning [63]. Distinct neurons respond to both positive and negative stimuli, but they do not group into clear anatomical nuclei [64]. Studies report that infusions of DA receptor antagonists into the BLA prevented CS-induced reinstatement of responding after extinction [65]. This could mean a special involvement of DA-ergic transmission in the BLA in drug-seeking/taking behavior. Consistent with these observations, NA core DA efflux was not increased during the response-dependent presentation of conditioned stimuli in a reinstatement procedure [66, 67], whereas glutamate (GLU) efflux was increased in the NA core of animals engaged in active cocaine seeking [68]. Finally, combined "cues + drug-primed" reinstatement conditions showed that increased DA and GLU efflux in the medial pfF (mpfC) and NA plays a role in promoting reinstatement, and may be an important mediator of drug-seeking behavior primed by multiple relapse triggers [69]. Together, these findings suggest that drug seeking maintained by drug-associated conditioned reinforcers may depend on DA-ergic mechanisms in the BLA and GLU-ergic mechanisms in the NAc core, and together in the mpFC.

This raises the question of whether these selective neurochemical mechanisms in the BLA and NA core are components of a neuroanatomical subsystem within limbic cortical–ventral striato-pallidal circuitry [70]. In part, the technique of the so-called "disconnection" indicated that the dorsal striatum (DS) and VS interact with each other serially, in a wide range of functional settings, such as PIT on goal-directed behavior [55]. Specific PIT involves the BLA and NA shell. General PIT involves the central amygdala (CeA) and NA core [59]. The VS has long been suggested to be the interface between emotion, motivation, and action on the basis of its major inputs from the limbic cortices such as the BLA, the orbitofrontal cortex (oFC), and the LHyp [55, 70, 71]. The NA core has important functions in Pavlovian conditioning, and in the interactions between Pavlovian and instrumental learning mechanisms involved in involuntary behavior [55, 57, 66]. Conversely, the role of DS in both cognitive and motor functions is well established, providing the neurobiological substrate of both goal-directed and habitual control of "instrumental learning" [72–75]. Sequential phases of Pavlovian and instrumental learning could be especially relevant for the transition from casual drug use to substance abuse, involving compulsive drug-seeking/taking behavior [49].

Recently, several experimental and functional observations support the idea of common neural circuitry forming a distinct entity into the basal forebrain, termed the "extended amygdala". This circuit may be delegated to act on the motivational, emotional, and habitual effects of drug addiction [76–79]. The extended amygdala represents a macro-structure composed of several basal forebrain structures: the bed nucleus of the stria terminalis (BNST), the central medial amygdala (CeA), and the NA shell [76, 77]. These structures have similarities in morphology, immunohistochemistry, and connectivity [78, 79], and they receive afferent connections from limbic structures such as the hippocampus (HP), BLA, and LHyp. Key elements of the extended amygdala include not only neurotransmitters

associated with the positive reinforcing effects of drugs of abuse, but also major components of the brain stress systems associated with the negative reinforcement of dependence [76].

A New Perspective on Rewarding Memories in Drug Addiction

The "Habit-Memory" Hypothesis

The implications of the psychological/neural mechanisms of drug-seeking behavior have an important role in addressing drug addiction therapies. Interestingly, recent evidence indicates that different memory systems are also used in the new learning occurring during behavioral extinction [80]. The passage from initial casual drug use to eventual addiction could involve, at least in part, a compulsive drug-seeking/taking behavior guided by dorsal striatal-dependent habit-learning mechanisms [49, 52, 72]. This suggests that when "habit-like" drug-seeking behavior is firmly acquired, the extinction of such behavior may be differentially influenced by engaging both habit and memory systems. Furthermore, a dissociation has been defined between cognitive (hippocampus-dependent) and habit (DS-dependent) memory systems, during an initial acquisition of learned behavior [81–83]. Recently, it has been tested whether habit and cognitive memory systems are involved in the extinction of such behaviors [84]. In the response extinction condition, rats performed a runway approach response to an empty fluid well. In the latent extinction condition, rats were placed at the empty fluid well without performing a runway approach response. Subsequently, it has been shown that the relative effectiveness of multiple memory systems was altered by oral cocaine self-administration, during extinction training [84]. Finally, it has been found that an abnormal stimulus–response habit guiding acquired approach response can affect the cocaine-induced impairment of latent extinction, thus rendering cognitive learning mechanisms inefficient during latent extinction training. Consistent with these results, drug-seeking behaviors underlying addiction may involve, at least in part, a transition from goal-directed behaviors to habitual behaviors that characterize the function of the DS memory system [49, 52, 72, 85–87].

The Neural Basis of Habit Memory

The BLA plays a crucial role in emotion and memory [88, 89]. Numerous studies have implicated the BLA in the effects of emotional arousal on memory, mediated by HP and DS [90–92]. Moreover, recent evidence indicates that the relative use of cognitive and habit memory can be influenced by an organism's emotional state [93]. On the basis that anxiety and/or stress can promote relapse into previously acquired habitual and maladaptive human behaviors such as addiction [4, 76], recent data has highlighted the mechanisms by which emotional arousal can produce a clinically significant propensity to the use of habit memory [94]. In the rat, HP and DS each receive anatomical projections from the BLA [95, 96]. Moreover, in the dual-solution plus maze task [97], BLA infusion of anxiogenic drugs may produce an inclination towards the use of habit memory by directly facilitating DS-dependent response learning. Alternatively, the infusions may indirectly bias rats towards response learning by impairing HP-dependent place learning [93, 94, 97]. Extensive evidence indicates that competitive interference between cognitive and habit memory systems can exist in some learning situations [98–100]. The emotional processes mediated by the BLA may also impact learning and memory by influencing the degree and nature of competitive interference among multiple memory systems. Finally, taking the considerable impact of emotion and memory on adaptive behavior, it is not surprising that the role of the amygdala in human psychopathology has received considerable empirical and theoretical attention [92, 101–104]. Recently, it has been suggested that a modulatory action may potentially provide a mechanism whereby stress or anxiety could release habit-learning systems from the competing and/or inhibitory influences

of cognitive memory systems, promoting relapse into previously acquired habitual and maladaptive behaviors, as occur in drug addiction or obsessive-compulsive disorder [94].

Pre-frontal Cortical Norepinephrine Transmission in Drug Addiction

Drug addiction research is focused on the regulation of mesoaccumbens DA-ergic transmission in response to pleasant or aversive stimuli. However, recently it has been shown that mesoaccumbens DA-ergic transmission seems to be modulated by the mesocortical DA-ergic system in an inhibitory way, suggesting an inverse response relationship between them [8]. Moreover, a growing body of data considered the idea of a "hypofrontality" in drug addiction, considering that a prolonged drug use can induce an inability to inhibit responding toward the stimuli previously paired with a reward, resembling the focused and persistent drug-seeking behavior observed in drug addicts [105, 106].

It has been demonstrated that norepinephrine (NE) in the mpFC has a crucial role in NA DA release, induced by systemic amphetamine, morphine, or lithium administration [8–10]. Hence, studies on the involvement of brain NE-ergic systems in behavior control mostly focus on emotional memory regulation by the amygdala (AMY, [92, 104, 107]). Finally, a possible mpFC NE/DA counteractive action on subcortical DA transmission has been suggested [8–10]. Psychobiological studies, which investigated different genetic backgrounds and used a useful strategy for investigating the neural basis of drug effects, have identified relationships between catecholaminergic neurotransmission and maladaptive behavior [108]. Using two well-known inbred strains of mice (DBA/2J, DBA and C57BL6/J, C57), it has been shown that DBA mice are poorly responsive to the enhancing extracellular DA induced by both natural and pharmacological substances in the NA [109–112]. Oppositely, C57 mice are highly responsive to stimulating/reinforcing effects of both natural and pharmacological substances, as shown by increased locomotor activity in amphetamine-induced conditioned place preference (CPP, [109, 110]) or by increased seeking/taking chocolate behavior in a conditioned suppression paradigm [111–113]. Since DA mpFC has an inhibitory role on DA NA, while NE is excitatory [7], it has been hypothesized that the NE/DA balance in the mpFC controls DA in the NA and related behavioral outcomes, making the C57 strain more responsive than DBA [8, 30]. This hypothesis was confirmed by experiments showing that selective mpFC NE depletion abolished the effects of amphetamine on DA in the NA of C57 mice [8], while selective mpFC DA depletion (preventing NE) led to DA outflow in the NAc and behavioral outcomes in DBA mice which are entirely similar to those of C57 [9, 108]. These data suggested that DA in the NA is controlled by mpFC NE, which enhances it, and by mpFC DA, which inhibits it.

Prefrontal NE transmission is known to play a critical role in regulating many cortical functions, including arousal, attention, motivation, learning, memory, and behavioral flexibility [113–118]. Moreover, both rewarding/reinforcing and aversive stimuli have been shown to increase NE release in mpFC [10, 112, 119, 120]. Furthermore, it has been shown that novel stressful experiences enhance DA release in the NA through activation of mpFC alpha-1 adrenergic receptors by high levels of released NE [121, 122].

There is some evidence to indicate that mpFC NE/DA transmission controls DA release in the NA [8, 9, 30, 117, 123]. Thus, mpFC-NA regulation partially regulates the response to rewarding (amphetamine) or aversive (stress) stimuli [10]. Further studies have provided substantial support for this view, through experimental evidence that the mpFC NE is crucial in the effects of other addictive drugs [8, 9], palatable food [11, 111–113], and aversive pharmacological or physical stimuli [10].

It is already known that the BLA is involved in forming associations between neutral and aversive stimuli [61, 62, 124–126]. The BLA receives stress-related DA projection from the VTA, suggesting that the BLA is involved in the modulation of affective stress responses, along with the

NA and mpFC [127–129]. It has been shown that intra-BLA infusions of DA-ergic receptor antagonists enhanced DA release in NA in stressed rats, while it reduced the DA stress response in the mpFC [127]. Thus, these findings suggest that increased BLA DA-ergic transmission has opposite effects on the NAc and mpFC DA responses to stress. Moreover, the anxiety induced by withdrawal is a significant factor contributing to continued drug abuse in addicted people, and the BLA is a major brain emotional center regulating the expression of fear and anxiety [76, 130–133]. Furthermore, recent studies have suggested that central NE-ergic systems are activated during acute withdrawal from ethanol, and may have a motivational significance [134]. Moreover, electrophysiological studies have shown that interneuronal GABA-ergic activity in the "extended amygdala" may reflect the negative emotional state of motivational drug-seeking [76]. Furthermore, evidence suggests that NE enhances GABA-ergic neurotransmission via the α1 receptors [135]. Acute withdrawal from all major substances of abuse increases reward thresholds, anxiety-like responses, and AMY neurotransmission [76, 136]. Compulsive drug use associated with dependence is mediated not only by loss of function of reward systems, but also by recruitment of brain stress systems such as NE in the "extended amygdala" [76]. Finally, brain arousal/stress systems in the extended amygdala may be key components of the negative emotional states that drive dependence on drugs of abuse, and may overlap with the negative emotional components of other psychopathologies [77].

Conclusions

A few interesting questions are raised in the light of all the converging evidences presented here, starting from the theoretical/psycho-bio-physiological conceptualizations of drug addiction to the last findings about a possible conceptual framework linking pathological learning and memory with drug addiction.

The first question is whether the three theoretical conceptualizations, the "incentive-salience theory", the "hedonic dysregulation theory", and the "habit-based learning theory" are able to individually explain the psychopathological features of drug addiction. It is more likely, though, that these three theories can be considered as parts of a single general conceptualization that can better explain the psychopathological features of drug addiction. The hypothesis that an "aberrant motivation", a "hedonic dysregulation" and "aberrant learning" can be individual features which can be included in the complex of psychopathological behavior should be considered.

The passage from occasional drug use to abuse is related to a change from a positive reinforcement to a negative one, with changes on motivational baseline. Drug reward is comprised of two components: one appetitive (orienting towards food) and the other consummatory (hedonic evaluation), which are also referred to as "wanting" and "liking" respectively. It has been explained that "wanting" and "liking" could act independently, defining a psychological and neuroanatomical separation between them [5, 12]. Moreover, it has been defined that craving (intense needing) and continuous neuro-plastic changes are involved in the passage from casual drug use to addiction [26]. Finally, it has been argued that only maladaptive habit-based learning could trigger drug-seeking behavior [6]. However, these three hypotheses are able to explain singular parts of the entire complex of drug addiction. Finally, motivation, hedonic dysregulation, and habit-based learning can be considered parts of the complex of the drug-addicted behavior; and neuroanatomical and neurobiological evidence discussed here are in line with this idea. However, although several studies have investigated how and when these three characteristics are involved in drug addiction, little is known about their possible temporal interpolation. Several human and animal studies have shown that the time of reward has an important role in reward processing [137, 138]. Furthermore, time intervals and rates of reward are of crucial importance for conditioning, and DA neurons

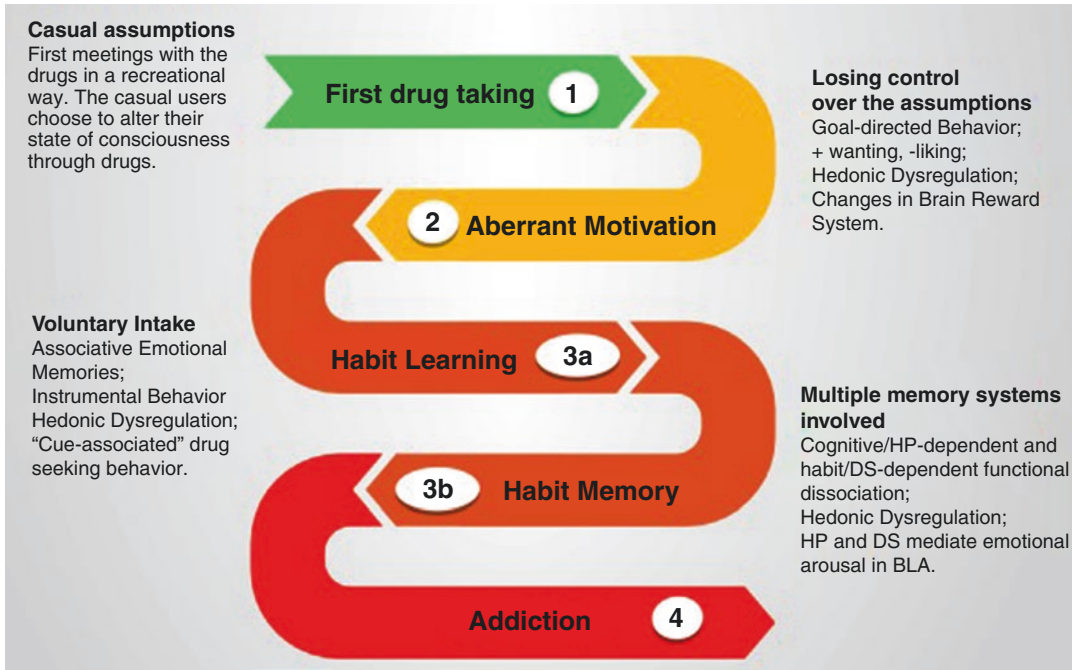


Fig. 16.1 Hypothetical timeline of the temporal interpolation. Figure describes a hypothetical timeline where the major features are defined in a single temporal interpolation from the first drug taking to the addiction. During this time, neurobehavioral changes such as the passage form a goal-directed behavior to an instrumental behavior and a functional dissociation between cognitive/HP-dependent memory and a habit/DS-dependent memory act on the hedonic dysregulation, and on the representation of the value of the drug, drastically inducing the addiction

are crucially involved in the processing of temporal information about the rewards. DA-ergic neurons in the meso-cortico-limbic system show reward-related responses that are sensitive to the predicted time of reward and the instantaneous reward probability [137]. This suggests a possible temporal interpolation from occasional use to abuse of substances, mediated by a meso-cortico-limbic DA-ergic circuit (Fig. 16.1). At a clinical level, this would also help to understand how and when to intervene along the continuum from occasional use to abuse of pharmacological substances.

A growing body of data hypothesizes the possibility of a conceptual framework linking the pathological learning, memory, and drug addiction. Recently, it has been hypothesized that when "habit-like" drug-seeking behavior is firmly acquired, the extinction of such behavior may be differentially influenced by engaging both habit and memory systems. Furthermore, a dissociation has been defined between cognitive (hippocampus-dependent) and habit (DS-dependent) memory systems, during an initial acquisition of learned behavior [81–83].

The second question is whether the three features presented above (aberrant motivation, hedonic dysregulation, and aberrant learning) underlying drug-addicted behavior could also be evaluated from a multi-emotional memory system point of view, highlighting a possible major role of aberrant learned associations between drug-associated stimuli and environmental factors, such as stress, driving the maladaptive compulsive seeking/taking behavior that is a main feature of drug addiction. Although there are emergent studies about the possible role of multi-emotional memory systems in drug addiction, little is known about the possible role of "habit memory" in

psychopathological behavior characterizing drug addiction.

Finally, taken together, these four theories could contribute to better understanding the psychopathological features of drug addiction, such as the compulsive use of substances of abuse as well as the relapse. Thus, future works could aim to better understand the key elements characterizing the psycho-physiopathological aspects of drug addiction.

References

1. United Nations Office on Drug and Crime. World drug report 2014. Vienna: United Nations Publications; 2014.
2. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Washington, DC: American Psychiatric Association; 2013.
3. Nestler EJ. Molecular mechanisms of opiate and cocaine addiction. Curr Opin Neurobiol. 1997;7(5):713–9.
4. Koob GF, LeMoal M. Drug abuse: hedonic homeostatic dysregulation. Science. 1997;278(5335):52–8.
5. Berridge KC. Motivational concepts in behavioral neuroscience. Physiol Behav. 2004;81(2):179–209.
6. Robbins TW, Everitt BJ, Nutt DJ. Introduction: the neurobiology of drug addiction: new vistas. Philos Trans R Soc Lond B Biol Sci. 2008;363(1507):3109–11. doi:10.1098/rstb.2008.0108.
7. Darracq L, Blanc G, Glowinski J, Tassin JP. Importance of the noradrenaline-dopamine coupling in the locomotor activating effects of D-amphetamine. J Neurosci. 1998;18(7):2729–39.
8. Ventura R, Cabib S, Alcaro A, Orsini C, Puglisi-Allegra S. Norepinephrine in the prefrontal cortex is critical for amphetamine-induced reward and mesoaccumbens dopamine release. J Neurosci. 2003;23(5):1879–85.
9. Ventura R, Alcaro A, Puglisi-Allegra S. Prefrontal cortical norepinephrine release is critical for morphine-induced reward, reinstatement and dopamine release in the nucleus accumbens. Cereb Cortex. 2005;15(12):1877–86.
10. Ventura R, Morrone C, Puglisi-Allegra S. Prefrontal/accumbal catecholamine system determines motivational salience attribution to both reward- and aversion-related stimuli. Proc Natl Acad Sci U S A. 2007;104(12):5181–6.
11. Latagliata EC, Patrono E, Puglisi-Allegra S, Ventura R. Food seeking in spite of harmful consequences is under prefrontal cortical noradrenergic control. BMC Neurosci. 2010;11:15. doi:10.1186/1471-2202-11-15.
12. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev. 1998;28(3):309–69.
13. Adams E, Klug J, Quast M, Stairs DJ. Effects of environmental enrichment on nicotine-induced sensitization and cross-sensitization to d-amphetamine in rats. Drug Alcohol Depend. 2013;129(3):247–53. doi:10.1016/j.drugalcdep.2013.02.019.
14. Harb MR, Almeida OFX. Pavlovian conditioning and cross-sensitization studies raise challenges to the hypothesis that overeating is an addictive behavior. Transl Psychiatry. 2014;4:e387. doi:10.1038/tp.2014.28.
15. Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev. 1993;18(3):247–91.
16. Koob GF. Animal models of craving for ethanol. Addiction. 2000;95(Suppl 2):S73–81.
17. Parylak SL, Koob GF, Zorrilla EP. The dark side of food addiction. Physiol Behav. 104(1):149–56. doi:10.1016/j.physbeh.2011.04.063.
18. Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology. 2010;35(1):217–38. doi:10.1038/npp.2009.110.
19. Piazza PV, Deroche-Gamonet V. A multistep general theory of transition to addiction. Psychopharmacology (Berl). 2013;229(3):387–413. doi:10.1007/s00213-013-3224-4.
20. Volkow ND, Wise RA. How can drug addiction help us understand obesity? Nat Neurosci. 2005;8(5):555–60.
21. Volkow ND, Wang GJ, Fowler JS, Telang F. Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. Philos Trans R Soc Lond B Biol Sci. 2008;363(1507):3191–200. doi:10.1098/rstb.2008.0107.
22. Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. Trends Cogn Sci. 2011;15(1):37–46. doi:10.1016/j.tics.2010.11.001.
23. Di Chiara G, Imperato A. Drugs abused by humans preferntially increase synaptic dopamine concentrations in the mesolimbico system of freely moving rats. Proc Natl Acad Sci U S A. 1988;85(14):5274–8.
24. Wise RA, Rompre PP. Brain dopamine and reward. Annu Rev Psychol. 1989;40:191–225.
25. Pontieri FE, Tanda G, Di Chiara G. Intravenous cocaine, morphine and amphetaemine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. Proc Natl Acad Sci U S A. 1995;92(26):12304–8.
26. Koob GF. A role for brain stress systems in addiction. Neuron. 2008;59(1):11–34. doi:10.1016/j.neuron.2008.06.012.
27. Singh T, McDannald MA, Haney RZ, Cerri DH, Schoenbaum G. Nucleus accumbens core and shell are necessary for reinforcer devaluation effects on pavlovian conditioned responding. Front Integr Neurosci. 2010;4:126. doi:10.3389/fnint.2010.00126.
28. Castro DC, Cole SL, Berridge KC. Lateral hypothalamus, nucleus accumbens, and ventral pallidum

roles in eating and hunger: interactions between homeostatic and reward circuitry. Front Syst Neurosci. 2015;9:90. doi:10.3389/fnsys.2015.00090.
29. Peciňa S, Smith KS, Berridge KC. Hedonic hot spots in the brain. Neuroscientist. 2006;12(6):500–11.
30. Puglisi-Allegra S, Ventura R. Prefrontal/accumbal cathecolamine system processes high motivational salience. Front Behav Neurosci. 2012;6:31. doi:10.3389/fnbeh.2012.00031.
31. Di Marzo V, Ligresti A, Cristino L. The endocannabinoid system as a link between homoeostatic and hedonic pathways involved in energy balance regulation. Int J Obes (Lond). 2009;33(Suppl 2):S18–24. doi:10.1038/ijo.2009.67.
32. Jay TM. Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. Prog Neurobiol. 2003;69(6):375–90.
33. Schultz W. Predictive reward signal of dopamine neurons. J Neurophysiol. 1998;80(1):1–27.
34. Torregrosa MM, Gordon J, Taylor JR. Double dissociation between the anterior cingulate cortex and nucleus accumbens core in encoding the context versus the content of pavlovian cocaine cue extinction. J Neurosci. 2013;33(19):8370–7. doi:10.1523/JNEUROSCI.0489-13.2013.
35. Saddoris MP, Carelli RM. Cocaine self-administration abolishes associative neural encoding in the nucleus accumbens necessary for higher-order learning. Biol Psychiatry. 2014;75(2):156–64. doi:10.1016/j.biopsych.2013.07.037.
36. Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry. 2005;162(8):1403–13.
37. Haber SN, Fudge JL, McFarland NR. Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci. 2000;20(6):2369–82.
38. Haber SN. The primate basal ganglia: parallel and integrative networks. J Chem Neuroanat. 2003;26(4):317–30.
39. Berke JD. Fast oscillations in cortical-striatal networks switch frequency following rewarding events and stimulant drugs. Eur J Neurosci. 2009;30(5):848–59. doi:10.1111/j.1460-9568.2009.06843.x.
40. Ren X, Ferreira JG, Zhou L, Shammah-Lagnado SJ, Jeckel CW, de Araujo IE. Nutrient selection in the absence of taste receptor signaling. J Neurosci. 2010;30(23):8012–23. doi:10.1523/JNEUROSCI.5749-09.2010.
41. Wiltschko AB, Pettibone JR, Berke JD. Opposite effects of stimulant and antipsychotic drugs on striatal fast-spiking interneurons. Neuropsychopharmacology. 2010;35(6):1261–70. doi:10.1038/npp.2009.226.
42. Meredith GE. The synaptic framework for chemical signaling in nucleus accumbens. Ann N Y Acad Sci. 1999;877:140–56.
43. Matsumoto J, Urakawa S, Hori E, de Araujo MF, Sakuma Y, Ono T, et al. Neuronal responses in the nucleus accumbens shell during sexual behavior in male rats. J Neurosci. 2012;32(5):1672–86. doi:10.1523/JNEUROSCI.5140-11.2012.
44. Tepper JM, Plenz D. Microcircuits in the striatum: striatal cell types and their interaction. In: Grillner S, Graybiel AM, editors. Microcircuits: the interface between neurons and global brain function. Cambridge: MIT; 2006. p. 127–48.
45. Lansink CS, Goltstein PM, Lankelma JV, Pennartz CM. Fast-spiking interneurons of the rat ventral striatum: temporal coordination of activity with principal cells and responsiveness to reward. Eur J Neurosci. 2010;32(3):494–508. doi:10.1111/j.1460-9568.2010.07293.x.
46. Cacciapaglia F, Wightman RM, Carelli RM. Rapid dopamine signaling differentially modulates distinct microcircuits within the nucleus accumbens during sucrose-directed behavior. J Neurosci. 2011;31(39):13860–9. doi:10.1523/JNEUROSCI.1340-11.2011.
47. Nishijo H, Uwano T, Ono T. Representation of taste stimuli in the brain. Chem Senses. 2005;30(Suppl 1):i174–5.
48. Shimura T, Imaoka H, Okazaki Y, Kanamori Y, Fushiki T, Yamamoto T. Involvement of the mesolimbic system in palatability-induced ingestion. Chem Senses. 2005;30(Suppl 1):i188–9.
49. Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci. 2005;8(11):1481–9.
50. Alderson HL, Robbins TW, Everitt BJ. Heroin self-administration under a second-order schedule of reinforcement: acquisition and maintenance of heroin-seeking behaviour in rats. Psychopharmacology (Berl). 2000;153(1):120–33.
51. Arroyo M, Markou A, Robbins TW, Everitt BJ. Acquisition, maintenance and reinstatement of intravenous cocaine self-administration under a second-order schedule of reinforcement in rats: effects of conditioned cues and continuous access to cocaine. Psychopharmacology (Berl). 1998;140(3):331–44.
52. Everitt BJ, Dickinson A, Robbins TW. The neuropsychological basis of addictive behaviour. Brain Res Brain Res Rev. 2001;36(2-3):129–38.
53. Gasbarri A, Pompili A, Packard MG, Tomaz C. Habit learning and memory in mammals: behavioral and neural characteristics. Neurobiol Learn Mem. 2014;114:198–208. doi:10.1016/j.nlm.2014.06.010.
54. Dickinson A, Smith S, Mirenowicz J. Dissociation of Pavlovian and instrumental incentive learning under dopamine antagonists. Behav Neurosci. 2000;114(3):468–83.
55. Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev. 2002;26(3):321–52.
56. Dalley JW, Everitt BJ, Robbins TW. Impulsivity, compulsivity and top-down cognitive control. Neuron. 2011;69(4):680–94. doi:10.1016/j.neuron.2011.01.020.

57. Parkinson JA, Cardinal RN, Everitt BJ. Limbic cortical–ventral striatal systems underlying appetitive conditioning. Prog Brain Res. 2000;126:263–85.
58. Di Ciano P, Everitt BJ. Direct interactions between basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. J Neurosci. 2004;24(32):7167–73.
59. Corbit LH, Balleine BW. Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. J Neurosci. 2005;25(4):962–70.
60. Hyman SE, Malenka RC. Addiction and the brain: the neurobiology of compulsion and its persistence. Nat Rev Neurosci. 2001;2(10):695–703.
61. Tomaz C, Dickinson-Anson H, McGaugh JL. Basolateral amygdala lesions block diazepam-induced anterograde amnesia in an inhibitory avoidance task. Proc Natl Acad Sci U S A. 1992;89(8):3615–9.
62. Tomaz C, Dickinson-Anson H, McGaugh JL, Souza-Silva MA, Viana MB, Graeff EG. Localization in the amygdala of the amnestic action of diazepam on emotional memory. Behav Brain Res. 1993;58(1-2):99–105.
63. Milton AL, Lee JL, Everitt BJ. Reconsolidation of appetitive memories for both natural and drug reinforcement is dependent on β-adrenergic receptors. Learn Mem. 2008;15(2):88–92. doi:10.1101/lm.825008.
64. Paton JJ, Belova MA, Morrison SE, Salzman CD. The primate amygdala represents the positive and negative value of visual stimuli during learning. Nature. 2006;439(7078):865–70.
65. See RE, Kruzich PJ, Grimm JW. Dopamine, but not glutamate, receptor blockade in the basolateral amygdala attenuates conditioned reward in a rat model of relapse to cocaine-seeking behavior. Psychopharmacology (Berl). 2001;154(3):301–10.
66. Di Ciano P, Everitt BJ. Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. Neuropsychopharmacology. 2001;25(3):341–60.
67. Neisewander JL, O'Dell LE, Tran-Nguyen LT, Castañeda E, Fuchs RA. Dopamine overflow in the nucleus accumbens during extinction and reinstatement of cocaine self-administration behavior. Neuropsychopharmacology. 1996;15(5):506–14.
68. McFarland K, Davidge SB, Lapish CC, Kalivas PW. Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. J Neurosci. 2004;24(7):1551–60.
69. Parsegian A, See RE. Dysregulation of dopamine and glutamate release in the prefrontal cortex and nucleus accumbens following methamphetamine self-administration and during reinstatement in rats. Neuropsychopharmacology. 2014;39(4):811–22. doi:10.1038/npp.2013.231.
70. Belin D, Belin-Rauscent A, Murray JE, Everitt BJ. Addiction: failure of control over maladaptive incentive habits. Curr Opin Neurobiol. 2013;23(4):564–72. doi:10.1016/j.conb.2013.01.025.
71. Bechara A, Damasio H, Damasio AR. Emotion, decision making and the orbitofrontal cortex. Cereb Cortex. 2000;10(3):295–307.
72. Belin D, Everitt BJ. Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. Neuron. 2008;57(3):432–41. doi:10.1016/j.neuron.2007.12.019.
73. Faure A, Haberland U, Conde F, El Massioui N. Lesion to the nigrostriatal dopamine system disrupts stimulus–response habit formation. J Neurosci. 2005;25(11):2771–80.
74. Yin HH, Knowlton BJ, Balleine BW. Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci. 2004;19(1):181–9.
75. Yin HH, Ostlund SB, Knowlton BJ, Balleine BW. The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci. 2005;22(2):513–23.
76. Koob GF. Brain stress systems in the amygdala and addiction. Brain Res. 2009;1293:61–75. doi:10.1016/j.brainres.2009.03.038.
77. Koob GF. Addiction is a reward deficit and stress surfeit disorder. Front Psych. 2013;4:72. doi:10.3389/fpsyt.2013.00072.
78. Jennings JH, Sparta DR, Stamatakis AM, Ung RL, Pleil KE, Kash TL, et al. Distinct extended amygdala circuits for divergent motivational states. Nature. 2013;496(7444):224–8. doi:10.1038/nature12041.
79. Stamatakis AM, Sparta DR, Jennings JH, McElligott ZA, Decot H, Stuber GD. Amygdala and bed nucleus of the stria terminalis circuitry: implications for addiction-related behaviors. Neuropharmacology. 2014;76Pt B:320–8. doi:10.1016/j.neuropharm.2013.05.046.
80. Gabriele A, Packard MG. Evidence of a role for multiple memory systems in behavioral extinction. Neurobiol Learn Mem. 2006;85(3):289–99.
81. Packard MG, Knowlton BJ. Learning and memory functions of the basal ganglia. Annu Rev Neurosci. 2002;25:563–93.
82. White NM, McDonald RJ. Multiple parallel memory systems in the brain of the rat. Neurobiol Learn Mem. 2002;77(2):125–84.
83. Squire LR. Memory systems of the brain: a brief history and current perspective. Neurobiol Learn Mem. 2004;82(3):171–7.
84. Gabriele A, Setlow B, Packard MG. Cocaine self-administration alters the relative effectiveness of multiple memory systems during extinction. Learn Mem. 2009;16(5):296–9. doi:10.1101/lm.1253409.
85. Tiffany ST. A cognitive model of drug urges and drug-use behavior: role of automatic and nonautomatic processes. Psychol Rev. 1990;97(2):147–68.
86. Packard MG. Glutamate infused posttraining into the hippocampus or caudate-putamen differentially

strengthens place and response learning. Proc Natl Acad Sci U S A. 1999;96(22):12881–6.
87. Porrino LJ, Lyons D, Smith HR, Daunais JB, Nader MA. Cocaine self-administration produces a progressive involvement of limbic, association, and sensorimotor striatal domains. J Neurosci. 2004;24(14):3554–62.
88. Davis M, Whalen PJ. The amygdala: vigilance and emotion. Mol Psychiatry. 2001;6(1):13–34.
89. Charney DS. Neuroanatomical circuits modulating fear and anxiety behaviors. Acta Psychiatr Scand Suppl. 2003;417:38–50.
90. Packard MG, Cahill L, McGaugh JL. Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. Proc Natl Acad Sci U S A. 1994;91(18):8477–81.
91. Packard MG, Teather LA. Amygdala modulation of multiple memory systems: hippocampus and caudate–putamen. Neurobiol Learn Mem. 1998;69(2):163–203.
92. McGaugh JL. The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci. 2004;27:1–28.
93. Packard MG, Wingard JC. Amygdala and "emotional" modulation of the relative use of multiple memory systems. Neurobiol Learn Mem. 2004;82(3):243–52.
94. Wingard JC, Packard MG. The amygdala and emotional modulation of competition between cognitive and habit memory. Behav Brain Res. 2008;193(1):126–31. doi:10.1016/j.bbr.2008.05.002.
95. Pitkänen A, Pikkarainen M, Nurminen N, Ylinen A. Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Ann N Y Acad Sci. 2000;911:369–91.
96. Jolkonnen E, Pikkarainen M, Kemppainen S, Pitkänen A. Interconnectivity between the amygdaloid complex and the amygdalostriatal transition area: a PHA-L study in rat. J Comp Neurol. 2001;431(1):39–58.
97. Packard MG, McGaugh JL. Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. Neurobiol Learn Mem. 1996;65(1):65–72.
98. Poldrack RA, Packard MG. Competition among multiple memory systems: converging evidence from animal and human brain studies. Neuropsychologia. 2003;41(3):245–51.
99. Gold PE. Coordination of multiple memory systems. Neurobiol Learn Mem. 2004;82(3):230–42.
100. McDonald RJ, Hong NS, Devan BD. The challenges of understanding mammalian cognition and memory-based behaviours: an interactive learning and memory systems approach. Neurosci Biobehav Rev. 2004;28(7):719–45.
101. Lang PJ, Davis M, Ohman A. Fear and anxiety: animal models and human cognitive psychophysiology. J Affect Disord. 2000;61(3):137–59.
102. LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci. 2000;23:155–84.
103. McIntyre CK, Power AE, Roozendaal B, McGaugh JL. Role of the baso-lateral amygdala in memory consolidation. Ann N Y Acad Sci. 2003;985:273–93.
104. Roozendaal B, McEwen BS, Chattarij S. Stress, memory and the amydgala. Nat Rev Neurosci. 2009;10(6):423–33. doi:10.1038/nrn2651.
105. Sun W, Rebec GV. Repeated cocaine self-administration alters processing of cocaine-related information in rat prefrontal cortex. J Neurosci. 2006;26(30):8004–8.
106. Ary AW, Lominac KD, Wroten MG, Williams AR, Campbell RR, Ben-Shahar O, et al. Imbalances in prefrontal cortex CC-Homer1 versus CC-Homer2 expression promote cocaine preference. J Neurosci. 2013;33(19):8101–13. doi:10.1523/JNEUROSCI.1727-12.2013.
107. Mahler SV, Berridge KC. What and when to "want"? Amygdala-based focusing of incentive salience upon sugar and sex. Psychopharmacology (Berl). 2012;221(3):407–26. doi:10.1007/s00213-011-2588-6.
108. Ventura R, Alcaro A, Cabib S, Conversi D, Mandolesi L, Puglisi-Allegra S. Dopamine in the medial prefrontal cortex controls genotype-dependent effects of amphetamine on mesoaccumbens dopamine release and locomotion. Neuropsychopharmacology. 2004;29(1):72–80.
109. Zocchi A, Orsini C, Cabib S, Puglisi-Allegra S. Parallel strain-dependent effect of amphetamine on locomotor activity and dopamine release in the nucleus accumbens: an in vivo study in mice. Neuroscience. 1998;82(2):521–8.
110. Cabib S, Orsini C, Le Moal M, Piazza PV. Abolition and reversal of strain differences in behavioral responses to drugs of abuse after a brief experience. Science. 2000;289(5478):463–5.
111. Di Segni M, Patrono E, Patella L, Puglisi-Allegra S, Ventura R. Animal models of compulsive eating behavior. Nutrients. 2014;6(10):4591–609. doi:10.3390/nu6104591.
112. Patrono E, Di Segni M, Patella L, Andolina D, Valzania A, Latagliata EC, et al. When chocolate seeking becomes compulsion: gene–environment interplay. PLoS One. 2015;10(3):e0120191. doi:10.1371/journal.pone.0120191.
113. Tassin JP. Norepinephrine–dopamine interactions in the prefrontal cortex and the ventral tegmental area: relevance to mental diseases. Adv Pharmacol. 1998;42:712–6.
114. Feenstra MG, Botterblom MH, Mastenbroek S. Dopamine and noradrenaline efflux in the prefrontal cortex in the light and dark period: effects of novelty and handling and comparison to the nucleus accumbens. Neuroscience. 2000;100(4):741–8.
115. Dalley JW, Cardinal RN, Robbins TW. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. Neurosci Biobehav Rev. 2004;28(7):771–84.
116. Mingote S, de Bruin JP, Feenstra MG. Noradrenaline and dopamine efflux in the prefrontal cortex in rela-

tion to appetitive classical conditioning. J Neurosci. 2004;24(10):2475–80.
117. Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus–norepinephrine function: adaptive gain and optimal performance. Annu Rev Neurosci. 2005;28:403–50.
118. Robbins TW, Arnsten AF. The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. Annu Rev Neurosci. 2009;32:267–87. doi:10.1146/annurev.neuro.051508.135535.
119. Page ME, Lucki I. Effects of acute and chronic reboxetine treatment on stress-induced monoamine efflux in the rat frontal cortex. Neuropsychopharmacology. 2002;27(2):237–47.
120. van der Meulen JA, Joosten RN, de Bruin JP, Feenstra MG. Dopamine and noradrenaline efflux in the medial prefrontal cortex during serial reversals and extinction of instrumental goal-directed behavior. Cereb Cortex. 2007;17(6):1444–53.
121. Nicniocaill B, Gratton A. Medial prefrontal cortical alpha1 adrenoreceptor modulation of the nucleus accumbens dopamine response to stress in Long–Evans rats. Psychopharmacology (Berl). 2007;191(3):835–42.
122. Mitrano DA, Schroeder JP, Smith Y, Cortright JJ, Bubula N, Vezina P, et al. α-1 Adrenergic receptors are localized on presynaptic elements in the nucleus accumbens and regulate mesolimbic dopamine transmission. Neuropsychopharmacology. 2012;37(9):2161–72. doi:10.1038/npp.2012.68.
123. Devoto P, Flore G, Pira L, Diana M, Gessa GL. Co-release of noradrenaline and dopamine in the prefrontal cortex after acute morphine and during morphine withdrawal. Psychopharmacology (Berl). 2002;160(2):220–4.
124. Greba Q, Gifkins A, Kokkinidis L. Inhibition of amygdaloid dopamine D2 receptors impairs emotional learning measured with fear-potentiated startle. Brain Res. 2001;899(1-2):218–26.
125. Guarraci FA, Frohardt RJ, Young SL, Kapp BS. A functional role for dopamine transmission in the amygdala during conditioned fear. Ann N Y Acad Sci. 1999;877:732–6.
126. Rosenkranz JA, Grace AA. Cellular mechanisms of infralimbic and prelimbic prefrontal cortical inhibition and dopaminergic modulation of basolateral amygdala neurons in vivo. J Neurosci. 2002;22(1):324–37.
127. Stevenson CW, Gratton A. Basolateral amygdala modulation of the nucleus accumbens dopamine response to stress: role of the medial prefrontal cortex. Eur J Neurosci. 2003;17(6):1287–95.
128. Floresco SB, Tse MT. Dopaminergic regulation of inhibitory and excitatory transmission in the basolateral amygdala–prefrontal cortical pathway. J Neurosci. 2007;27(8):2045–57.
129. Ito R, Canseliet M. Amphetamine exposure selectively enhances hippocampus-dependent spatial learning and attenuates amygdala-dependent cue learning. Neuropsychopharmacology. 2010;35(7):1440–52. doi:10.1038/npp.2010.14.
130. Läck AK, Diaz MR, Chappell A, DuBois DW, McCool BA. Chronic ethanol and withdrawal differetially modulate pre- and post-synaptic function at glutamatergic synapses in rat basolateral amygdala. J Neurophysiol. 2007;98(6):3185–96.
131. Smith RJ, Aston-Jones G. Noradrenergic transmission in the extended amygdala: role in increased drug-seeking and relapse during protracted drug abstinence. Brain Struct Funct. 2008;213(1-2):43–61. doi:10.1007/s00429-008-0191-3.
132. Barros M, Giorgetti M, Souto AA, Vilela G, Santos K, Boas NV, et al. Persistent anxiety-like behavior in marmosets following a recent predatory stress condition: reversal by diazepam. Pharmacol Biochem Behav. 2007;86(4):705–11.
133. Barros M, Maior RS, Houston JP, Tomaz C. Predatory stress as an experimental strategy to measure fear and anxiety-related behaviors in non-human primates. Rev Neurosci. 2008;19(2-3):157–69.
134. Brady KT, Sinha R. Co-occurring mental and substance use disorders: the neurobiological effects of chronic stress. Am J Psychiatry. 2005;162(8):1483–93.
135. Dumont EC, Williams JT. Noradrenaline triggers GABAA inhibition of bed nucleus of the stria terminalis neurons projecting to the ventral tegmental area. J Neurosci. 2004;24(38):8198–204.
136. Gilpin NW, Koob GF. Effects of β-adrenoceptor antagonists on alcohol drinking by alcohol-dependent rats. Psychopharmacology (Berl). 2010;212(3):431–9. doi:10.1007/s00213-010-1967-8.
137. Bermudez MA, Schultz W. Timing in reward and decision processes. Philos Trans R Soc Lond B Biol Sci. 2014;369(1637):20120468. doi:10.1098/rstb.2012.0468.
138. Bermudez MA, Göbel C, Schultz W. Sensitivity to temporal structure in amygdala neurons. Curr Biol. 2012;22(19):1839–44. doi:10.1016/j.cub.2012.07.062.

17 Stress and the Dynamic Fear Memory: Synaptic–Cellular Bases and Their Implication for Psychiatry Disorders

Gastón Diego Calfa, Marcelo Giachero, and Víctor Alejandro Molina

Abstract

There is consensus that the acquisition and storage of relevant aversive information allows organisms to cope with threat situations. Such a mnemonic process is supported by lasting modifications in the aversive neuronal circuitry, resulting in changes in the behavioral response. In this way, the capacity to form long-lasting emotional memories makes it possible to predict and anticipate a potential threat in future situations, thus favoring, from an evolutionary point of view, survival conditions.

In this context, one of the relevant questions is how the perturbations to the modulatory mechanism involved in the adaptive response result in an excessive and inappropriate state of fear and anxiety.

Associative learning related to the emergence of a long-lasting fear memory is critically implicated in the pathogenesis of anxiety disorders, including post-traumatic stress disorder, phobia, and panic. Consonant with such a view, most of the symptoms of these psychiatry entities are due to the persistence and the re-experience of traumatic memories.

Consequently, understanding the neurobiological changes associated with the formation of long-lasting fear memory under particular negative emotional states is relevant for the comprehension of the underlying mechanisms involved in the occurrence of traumatic and persistent memories, as well as for the rebuilding of potential therapeutic tools that could reestablish the adaptive dynamic of the fear memory trace.

G.D. Calfa, PhD (✉) • V.A. Molina
IFEC-CONICET, Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, Córdoba 5000, Argentina
e-mail: gcalfa@fcq.unc.edu.ar

M. Giachero, PhD
Laboratory of Behavioral Neurobiology, Center of Biological Sciences, Federal University of Santa Catarina, Florianopolis, SC, Brazil

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_17

In this chapter, we focus on the relevant outcomes observed in animal models of fear learning and memory and their interaction with stressful experiences, along with the observations performed in humans suffering the psychiatric illnesses previously mentioned.

Keywords
Stress • Fear Memory • Psychiatry Disorders • Structural Plasticity

Introduction

The formation of a long-term memory has a crucial role in the adaptive behavioral repertoire of different organisms to subsist in a complex environment and to anticipate future encounters with potential threat events. Following this line of reasoning, fear learning constitutes the mechanism that promotes, from an evolutionary point of view, survival conditions [1]. However, fear-promoting experience might lead to a negative emotional state [2]. This state is evident in individuals suffering from anxiety disorders such as phobia, panic disorder, or posttraumatic stress disorder (PTSD) in which the characteristic symptomatology is the re-experiencing of the aversive events, the avoidance of such events, and hyper-arousal.

Classically, one of the animal models widely used in the study of fear memory formation is Pavlovian contextual fear conditioning. In this, an animal learns to associate an unconditional stimulus (US; e.g., electric shock) with another stimulus that has no biological relevance by itself (conditional stimulus [CS]; e.g., light, sound, context). Once the memory is formed, simple re-exposure to the CS elicits a fear response [3]. It is important to highlight that the term "fear" used for animal models refers specifically to the measurable behavior induced by the threat [4]. The classic behavioral output that is evaluated as fear response is freezing, characterized by a crouching position with the absence of any movement except for those which are respiratory-related [5].

One of the main questions in the study of neuroscience is how the organism processes and stores any biologically relevant information for survival purposes.

In this respect, any situation or stimulus that represents a threat or any other potentially dangerous stimuli is processed by different and particular brain areas [6]. Thus, it is postulated that neurobiological changes induced by threat experiences, together with those associated with processes of acquisition/consolidation of the fear memory, underlie the psychopathologies mentioned above.

The Dynamic Fear Memory

As stated several times, "…we are mainly the consequence of our past experiences...." In other words, memories shape our identity and critically influence our emotional reactions to the changing environment. Thus, the different situations to which an organism is exposed along its vital cycle underlie its individual characteristics. Learning and memory processes are built on the basis of such a statement. During this cycle, the organism acquires diverse behavioral and biological responses to cope with future threat events.

The concept of learning and memory has received a dedicated analysis through the history of science. Both behavioral and neurobiological scientists have built the foundations of the actual concepts, nevertheless always under continuous debate and far from achieving a serene consensus.

From a cognitive "psychological" framework, *learning* refers to the acquisition of information about the environment, with the subsequent formation of a memory trace [7].

From a "neurobiological" point of view, this term refers to the neuronal change that encodes the memory trace.

In relation to memory, the most common definitions refer to the "lasting changes in the behavior of an individual as a result of their past experiences." Currently, the most widely used and accepted concept of memory is of the "internal representation." Basically, this concept refers to the structured information from previous experiences encoded on particular neuronal circuits [8]. Therefore, memory is the retention over time of experience-dependent internal representations that could take control of behavior [7].

From the point of view of memory as *remembering*, Tulving and Thompson argue that memory is similar to the perception, in the sense that it involves understanding of the current stimuli based on past experiences. Consequently, a new memory to be remembered in a meaningful manner should be consolidated within a related preexisting memory [9].

The Different Phases of Fear Memory

A large body of evidence has maintained that following an initial learning experience, the recently acquired information is in an unstable state, which is stabilized by a process termed "consolidation." This process is commonly addressed at two levels of description and analysis: the cellular/synaptic level and the brain system level. Synaptic consolidation refers to the post-encoding transformation of the information acquired into a long-term form at local synaptic and cellular nodes in the brain circuitry that encodes the memory trace. System consolidation refers to the temporal post-encoding reorganization of long-term memory over distributed brain circuits. In this work, we focus on the synaptic consolidation since system consolidation is beyond the scope of this chapter. In contrast to the view that following consolidation memory is static or fixed, considerable behavioral and neurobiological evidence now indicates that memory is quite malleable. In fact, memory recall by filing a reminder stimulus (a key or signal associated with the original learning) can induce an additional unstable phase requiring an active process to stabilize such a memory trace again. This process has been defined as reconsolidation, and has been proposed as an important component of long-term memory processing.

Currently, it is well accepted that memory is primarily a dynamic encoding process. To understand the mechanisms involved in the formation of long-term memory, it is essential to elucidate the nature and the temporal evolution of the biological changes that accompany the process of acquisition, storage, retrieval, and reconsolidation [7].

Memory Consolidation

In 1900, Müller and Pilzecker, performing studies on human behavior, found that memory of recently learned information was interrupted by the learning of a different piece of information shortly after the original one [10]. This finding suggested that the process underlying new memories initially remains in a fragile state, and that consolidation occurs over time. Meanwhile, several years earlier, the French psychologist Théodule-Armand Ribot had already reported that the probability of amnesia by brain trauma was greater for recent than for more remote memories [11]. These studies, along with many others which came years later, constituted the main foundation of the phenomenon of retrograde amnesia, giving rise to two memory models, the synaptic and the systemic consolidation models.

Synaptic consolidation includes cellular and molecular phenomena accompanying changes in synaptic efficacy after a learning experience [8, 12]. In fact, learning experience leads to the activation of intracellular signaling cascades, resulting in post-translational modifications, the modulation of gene expression and the synthesis of gene products leading to changes in synaptic efficacy. According to this model, the higher the progress of these processes is, the lower is the probability that a memory change occurs by applying an amnesic treatment [13]. Different pharmacological, genetic, and lesion strategies have made possible to clarify, in part, the neuronal and the molecular processes underlying

memory consolidation [14]. Diverse molecular events closely associated to synaptic consolidation have been used to describe this phenomenon. Accordingly, researchers have described the involvement of different glutamate receptors, transcription factors [cAMP-responsive element binding protein (CREB), transcription factor CCAAT enhancer binding protein (C/EBPβ), c-fos, nuclear factor-kappa B (NF-kB), Zif-268, and brain-derived neurotrophic factor (BDNF)], and kinases (protein kinase A, PKA). In addition, other effectors such as activity-regulated cytoskeletal protein (Arc) and tissue plasminogen activator (t-PA) were also used [15–17].

Memory Retrieval

Memory retrieval is a complex process by which the previously acquired information can be used. In other words, it is the only evidence that memory exists.

From a cognitive framework, Endel Tulving proposed that the retrieval can be subdivided into two separate processes: reactivation and conversion. Reactivation (Tulving called this "ecphory") refers to the process whereby retrieval information, provided by a cue, is correlated with information stored in a memory trace. The product of this correlation enables the second stage of retrieval: conversion. This stage covers the subjective experience of reminder and the corresponding memory performance [9]. From a neurobiological point of view, reactivation is also considered as the initial stage of retrieval, and corresponds to the activation of the neural systems that encode the memory trace. Operationally, this phase results from the re-exposure to the information acquired during training. Thus, the reactivation involves the passage of a memory from an inactive to an active state, which in some instances can be manifested behaviorally [18]. In this context, the behavioral performance should not serve as a defining attribute for relevant memory formation.

As discussed below, reconsolidation researchers propose that retrieval of consolidated memories can destabilize the memory and thus induce an unstable state. In order to persist, a restabilization process termed reconsolidation is required, which is dependent on new protein synthesis [13, 19] and permits memory updating [20, 21]. Accordingly, memory retrieval has been used primarily as a tool to study the reconsolidation process. However, it is appropriate to reconsider retrieval as a process by itself, that is, not only as a preliminary stage of reconsolidation, but as a different stage of memory processing that plays a key role with respect to preexisting memories.

As previously noted [13, 22], retrieval is not a passive readout of prior experiences; rather, it can turn memory into a transient plasticity enabling dynamic modifications of the established memory trace [23–26]. However, it should be noted that retrieval does not always lead to a reconsolidation process [27]. Under certain circumstances, for instance, new information can be updated into the original trace and its strength can be significantly modified [21, 28, 29].

Memory Reconsolidation

It has long been argued that a newly acquired memory irreversibly passes over time from a labile to a stable state where it remains resistant to the effects of any manipulation, pointing to consolidation as a unitary process [20]. As noted above, this standard theory of consolidation was challenged when Donald Lewis revealed post-retrieval amnesia, a phenomenon referred to as "cue-dependent amnesia" [18]. Lewis and colleagues observed that memory was susceptible to the amnesic effects of electroconvulsive shock after the presentation of a signal associated with learning [30].

This additional labile retrieval-induced phase did not receive attention until 1997, when it was described that the retrieval of a well-established spatial memory was dependent on NMDA receptors (N-methyl-D-aspartate) to maintain stability [31]. Then, the "reconsolidation" concept was introduced to explain the process by which previously consolidated memories are stabilized again after retrieval [23]. In recent years, the reconsolidation

theory has received greater theoretical attention and further experimental confirmation. This particular memory phase has been shown in a wide range of species (including humans), in different behavioral tasks and brain regions and by using diverse amnesic and memory-enhancing agents [32–37]. For instance, Bustos and colleagues among others demonstrated that systemic administration of the benzodiazepine midazolam after retrieval resulted in memory deficit in a subsequent test [38]. No memory disturbances were observed in the absence of retrieval. Moreover, no interfering effect was noticeable after the reconsolidation/stabilization window was closed. Reconsolidation does not seem to occur every time that a consolidated memory is reactivated. It is more likely to occur when new information becomes available in the retrieval situation. Even more, there are certain conditions where this process either does not appear to occur or is highly limited. These boundary conditions place constraints on the emergence of both retrieval-induced instability and the restabilization process. In other words, when retrieved, and under certain conditions, they can return to an unstable state and need to be reconsolidated to persist. For instance, older [39, 40] and stronger [41] memories, as well as those reactivated for a short period [39, 42] are less susceptible to engage the labilization/reconsolidation process.

Importantly, it has been proposed that memory reconsolidation may serve as an adaptive function. In fact, a malleable phase following retrieval could be critical for maintaining memory relevance in a changing environment, allowing the incorporation of new or additional information and thus enabling an immediate re-calibration of the existing memory [26]. However, as discussed above, it is suggested that retrieval could generate a transient plasticity state during which a memory would be modified and/or updated without requiring a reconsolidation process [27].

Despite the evidence suggesting that memories could be updated after retrieval, it is not entirely clear whether this update depends on the consolidation or reconsolidation process. It has been indicated that reconsolidation occurs only in conjunction with the incorporation of new information, i.e., accumulating findings support the notion that the availability of new information during retrieval is a requirement for the occurrence of reconsolidation [28].

A method for examining the reconsolidation role in memory updating is to identify differential mechanisms of reconsolidation and consolidation (see section "Consolidation vs Reconsolidation"). In this regard, Lee (2010) showed that reconsolidation supports the update of a hippocampal-dependent contextual memory [21].

Thus, understanding the molecular mechanisms of reconsolidation could provide crucial clues about the dynamic aspects of normal mnemonic function. In this context, psychiatric disorders can be characterized by exceptionally robust and persistent emotional memories.

Memory recall can induce a state in which synapses seem to require the synthesis of new proteins, as inhibition of this post-retrieval process results in a degree of amnesia for retrieved memory [32].

With regard to biological events, several transcription factors have been implicated in memory reconsolidation: CREB, NF-kB, EtsLiKe gene1 (ELK1), Zif268, and C/EBPβ [43–47]. Transcription factors are phosphorylated by kinases. The extracellular signal-regulated kinase (ERK) is required for the reconsolidation of conditioned fear [48], object-recognition [49], and conditioned place preference memories [45]. PKA is also necessary for the reconsolidation of conditioned fear memories [50].

As previously suggested, the reconsolidation process is composed of two distinctive and mechanistically different phases; namely, a reactivation-induced destabilization and a subsequent restabilization process. Currently, there is intensive research aimed at identifying the molecular mechanisms involved in the induction of a state from consolidated to labile, essential for the occurrence of memory reconsolidation. In this respect, NMDA receptors containing GluN2B subunits in baso-lateral amygdala (BLA) have been reported to be crucial for memory destabilization. Consistent with the fact that GluN2B subunits are required for memory destabilization, intra-BLA administration of a selective antagonist of this NMDA subtype prevents the

instability induced by fear memory reactivation [51, 52]. Furthermore, this particular NMDA receptor subtype is critically involved in the protein degradation by the ubiquitin/proteasome required for memory destabilization after reactivation [53–55]. Consistently, inhibitors of proteasome activity block reactivation-induced destabilization.

In sum, subunits of the NMDA receptors such as GluN2B in the amygdala [51, 52], protein degradation [55] , and cannabinoid receptors CB1 in the hippocampus [56] have been identified as crucial for retrieval-induced labilization of consolidated fear memory.

Consolidation vs Reconsolidation

Despite its name, reconsolidation is not a faithful recapitulation of consolidation. Although there are common mechanisms at the cellular and molecular level, recent evidence has shown specific ones for consolidation and reconsolidation.

Clear differences between consolidation and reconsolidation are presented in the activation profile of transcription factors. In the dorsal hippocampus (DH), there is a double dissociation between brain-derived neurotrophic factor (BDNF), selectively required for consolidation, and Zif-268, selectively required for reconsolidation of contextual fear memory [46]. In this respect, it has been established that this separation at gene expression level is reflected in parallel and independent signaling pathways upstream of transcriptional activation: the NMDA receptor-ERK1-BDNF path is functional for consolidation, whereas NMDA receptor-inhibitor NFkB-kinase (IKK)-Zif-268 is functional for reconsolidation [57].

Extinction Process

As stated by Dr. Rescorla: “Extinction is one of the most basic findings in the study of Pavlovian conditioning. It is routinely observed that a CS which has previously paired with an US loses its power to evoke the learned response when the US is removed” [58]. Three theoretical models have been postulated for the development of the extinction process:

1. Devaluation of the meaning of the US [59];
2. Unlearning or elimination of the association CS–US [60];
3. The formation of a new inhibitory association named, for example: CS/no–US [61].

It is important to remark that the extinguished conditioned response can be observed again at different times after the extinction training has finished through different processes, those which are considered as definers of extinction properties:

(a) A mechanism called spontaneous recovery, in which the expression of the extinction decays with time;
(b) A mechanism called renewal, in which the original fear response reappears in a different context where the extinction process occurred;
(c) A mechanism called reinstatement, in which the return of an extinguished conditioned response is the consequence of the re-exposure to the unconditioned stimulus,

This evidence supports the notion that extinction is a new learning process that inhibits or reduces temporarily the original association [3]. Thus, as with other learning processes, the extinction takes place in three steps: acquisition, consolidation, extinction retrieval or recall [62].

The acquisition of the extinction is based on the initial learning in which the CS does not predict a threat, thus the conditional response decrease. Afterwards, different physiological and molecular events stabilize the extinction memory through the consolidation phase. Subsequently, the presentation of the conditional stimulus promotes the recall of the extinction, which would be expressed as the suppression or attenuation of the original conditioned response.

With regard to the signaling pathways and network mechanisms involved in the formation of the extinction memory, different results have emphasized a critical role of NMDA receptors and

particularly the NR2B subunit in the lateral amygdala (LA) and ventro-medial pre-frontal cortex (vmPFC). In this way, the antagonism of NMDA receptors using MK-801 [63], AP-5 [64, 65] or the NR2B blocker ifenprodil [66] prevents the formation of the extinction memory. Interestingly, the administration before or after extinction of a partial agonist acting at the glycine-recognition site of the NMDA receptor, D-cycloserine, in a fear-potentiated startle paradigm, and fear conditioning facilitates extinction [67].

In relation to kinase pathways, many of them including PKA, MAPK, PI-3-K, and CAMK are also involved in extinction memory. In this respect, an upregulation of phosphorylated MAPK/ERK within the BLA was observed in the auditory fear-conditioning paradigm in a time-dependent manner at late extinction periods [68]. Similarly, the facilitating effects of D-cycloserine on fear extinction and MAPK activation was prevented by the blockade of MAPK in BLA (PD98059 and U0–126) or PI-3-Kinase inhibitor (wortmannin) preventing also the PI-3-K activation [69].

Stress

The word "stress" was introduced in physiological and biomedical research by the Hungarian-Canadian scientist Hans Selye in 1936 to describe a non-specific syndrome in laboratory animals in response to different harmful agents. Later, the stress definition was modified to describe a potential or actual threat to homeostasis, imposed by internal or external adverse forces, called "stressors." Homeostasis is restored by a complex repertoire of adaptive behavioral and physiological responses of the body, named "stress response" mediated by the "stress system."

With the increasing number of publications in the field of stress research, the use of a conventional definition of stress such as "the body's response to any actual or threatened homeostasis disturbance" has become evident. This definition brings considerable problems, since it argues that any threat to homeostasis is a stressor [70]. In conclusion, it is inappropriate to use the term "stress" for conditions ranging from the lightest challenging stimulation to severely aversive conditions [71].

Stress, as a complex process, involves several components: the stimulus or *stressor*, cognitive appraisal of this stimulus, which assesses how far it can be considered as a threat, and the behavioral and physiological response or the *stress response* [72]. Clearly, at the present time, the main problem with the stress concept is that most existing definitions include only some of these components.

In recent years, the introduction of the terms "controllability" and "predictability" to stress concept has led to a more precise definition of this phenomenon. In this context, it is considered that the induction of a stress condition depends more on the extent to which the stimulus can be predicted and controlled than the physical nature of the stressor [73].

Koolhaas and co-workers (2011) proposed an interesting characterization of stress: "the term should be limited to conditions where an environmental demand exceeds the natural regulatory capacity of an organism, particularly in situations which include the lack of prediction and control" [71]. Thus, this more precise definition could avoid confusion with normal physiological reactions that are required to sustain the behavior.

There is a close association between stress and different psychiatric disorders including PTSD, depression, and schizophrenia. The hypothesis under consideration is that the symptomatology observed in those patients represents the behavioral manifestation of the stress-induced changes in brain structure and function. Consistent with this idea, the exposure to traumatic events results in acute or chronic neurobiological changes in specific brain areas involved in such responses, with the possibility of suffering long-term changes in brain circuits [74].

The Brain Circuit Involved in Fear Memory Process and Stress

The brain does not learn or remember as a unitary simple structure: different central circuits are specialized and code different types of information

[75]. The critical information is controlled by a hierarchical neuronal system that determines the efficacy of the behavioral response and in consequence, the dynamic adaptation to the threatening situation [3]. The majority of the researchers propose a processing model for fear memories in which the amygdaloid nucleus, the hippocampus and the medial-PFC play differential but pivotal roles. In the same way, each brain area presents subdivisions with particular functional characteristics [76, 77]. It is important to remark that even though other brain areas participate in the processing of fear memories, advances in the knowledge of the brain circuits in fear memory were mostly achieved in these brain areas.

Under this scenario, a vast literature suggests that the amygdala is essential for the formation of emotional memories [78], and it is also critically involved in stress-induced anxiety and fear reaction [79–82]. This pivotal contribution to fear memory and stress implies that these processes can be influenced by each other and thus, be interrelated. Thus, the amygdala is a major contributor to the interaction between stress and fear memory [78, 83], which is consistent with studies reported in humans. In this respect, memory deficits in amygdala-lesioned patients [84], and functional imaging experiments, revealed a heightened amygdala activity during emotional encoding [85], supporting this notion.

The amygdaloid complex is located in the anterior medial portion of the temporal lobe, and is comprised of a very heterogeneous nuclear group divided on the basis of cytoarchitectonic, histochemical and immunocytochemical studies [86]. Considering the involvement of these nuclei in fear memory, the BLA, the central (CE), and the intercalated (ITC) cell masses are the principal sub-areas well defined in such a process.

The synaptic information from thalamic/somatosensory inputs and cortical and hippocampal afferents converges into the BLA, thus resuming in this sub-nuclei the information of the CS and the US from the conditioning paradigm, which promote synaptic plasticity events for the learning and memory processes [78]. In this way, the BLA integrates the information that makes it possible to distinguish innate and learned environmental threats, playing a fundamental role in prompting relevant actions for survival purposes. The CE nucleus is the principal efferent area from the BLA, particularly for emotional responses and associated physiological responses [87] which control the expression of the fear response, including behavioral, anatomical, and endocrine responses through efferent projections, mainly to lateral hypothalamic, paraventricular nucleus of the hypothalamus, the periaqueductal grey area, and motor areas of the brainstem [78].

The hippocampal formation has a fundamental role in different processes of contextual fear memory, stress, and anxiety. Behavioral evidence as well as neuroanatomical studies have demonstrated clear different conclusions about efferent and afferent connectivity throughout its septo-temporal axis [88, 89]. Thus, the dorsal pole of the hippocampus (DH) would play a more selective role in the spatial representation of an event, while the ventral region (VH) would contribute to the modulation of fear and anxiety [90, 91].

Related to the spatial and contextual processing of the information, the DH might be downstream from the BLA neuronal activation involved in contextual fear [92]. However, the BLA rarely presents direct projections to DH, its principal interconnection being through the entorhinal cortex (EntC), mainly the dorso-lateral portion (dl-EntC) [86], which constitutes the principal source of afferent excitatory neurotransmission to DH [93]. Strictly, layer 2 of the dl-EntC receives dense excitatory projections from BLA, and in turn sends dense excitatory efferent through the perforant path to the dentate gyrus of the hippocampus.

The PFC is a brain area involved in the consolidation as well as in the expression of conditioned fear, and the acquisition, consolidation, and expression of extinction memory. Considering the cellular types and the pattern of its organization, the PFC can be divided into four regions taking into account the dorsal to ventral distribution: medial prefrontal, cingulate anterior, prelimbic (PL) and infralimbic (IL) [94]. As with the majority of the neocortical areas, the cellular organization presents six layers (I to VI), where layer VI is the deepest one and layer I is the

closest to the *piamatter*. Layers II/III and V/VI present pyramidal neurons with apical dendrites that project to layer I.

Different evidence from neuronal tracings and electrophysiological studies in PFC has revealed the existence of PL and IL interconnections, with functional physiological consequences when such connection is interrupted [95].

Stress, Fear Memory, and Psychopathology

It is important to make an observation with regard to the behavioral manifestations observed in psychological pathologies. In PTSD patients, it is commonly assumed that by definition a psychologically traumatic event caused the behavioral manifestation, and by consequence that any biological abnormality found to accompany the PTSD has to be traumatically induced. However, we should also consider that any exposure to a threat event which the individual fails to cope with would increase the risk of developing the psychological pathology [74].

It is known that patients suffering from PTSD, anxiety, or panic disorder present alterations in memory function [96] in which preclinical and clinical studies have shown changes in the brain circuitry critically involved in the memory process [74, 97]. Most of the psychophysiological studies, which include measures of heart rate, skin conductance, facial electromyogram (EMG), and cortical electroencephalographic event-related potentials (ERPs), showed alterations in those measures in PTSD patients when they were exposed to idiographic trauma cues [98]. Interestingly, these parameters decreased notably in an imagery test in patients that received post-trauma propranolol, a beta-adrenergic blocker that has been found to attenuate the consolidation of fear memories [99] compared to placebo.

In animal studies, it has been found that chronically stressed rats presented critical damage in CA3 pyramidal neurons (cornu ammonis 3) of the hippocampus, which was also associated with high levels of corticosterone, decrease of the neurotrophic factor BDNF, and inhibition of the neurogenesis [100–104]. Interestingly, in the same way as in this animal model, structural MRI studies in the hippocampus of PTSD patients revealed a reduced volume of this brain area. Whether smaller hippocampal size is a result of trauma exposure or rather represents a risk factor for PTSD remains to be determined. However, it has been suggested that the hippocampal volume serves as a pre-trauma risk factor for this pathology [74]. From a functional perspective, an interesting study performed in adult women revealed a functional deficit of this brain area that is associated to the failure to recall extinction memory [105].

Considering the cortex, particularly vmPFC and dorsal anterior cingulate cortext (dACC), a lower volume of such prefrontal regions has also been reported, but in contrast to the observations made in the hippocampus, such cortical reduction would represent an acquired feature of PTSD rather than a pre-existing vulnerability [106]. From a functional perspective, the activation of dACC is higher during fear conditioning in PTSD patients in comparison to control individuals [107]. Considering a critical role of these brain areas in fear learning and expression of the fear, such functional abnormalities are positively associated with severity of symptoms [107].

In relation to the amygdala, functional neuroimaging studies have revealed an exaggerated activation in response to trauma-related stimuli as well as generic ones in PTSD patients in comparison to control individuals [108]. This is in relation to the principal role that this brain area presents in the detection of threat, fear learning, fear expression, and heightening memory for emotional events [78].

The use of an animal model for these critical symptoms of PTSD, anxiety, panic disorder, or phobia has revealed that in addition to the hippocampus, the amygdala and the prefrontal cortex mentioned above were also implicated in the neuronal circuitry involved in stress and fear memory [109]. Lesion studies have demonstrated that the medial prefrontal cortex modulates emotional responsiveness through inhibition of amygdala function [110]. Studies show that neurons of the medial PFC play an active role in

inhibition of fear responses that are mediated by the amygdala [111, 112]. Conditioned fear responses are extinguished following repeated exposure to the conditioned stimulus [in the absence of the unconditioned stimulus (aversive; e.g., electric shock)]. This inhibition appears to be mediated by medial prefrontal cortical inhibition of amygdala responsiveness. Animal studies have also shown that early stress is associated with a decrease in branching of neurons in the medial PFC [113, 114].

As mentioned, there is wide consensus that stress promotes the emergence of aversive motivated memories, including fear memory. Our laboratory has observed in rats that exposure to a single stressful event facilitates the encoding of contextual fear memories [83] and the induction of neural plasticity such as long-term potentiation (LTP) in BLA, a cellular model proposed to be implicated in fear-memory formation [83]. In the same way, the interaction between an aversive experience and an established fear-memory trace result in a robust and persistent fear memory [79, 80]. This stress-induced promoting influence was shown to be prevented by midazolam (MDZ, a positive modulator of GABAa receptors) intra-BLA infusion before stress exposure. Such influence is related to the modulation of the GABAergic transmission in this brain area, since intra-BLA bicuculline, a GABAa blocker, introduced just before the encoding of the fear memory mimicked stress-induced facilitating effect on fear-memory formation [79, 83].

Similarly, an identical environmental challenge promoted exaggerated behavioral responses to new encounters with discrete aversive stimulus not previously related [82, 115]. Additionally, a similar stressful experience prior to fear memory encoding resulted in a memory trace that was insensitive to the interfering effect of MDZ on fear-memory reconsolidation, suggesting that previous stress can restrict the destabilization and the engagement of reconsolidation in later retrieval sessions [116, 117]. In support of this view, stress was seen to impede the elevation of both the expression of Zif-268 and the GluN2B sites in BLA, two molecular markers of the labilization/reconsolidation process, following reactivation [116]. All together, this evidence indicates that stress exposure prevents the onset of destabilization/reconsolidation process following reactivation. Hence, highly arousing experiences are determinant for the subsequent emergence of reconsolidation of the memory trace following reactivation.

Interestingly, the systemic and intra-BLA administration of D-cycloserine prior to reactivation, has been demonstrated to promote the destabilization of resistant memories such as those described in stressed animals [116, 117].

Finally, based on the proposal that memory is essentially flexible and malleable, and that the labilization/reconsolidation process is an expression of the dynamic nature of memory, as previously proposed [118], a previous history of stress would limit the flexibility of the memory trace following retrieval. This notion suggests that the lack of memory flexibility could be the basis of the emergence of traumatic memory. In line with this reasoning, highly arousing experiences would limit the memory's ability to incorporate new environmental information, even when the threat is not present anymore.

As stated before, alterations in the aversive information processing are associated to a persistent and exaggerated fear response, which is characteristic of psychiatric disorders [2, 119]. In this respect, exposure therapy in a clinical setting is based on the extinction of traumatic memories [120–122]. However, there are important studies revealing that the majority of those patients presented a re-establishment of the symptomatology. Thus, it has been suggested that changes in the characteristic dynamic of extinction-memory formation play a critical role in the development of the clinical symptoms of those disorders.

There are two fundamental concepts related to the complexity of performing extinction learning in psychiatry patients: (a) the patients could acquire the CS–US association much more intensely than normal people; thus, the emotional conditioned response would be reinforced in the absence of the US [123], and (b) it would be possible that there is a failure to inhibit the fear response in the presence of non-associated previously cues [124]. In addition to these behavioral

expressions, functional and structural imaging studies have demonstrated critical differences in the volume of PFC and amygdala, as well as patterns of activation of the mentioned areas in PTSD patients in comparison to voluntary controls [125, 126], which would predict vulnerability to develop the symptoms in anxiety disorders.

Structural Plasticity, Stress, and Fear Memory

The possibility that memory might involves structural changes in the nervous system was particularly hypothesized by Santiago Ramon y Cajal and Eugenio Tanzi, by the late nineteenth century. Most of the excitatory synapses in the brain terminate on dendritic spines [127–129]. Spines are the dendritic protrusions that contain the post-synaptic density protein (PSD) including receptors, channels, and signaling molecules [130].

It has been proposed that the modulation of the number of dendritic spines and/or their morphology may contribute to alterations in excitatory synaptic transmission [131]. Accordingly, changes in spine morphology are relevant to synaptic function and plasticity, as thin spines with long and narrow necks may isolate Ca^{2+} transients from the parent dendrite, whereas short and stubby spines may promote more coordinated and widespread Ca^{2+} transients in the parent dendrite, as well as synchronize Ca^{2+} signaling among adjacent spines [128, 132, 133]. In addition, large spines with conspicuous heads have a higher density of AMPA receptors, in addition to being more stable than thinner and longer spines [134]; accordingly, the ratio of AMPA to NMDA receptors increases linearly with the diameter of the PSD [135]. Thus, a higher number of dendritic spines might support an enhanced excitatory neurotransmission. In this respect, there is evidence that induction of LTP, a cellular mechanism of learning and memory [136], is associated with changes in the number and shape of dendritic spines [137, 138].

Animal models of fear learning and memory present a close association with structural plasticity at different brain areas. Twenty four hours after trace blink conditioning, an associative learning task that depends on the integrity of the hippocampus [139], the animals presented a higher number of multiple synaptic boutons that formed synapses onto dendritic spines [140] and an increase of the density of dendritic spines in which the antagonism of NMDA receptors prevented such change [141]. Following the same pattern, such a conditioning protocol induces an increase in the number of synapses in the cerebellum [142] and in the piriform cortex after olfactory learning [143].

In the anterior cingulate cortex, a prefrontal cortex region essential for the expression of remote memory [144], a higher number of dendritic spines was observed in a time-dependent manner after consolidation of the fear memory [145, 146]. Similarly, cue contextual fear conditioning significantly increased BLA dendritic spine numbers and the dendritic tree ramification up to 1 month after initial fear learning [147], highlighting that synaptic remodeling accompanies the formation of long-term memory [146].

As a consequence of exposure to emotional traumatic events, different brain areas present a remodeling of the dendritic tree. In particular, there is a critical regression of the number of dendritic spines in pyramidal CA3 hippocampal neurons after chronic restraint stress exposures in experimental animals [148]. In the same way, a shortened dendritic tree has also been observed in granular cells of the dentate gyrus and pyramidal cells of CA1 of rats exposed to chronic stress or with a prolonged corticosterone administration [149] , and even acute stress [150].

In mPFC, chronically stress-exposed animals presented a reduction of the dendritic tree ramification and the length of dendritic spines of pyramidal neurons of the layer II-III [151–153], and a reduction of the density of the spines [154].

These findings contrast with evidence in which chronic stress induced a higher dendritic tree in BLA complex [155] and in principal cells in LA [156]. Such a difference might be connected to the principal role that the amygdala presents in the long-term consolidation of fear memories, with persistent structural encoding of the aversive experience [78].

As stated before, stress affects several distinct cognitive processes [157], facilitating as such the emergence of fear memory [83, 158, 159]. We have observed in experimental animals that a single restraint stress exposure can facilitate the emergence of a robust and persistent contextual fear memory following a single training trial in a contextual fear conditioning. Interestingly, such training is incapable of yielding a full fear response at testing in unstressed rats [79, 160, 161]. In this respect, the total density, taking into account the number of mature and thin dendritic spines, was higher in fear-trained stressed animals in the dorsal hippocampus. It should be noted that neither the fear-conditioning protocol used nor the stress exposure per se was able to induce an increment in the number of dendritic spines [80]. The administration intra-BLA of MDZ, prior to the stress exposure, prevented such modifications [162], emphasizing the modulatory role of the GABAergic neurotransmission in the BLA to the structural plasticity in DH.

In sum, these results highlight the fact that the formation, enlargement, and even maturation of dendritic spines are relevant for synaptic rearrangement by the ongoing synaptic activity, which is assumed to contribute to the expression of fear memory.

Concluding Remarks

A vast literature supports the notion that the brain has a critical property that enables functional and structural plasticity which promotes the adaptation to threat and the coping response in a changing environment. In this scenario, memory is essentially flexible and malleable; in fact, the occurrence of the labilization/reconsolidation process following retrieval is an expression of the dynamic nature of memory, as previously noted [118].

However, based on our evidence, the previous exposure to unpredictable stress would limit the flexibility of the memory trace following retrieval. This notion suggests that the lack of memory flexibility could be on the basis of the emergence of traumatic memory. In line with this reasoning, highly arousing experiences would limit the memory's ability to incorporate new environmental information, even when the threat is not present anymore. The limitation to the capability to induce plasticity due to changes in the aversive information processing following intense aversive experiences could be associated with persistent and exaggerated fear responses, a hallmark of anxiety-related disorders. The knowledge of neurobiological mechanisms underlying memory plasticity in all its phases is relevant for developing novel treatments for stress-associated mental disorders.

References

1. Cahill L, McGaugh JL. Modulation of memory storage. Curr Opin Neurobiol. 1996;6(2):237–42.
2. Mahan AL, Ressler KJ. Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. Trends Neurosci. 2012;35(1):24–35.
3. Pape HC, Pare D. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol Rev. 2010;90(2):419–63.
4. Herry C, Johansen JP. Encoding of fear learning and memory in distributed neuronal circuits. Nat Neurosci. 2014;17(12):1644–54.
5. Blanchard RJ, Blanchard DC. Crouching as an index of fear. J Comp Physiol Psychol. 1969;67(3):370–5.
6. Joels M, Baram TZ. The neuro-symphony of stress. Nat Rev Neurosci. 2009;10(6):459–66.
7. Dudai YRI, Roediger HL, Tulvin E. Memory concepts. In: Roedinger HLD, Dudai Y, Fitzpatrick SM, editors. Science of memory: concepts. Oxford/New York: Oxford University Press; 2007. p. 446.
8. Dudai Y. The neurobiology of consolidations, or, how stable is the engram? Annu Rev Psychol. 2004; 55:51–86.
9. Tulving E, Thompson DM. Encoding specificity and retrieval processes in episodic memory. Psychological Review. 1973;80(5):352–73.
10. McGaugh JL. Memory—a century of consolidation. Science. 2000;287(5451):248–51.
11. Wixted JT. On Common Ground: Jost's (1897) law of forgetting and Ribot's (1881) law of retrograde amnesia. Psychol Rev. 2004;111(4):864–79.
12. Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. Science. 2001;294(5544):1030–8.
13. Hardt O, Einarsson EO, Nader K. A bridge over troubled water: reconsolidation as a link between cognitive and neuroscientific memory research traditions. Annu Rev Psychol. 2010;61:141–67.
14. Abel T, Lattal KM. Molecular mechanisms of memory acquisition, consolidation and retrieval. Curr Opin Neurobiol. 2001;11(2):180–7.

15. Maren S, Aharonov G, Fanselow MS. Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. Behav Neurosci. 1996;110(4):718–26.
16. Alberini CM. Transcription factors in long-term memory and synaptic plasticity. Physiol Rev. 2009;89(1):121–45.
17. Melchor JP, Strickland S. Tissue plasminogen activator in central nervous system physiology and pathology. Thromb Haemost. 2005;93(4):655–60.
18. Lewis DJ. Psychobiology of active and inactive memory. Psychol Bull. 1979;86(5):1054–83.
19. Tronson NC, Taylor JR. Molecular mechanisms of memory reconsolidation. Nat Rev Neurosci. 2007;8(4):262–75.
20. Alberini CM. The role of reconsolidation and the dynamic process of long-term memory formation and storage. Front Behav Neurosci. 2011;5:12.
21. Lee JL. Memory reconsolidation mediates the updating of hippocampal memory content. Front Behav Neurosci. 2010;4:168.
22. Dudai Y. Memory from A to Z. Keywords, concepts and beyond. Oxford: Oxford University Press; 2002.
23. Sara SJ. Retrieval and reconsolidation: toward a neurobiology of remembering. Learn Mem. 2000;7(2):73–84.
24. Dudai Y. Reconsolidation: the advantage of being refocused. Curr Opin Neurobiol. 2006;16(2):174–8.
25. Lee JL. Reconsolidation: maintaining memory relevance. Trends Neurosci. 2009;32(8):413–20.
26. Nader K, Hardt O. A single standard for memory: the case for reconsolidation. Nat Rev Neurosci. 2009;10(3):224–34.
27. Gisquet-Verrier P, Riccio DC. Memory reactivation effects independent of reconsolidation. Learn Mem. 2012;19(9):401–9.
28. Rodriguez-Ortiz CJ, et al. Intrahippocampal anisomycin infusions disrupt previously consolidated spatial memory only when memory is updated. Neurobiol Learn Mem. 2008;89(3):Psychological Review. 1973; 80(5): –9.
29. Lee JL. Memory reconsolidation mediates the strengthening of memories by additional learning. Nat Neurosci. 2008;11(11):1264–6.
30. Misanin JR, Miller RR, Lewis DJ. Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science. 1968;160(3827):554–5.
31. Przybyslawski J, Sara SJ. Reconsolidation of memory after its reactivation. Behav Brain Res. 1997;84(1–2):241–6.
32. Nader K, Schafe GE, Le Doux JE. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature. 2000;406(6797):722–6.
33. Pedreira ME, Perez-Cuesta LM, Maldonado H. Reactivation and reconsolidation of long-term memory in the crab Chasmagnathus: protein synthesis requirement and mediation by NMDA-type glutamatergic receptors. J Neurosci. 2002;22(18):8305–11.
34. Eisenberg M, et al. Stability of retrieved memory: inverse correlation with trace dominance. Science. 2003;301(5636):1102–4.
35. Anokhin KV, Tiunova AA, Rose SP. Reminder effects—reconsolidation or retrieval deficit? Pharmacological dissection with protein synthesis inhibitors following reminder for a passive-avoidance task in young chicks. Eur J Neurosci. 2002;15(11):1759–65.
36. Sangha S, et al. Intermediate and long-term memories of associative learning are differentially affected by transcription versus translation blockers in Lymnaea. J Exp Biol. 2003;206(Pt 10):1605–13.
37. Walker MP, et al. Dissociable stages of human memory consolidation and reconsolidation. Nature. 2003;425(6958):616–20.
38. Bustos SG, Maldonado H, Molina VA. Midazolam disrupts fear memory reconsolidation. Neuroscience. 2006;139(3):831–42.
39. Bustos SG, Maldonado H, Molina VA. Disruptive effect of midazolam on fear memory reconsolidation: decisive influence of reactivation time span and memory age. Neuropsychopharmacology. 2009;34(2):446–57.
40. Inda MC, Muravieva EV, Alberini CM. Memory retrieval and the passage of time: from reconsolidation and strengthening to extinction. J Neurosci. 2011;31(5):1635–43.
41. Suzuki A, et al. Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci. 2004;24(20):4787–95.
42. Alberini CM. Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? Trends Neurosci. 2005;28(1):51–6.
43. Merlo E, et al. Activation of the transcription factor NF-kappaB by retrieval is required for long-term memory reconsolidation. Learn Mem. 2005;12(1):23–9.
44. Kida S, et al. CREB required for the stability of new and reactivated fear memories. Nat Neurosci. 2002;5(4):348–55.
45. Miller CA, Marshall JF. Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. Neuron. 2005;47(6):873–84.
46. Lee JL, Everitt BJ, Thomas KL. Independent cellular processes for hippocampal memory consolidation and reconsolidation. Science. 2004;304(5672):839–43.
47. Tronel S, Milekic MH, Alberini CM. Linking new information to a reactivated memory requires consolidation and not reconsolidation mechanisms. PLoS Biol. 2005;3(9):293.
48. Duvarci S, Nader K, LeDoux JE. Activation of extracellular signal-regulated kinase–mitogen-activated protein kinase cascade in the amygdala is required for memory reconsolidation of auditory fear conditioning. Eur J Neurosci. 2005;21(1):283–9.

49. Kelly A, Laroche S, Davis S. Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. J Neurosci. 2003;23(12):5354–60.
50. Koh MT, Bernstein IL. Inhibition of protein kinase A activity during conditioned taste aversion retrieval: interference with extinction or reconsolidation of a memory? Neuroreport. 2003;14(3):405–7.
51. Milton AL, et al. Double dissociation of the requirement for GluN2B- and GluN2A-containing NMDA receptors in the destabilization and restabilization of a reconsolidating memory. J Neurosci. 2013;33(3):1109–15.
52. Ben Mamou C, Gamache K, Nader K. NMDA receptors are critical for unleashing consolidated auditory fear memories. Nat Neurosci. 2006;9(10):1237–9.
53. Jarome TJ, et al. Activity dependent protein degradation is critical for the formation and stability of fear memory in the amygdala. PLoS One. 2011;6(9):24349.
54. Jarome TJ, Helmstetter FJ. The ubiquitin–proteasome system as a critical regulator of synaptic plasticity and long-term memory formation. Neurobiol Learn Mem. 2013;105:107–16.
55. Lee SH, et al. Synaptic protein degradation underlies destabilization of retrieved fear memory. Science. 2008;319(5867):1253–6.
56. Suzuki A, et al. Activation of LVGCCs and CB1 receptors required for destabilization of reactivated contextual fear memories. Learn Mem. 2008;15(6):426–33.
57. Lee JL, Hynds RE. Divergent cellular pathways of hippocampal memory consolidation and reconsolidation. Hippocampus. 2013;23(3):233–44.
58. Rescorla RA. Protection from extinction. Learn Behav. 2003;31(2):124–32.
59. Rescorla RA. Effect of US habituation following conditioning. J Comp Physiol Psychol. 1973;82(1):137–43.
60. Rescorla RA. Inhibition of delay in Pavlovian fear conditioning. J Comp Physiol Psychol. 1967;64(1):114–20.
61. Bouton ME, King DA. Contextual control of the extinction of conditioned fear: tests for the associative value of the context. J Exp Psychol Anim Behav Process. 1983;9(3):248–65.
62. Quirk GJ, Mueller D. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology. 2008;33(1):56–72.
63. Baker JD, Azorlosa JL. The NMDA antagonist MK-801 blocks the extinction of Pavlovian fear conditioning. Behav Neurosci. 1996;110(3):618–20.
64. Falls WA, Miserendino MJ, Davis M. Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. J Neurosci. 1992;12(3):854–63.
65. Lee H, Kim JJ. Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. J Neurosci. 1998;18(20):8444–54.
66. Sotres-Bayon F, et al. Dissociable roles for the ventromedial prefrontal cortex and amygdala in fear extinction: NR2B contribution. Cereb Cortex. 2009;19(2):474–82.
67. Ledgerwood L, Richardson R, Cranney J. D-cycloserine and the facilitation of extinction of conditioned fear: consequences for reinstatement. Behav Neurosci. 2004;118(3):505–13.
68. Herry C, et al. Extinction of auditory fear conditioning requires MAPK/ERK activation in the basolateral amygdala. Eur J Neurosci. 2006;24(1):261–9.
69. Yang YL, Lu KT. Facilitation of conditioned fear extinction by d-cycloserine is mediated by mitogen-activated protein kinase and phosphatidylinositol 3-kinase cascades and requires de novo protein synthesis in basolateral nucleus of amygdala. Neuroscience. 2005;134(1):247–60.
70. Day TA. Defining stress as a prelude to mapping its neurocircuitry: no help from allostasis. Prog Neuro-Psychopharmacol Biol Psychiatry. 2005;29(8):1195–200.
71. Koolhaas JM, et al. Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav Rev. 2011;35(5):1291–301.
72. Levine S. Developmental determinants of sensitivity and resistance to stress. Psychoneuroendocrinology. 2005;30(10):939–46.
73. Salvador A. Coping with competitive situations in humans. Neurosci Biobehav Rev. 2005;29(1):195–205.
74. Pitman RK, et al. Biological studies of post-traumatic stress disorder. Nat Rev Neurosci. 2012;13(11):769–87.
75. Fanselow MS. From contextual fear to a dynamic view of memory systems. Trends Cogn Sci. 2010;14(1):7–15.
76. Herry C, et al. Neuronal circuits of fear extinction. Eur J Neurosci. 2010;31(4):599–612.
77. Sierra-Mercado D, Padilla-Coreano N, Quirk GJ. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology. 2011;36(2):529–38.
78. LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci. 2000;23:155–84.
79. Giachero M, et al. A BDNF sensitive mechanism is involved in the fear memory resulting from the interaction between stress and the retrieval of an established trace. Learn Mem. 2013;20(5):245–55.
80. Giachero M, Calfa GD, Molina VA. Hippocampal dendritic spines remodeling and fear memory are modulated by GABAergic signaling within the basolateral amygdala complex. Hippocampus. 2015;25(5):545–55.
81. Bignante EA, Paglini G, Molina VA. Previous stress exposure enhances both anxiety-like behaviour and p35 levels in the basolateral amygdala complex: modulation by midazolam. Eur Neuropsychopharmacol. 2010;20(6):388–97.
82. Martijena ID, et al. Gabaergic modulation of the stress response in frontal cortex and amygdala. Synapse. 2002;45(2):86–94.

83. Rodriguez Manzanares PA, et al. Previous stress facilitates fear memory, attenuates GABAergic inhibition, and increases synaptic plasticity in the rat basolateral amygdala. J Neurosci. 2005;25(38):8725–34.
84. Anderson AK, Phelps EA. Lesions of the human amygdala impair enhanced perception of emotionally salient events. Nature. 2001;411(6835):305–9.
85. LaBar KS, et al. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. Neuron. 1998;20(5):937–45.
86. de Olmos JS, Beltramino CA, Alheid G. Amygdala and extended amygdala of the rat: a cytoarchitectonical, fibroarchitectonical, and chemoarchitectonical survey. In: Paxinos G, editor. The rat nervous system. San Diego: Academic Press; 2004. p. 509–603.
87. Sah P, et al. The amygdaloid complex: anatomy and physiology. Physiol Rev. 2003;83(3):803–34.
88. Siegel A, Tassoni JP. Differential efferent projections from the ventral and dorsal hippocampus of the cat. Brain Behav Evol. 1971;4(3):185–200.
89. Swanson LW, Cowan WM. An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. J Comp Neurol. 1977;172(1):49–84.
90. Kjelstrup KG, et al. Reduced fear expression after lesions of the ventral hippocampus. Proc Natl Acad Sci U S A. 2002;99(16):10825–30.
91. Calfa G, Bussolino D, Molina VA. Involvement of the lateral septum and the ventral Hippocampus in the emotional sequelae induced by social defeat: role of glucocorticoid receptors. Behav Brain Res. 2007;181(1):23–34.
92. Maren S, Fanselow MS. Electrolytic lesions of the fimbria/fornix, dorsal hippocampus, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. Neurobiol Learn Mem. 1997;67(2):142–9.
93. Pitkanen A, et al. Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Ann N Y Acad Sci. 2000;911:369–91.
94. Heidbreder CA, Groenewegen HJ. The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neurosci Biobehav Rev. 2003;27(6):555–79.
95. Hoover WB, Vertes RP. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct. 2007;212(2):149–79.
96. Elzinga BM, Bremner JD. Are the neural substrates of memory the final common pathway in posttraumatic stress disorder (PTSD)? J Affect Disord. 2002;70(1):1–17.
97. Bremner JD, et al. Structural and functional plasticity of the human brain in posttraumatic stress disorder. Prog Brain Res. 2008;167:171–86.
98. Pole N. The psychophysiology of posttraumatic stress disorder: a meta-analysis. Psychol Bull. 2007;133(5):725–46.
99. Lonergan MH, et al. Propranolol's effects on the consolidation and reconsolidation of long-term emotional memory in healthy participants: a meta-analysis. J Psychiatry Neurosci. 2013;38(4):222–31.
100. Gould E, et al. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci U S A. 1998;95(6):3168–71.
101. Magarinos AM, et al. Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. J Neurosci. 1996;16(10):3534–40.
102. Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci. 1995;15(11):7539–47.
103. Sapolsky RM. Stress, glucocorticoids, and damage to the nervous system: the current state of confusion. Stress. 1996;1(1):1–19.
104. Sapolsky RM, et al. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. J Neurosci. 1990;10(9):2897–902.
105. Milad MR, et al. Estrous cycle phase and gonadal hormones influence conditioned fear extinction. Neuroscience. 2009;164(3):887–95.
106. Kasai K, et al. Evidence for acquired pregenual anterior cingulate gray matter loss from a twin study of combat-related posttraumatic stress disorder. Biol Psychiatry. 2008;63(6):550–6.
107. Rougemont-Bucking A, et al. Altered processing of contextual information during fear extinction in PTSD: an fMRI study. CNS Neurosci Ther. 2011;17(4):227–36.
108. Liberzon I, et al. Brain activation in PTSD in response to trauma-related stimuli. Biol Psychiatry. 1999;45(7):817–26.
109. McLaughlin KJ, Baran SE, Conrad CD. Chronic stress- and sex-specific neuromorphological and functional changes in limbic structures. Mol Neurobiol. 2009;40(2):166–82.
110. Morgan MA, Romanski LM, LeDoux JE. Extinction of emotional learning: contribution of medial prefrontal cortex. Neurosci Lett. 1993;163(1):109–13.
111. Milad MR, et al. Fear extinction in rats: implications for human brain imaging and anxiety disorders. Biol Psychol. 2006;73(1):61–71.
112. Milad MR, Quirk GJ. Neurons in medial prefrontal cortex signal memory for fear extinction. Nature. 2002;420(6911):70–4.
113. Brown SM, Henning S, Wellman CL. Mild, short-term stress alters dendritic morphology in rat medial prefrontal cortex. Cereb Cortex. 2005;15(11):1714–22.
114. Radley JJ, et al. Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. Neuroscience. 2004;125(1):1–6.
115. Calfa G, Volosin M, Molina VA. Glucocorticoid receptors in lateral septum are involved in the modulation of the emotional sequelae induced by social defeat. Behav Brain Res. 2006;172(2):324–32.
116. Espejo PJ, et al. Stress-induced resistance to the fear memory labilization/reconsolidation process.

Involvement of the basolateral amygdala complex. Neuropharmacology. 2016;109:349–56.
117. Bustos SG, et al. Previous stress attenuates the susceptibility to Midazolam's disruptive effect on fear memory reconsolidation: influence of pre-reactivation D-cycloserine administration. Neuropsychopharmacology. 2010;35(5):1097–108.
118. Nader K. Emotional memory. Handb Exp Pharmacol. 2015;228:249–70.
119. Cohen S, Janicki-Deverts D, Miller GE. Psychological stress and disease. JAMA. 2007;298(14):1685–7.
120. Hermans D, et al. Extinction in human fear conditioning. Biol Psychiatry. 2006;60(4):361–8.
121. McNally RJ. Mechanisms of exposure therapy: how neuroscience can improve psychological treatments for anxiety disorders. Clin Psychol Rev. 2007;27(6):750–9.
122. Hamm AO. Specific phobias. Psychiatr Clin North Am. 2009;32(3):577–91.
123. Eysenck HJ. Behavioral therapy and the philosophers. Behav Res Ther. 1979;17(5):511–4.
124. Davis M, Falls WA, Gewirtz J. Neural systems involved in fear inhibition: extinction and conditioned inhibition. In: Weiner I, Myslobodsky M, editors. Contemporary issues in modeling psychopathology. Boston: Kluwer Academic; 2000. p. 113–42.
125. Shin LM, et al. An fMRI study of anterior cingulate function in posttraumatic stress disorder. Biol Psychiatry. 2001;50(12):932–42.
126. Milad MR, et al. Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. Proc Natl Acad Sci U S A. 2005;102(30):10706–11.
127. Gray EG. Electron microscopy of synaptic contacts on dendrite spines of the cerebral cortex. Nature. 1959;183(4675):1592–3.
128. Nimchinsky EA, Sabatini BL, Svoboda K. Structure and function of dendritic spines. Annu Rev Physiol. 2002;64:313–53.
129. Harris KM. Structure, development, and plasticity of dendritic spines. Curr Opin Neurobiol. 1999;9(3):343–8.
130. Kennedy MB. The postsynaptic density at glutamatergic synapses. Trends Neurosci. 1997;20(6):264–8.
131. Bailey CH, Kandel ER. Structural changes accompanying memory storage. Annu Rev Physiol. 1993;55:397–426.
132. Yuste R, Majewska A, Holthoff K. From form to function: calcium compartmentalization in dendritic spines. Nat Neurosci. 2000;3(7):653–9.
133. Segal I, Korkotian I, Murphy DD. Dendritic spine formation and pruning: common cellular mechanisms? Trends Neurosci. 2000;23(2):53–7.
134. Matsuzaki M, et al. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. Nat Neurosci. 2001;4(11):1086–92.
135. Takumi Y, et al. Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. Nat Neurosci. 1999;2(7):618–24.
136. Huganir RL, Nicoll RA. AMPARs and synaptic plasticity: the last 25 years. Neuron. 2013;80(3):704–17.
137. Weiler IJ, Hawrylak N, Greenough WT. Morphogenesis in memory formation: synaptic and cellular mechanisms. Behav Brain Res. 1995;66(1–2):1–6.
138. Nikonenko I, et al. Activity-induced changes of spine morphology. Hippocampus. 2002;12(5):585–91.
139. Solomon PR, et al. Hippocampus and trace conditioning of the rabbit's classically conditioned nictitating membrane response. Behav Neurosci. 1986;100(5):729–44.
140. Geinisman Y, et al. Associative learning elicits the formation of multiple-synapse boutons. J Neurosci. 2001;21(15):5568–73.
141. Leuner B, Falduto J, Shors TJ. Associative memory formation increases the observation of dendritic spines in the hippocampus. J Neurosci. 2003;23(2):659–65.
142. Kleim JA, et al. Motor learning-dependent synaptogenesis is localized to functionally reorganized motor cortex. Neurobiol Learn Mem. 2002;77(1):63–77.
143. Knafo S, et al. Olfactory learning is associated with increased spine density along apical dendrites of pyramidal neurons in the rat piriform cortex. Eur J Neurosci. 2001;13(3):633–8.
144. Frankland PW, et al. The involvement of the anterior cingulate cortex in remote contextual fear memory. Science. 2004;304(5672):881–3.
145. Vetere G, et al. Spine growth in the anterior cingulate cortex is necessary for the consolidation of contextual fear memory. Proc Natl Acad Sci U S A. 2011;108(20):8456–60.
146. Restivo L, et al. The formation of recent and remote memory is associated with time-dependent formation of dendritic spines in the hippocampus and anterior cingulate cortex. J Neurosci. 2009;29(25):8206–14.
147. Heinrichs SC, et al. Dendritic structural plasticity in the basolateral amygdala after fear conditioning and its extinction in mice. Behav Brain Res. 2013;248:80–4.
148. Watanabe Y, Gould E, McEwen BS. Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res. 1992;588(2):341–5.
149. Sousa N, et al. Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. Neuroscience. 2000;97(2):253–66.
150. Chen Y, et al. Rapid loss of dendritic spines after stress involves derangement of spine dynamics by corticotropin-releasing hormone. J Neurosci. 2008;28(11):2903–11.
151. Liston C, et al. Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. J Neurosci. 2006;26(30):7870–4.
152. Shansky RM, et al. Stress-induced dendritic remodeling in the prefrontal cortex is circuit specific. Cereb Cortex. 2009;19(10):2479–84.
153. Shansky RM, Morrison JH. Stress-induced dendritic remodeling in the medial prefrontal cortex:

effects of circuit, hormones and rest. Brain Res. 2009;1293:108–13.
154. Radley JJ, et al. Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. Cereb Cortex. 2006;16(3):313–20.
155. Vyas A, et al. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J Neurosci. 2002;22(15):6810–8.
156. Suvrathan A, et al. Stress enhances fear by forming new synapses with greater capacity for long-term potentiation in the amygdala. Philos Trans R Soc Lond Ser B Biol Sci. 2014;369(1633):20130151.
157. Roozendaal B, McEwen BS, Chattarji S. Stress, memory and the amygdala. Nat Rev Neurosci. 2009;10(6):423–33.
158. Shors TJ, Weiss C, Thompson RF. Stress-induced facilitation of classical conditioning. Science. 1992;257(5069):537–9.
159. Cordero MI, et al. Prior exposure to a single stress session facilitates subsequent contextual fear conditioning in rats. Horm Behav. 2003;44(4):338–45.
160. Maldonado NM, et al. Activation of ERK2 in basolateral amygdala underlies the promoting influence of stress on fear memory and anxiety: influence of midazolam pretreatment. Eur Neuropsychopharmacol. 2014;24(2):262–70.
161. Maldonado NM, Martijena ID, Molina VA. Facilitating influence of stress on the consolidation of fear memory induced by a weak training: reversal by midazolam pretreatment. Behav Brain Res. 2011;225(1):77–84.
162. Giachero M, Calfa GD, Molina VA. Hippocampal structural plasticity accompanies the resulting contextual fear memory following stress and fear conditioning. Learn Mem. 2013;20(11):611–6.

18 The Relationship Between Drugs of Abuse and Palatable Foods: Pre-clinical Evidence Towards a Better Understanding of Addiction-Like Behaviors

Renata B.M. Duarte, Aline Caron Borges, and Marilia Barros

Abstract

Food is essential for the survival of all animals, yet ingestive behavior varies significantly between species. In humans, obesity and related pathologies are currently considered a public health issue, having attained global epidemic proportions. Therefore, a better understanding of its etiology may help improve treatment strategies, as well as promote large-scale social changes. In this sense, this chapter discusses mainly "food addiction" within the current framework of eating-related disorders. We first review the two main neurophysiological mechanisms that regulate ingestive behaviors: (i) the homeostatic drive, which, via activation of specific hormones, increases or inhibits food intake according to endogenous energy deposits; and (ii) the hedonic drive, which is related to the subjective pleasurable experiences associated with food and acts independent of the body's energy stores. We then focus on the main concepts and characteristics of "food addiction," with the development of food-related binge-like and craving behaviors that may be induced when the hedonic drive "overrides" the homeostatic system. Several behavioral criteria currently used to define drug addiction can be readily transposed to those related to eating disorders. At the neurobiological level, similar underlying neural pathways are activated and/or altered by compulsive-like drug and food intake. The behavioral and neurobiological overlap is discussed, with an emphasis on pre-clinical evidence, particularly between binge-eating disorders and drug addiction. Different animal models, their advantages and translational limitations to human pathologies are then discussed.

R.B.M. Duarte • M. Barros (✉)
Department of Pharmaceutical Sciences, School of Health Sciences, University of Brasilia, 70910-900, Brasilia, DF, Brazil

Primate Center, Institute of Biology, University of Brasilia, 70910-900, Brasilia, DF, Brazil
e-mail: mbarros@unb.br

A.C. Borges
Department of Pharmaceutical Sciences, School of Health Sciences, University of Brasilia, 70910-900, Brasilia, DF, Brazil

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_18

Keywords
Food addiction • Binge eating • Reward • Animal models

Introduction

Feeding is imperative for the survival of all animal species and is influenced by both genetic and environmental factors [1]. Eating patterns, however, may vary significantly among species, with some dedicating most of their time to foraging-related activities, whereas others spend months without ingesting food. Nonetheless, all ingestive behaviors have a common goal — to maintain energy homeostasis and ensure one's survival [2, 3].

However, our current society has generated a very particular problem: pathological eating behaviors. Together with our sedentary lifestyle, modern humans are constantly exposed to an "obesogenic" environment, characterized by a widespread availability of cheap and highly palatable foods with elevated salt, sugar, and/or fat content [4]. The consumption of such items can easily exceed an individual's daily nutritional needs and consequently increase the risk of becoming obese and/or developing other related pathologies, such as type 2 diabetes, cardiovascular diseases, and metabolic syndrome [5]. According to the Centers for Disease Control (2006), since the 1950s there has been a four-fold increase in meal size at American restaurants and a 12-kg increase in adults' average body mass.

People have been characterizing themselves as "food addicts". Chocolate is the item most commonly associated with reports of food craving or "addiction", although other energy-dense foods, such as sweet treats (i.e., cookies) and salty "snacks" (i.e., chips) are highly craved as well [6]. However, the high prevalence of obesity, its known effects on our health [7] and its high healthcare costs [8] have also increased the scientific/medical community's interest on "food addiction" and possible treatment strategies [9].

This new-found perspective and interest on the subject has led different researchers to not only suggest that obesity and some eating-related disorders can be directly related to addiction, but also that studying drug addiction can make significant contributions towards a better understanding of food addiction, obesity, and eating-related disorders [1]. In general terms, the latter are non-adaptive eating patterns that occur when food intake does not correspond to the desire to eat (i.e., food is consumed when satiated or physiological signs of hunger are ignored) [10], inducing a significant energy imbalance [11]. For example, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) [12], binge-eating disorder (BED) is defined as "recurring episodes of eating significantly more food in a short period of time than most people would eat under similar circumstances, with episodes marked by feelings of lack of control and distress" [12].

The issue of considering overeating as an actual type of addiction has been under debate for some time. Many have argued that foods or their macronutrients have addictive qualities similar to those of drugs of abuse [13–18]. Such view is based on neural circuits, and behavioral and clinical similarities between overeating and addiction.

In this chapter we discuss the relationship between palatable foods and drugs of abuse, based on the view that excessive intake of highly palatable foods and the pattern in which they are ingested may lead to addiction-like behaviors. As previously argued by Davis and Carter [14], the BED phenotype fits the concept of addiction particularly well, as they both refer to loss of control, tolerance, withdrawal, and craving. Within this framework, we first review the two main neurophysiological mechanisms that regulate ingestive behaviors: (i) the homeostatic drive that, via activation of specific hormones, increases or inhibits food intake according to endogenous energy deposits; and (ii) the hedonic drive, which is related to the subjective pleasurable experiences associated with food and acts independent

of the body's energy stores. We then focus on the main concepts and characteristics of "food addiction" and finally discuss different animal models for this type of addiction, including their advantages and translational limitations to human pathologies.

Neurophysiological Mechanisms Regulating Ingestive Behaviors

As mentioned above, many individuals no longer seem to eat only when physiologically hungry, at least when highly palatable foods are involved. However, eating patterns can generally be divided into two main types. Eating when energy-depleted, and not eating when energy needs have been met, is the main "homeostatic" mode that regulates our energy balance [1]. All other types of food intake may be considered a "non-homeostatic" or "hedonic" eating pattern, as they are not regulated or compensated by metabolic feedback mechanisms.

Therefore, ingestive behaviors are not coordinated by isolated areas of the brain or any one particular system. Complex neural circuits related to executive, reward, and autonomic functions are connected at different levels to the digestive system and circulating homeostatic signals from energy stores in the body [19–22]. These, in turn, respond to various environmental, social, circadian, and contextual factors [23, 24].

The main areas in the central nervous system (CNS) that comprise the appetitive and energy expenditure circuits (i.e., anabolic and catabolic responses to an increase or decrease in fat stores, respectively) include the prefrontal cortex (PFC), anterior cingulate cortex (ACC), insula, ventral striatum, amygdala, hippocampus, *substantia nigra* (SN), ventral tegmental area (VTA) and hypothalamus [22, 25].

In general, the cortical executive circuit exerts a key cognitive control over eating patterns, influencing the decision-making process that induces or represses food ingestion. Adequate executively-mediated self-control over food intake and/or energy expenditure is essential to maintain an overall energy homeostasis [26], particularly in "obesogenic" environments [27]. In fact, self-reported impulsivity in obese individuals has been associated with a higher detection rate of palatable foods [28], while compulsive-like eating patterns are induced by changes in PFC or ACC function, or even impaired connectivity between executive and reward circuits [27], similar to drug addicts [29–32].

Homeostatic Drive for Ingestive Behavior

The homeostatic control over ingestive behavior is primarily related to maintaining an energy balance. A hunger sensation can be triggered by an interaction between physiological signals from the digestive system and/or endogenous energy deposits, with different environmental, social, emotional, contextual, and circadian factors [23, 24].

The neural control of homeostasis was initially attributed to two nuclei in the hypothalamus. The lateral hypothalamus (LH) seems mainly involved in triggering food intake [2]. When stimulated, its neurons synthesize and release neuropeptide Y (NPY) and agouti-related protein (AgRP), leading to an increase in ingestive behaviors, whereas lesions result in hypophagia [33–35]. The ventromedial hypothalamus (VMH), on the other hand, is related to satiety [2]. Neurons in this nucleus express pro-opio-melanocortin (POMC) and cocaine-and-amphetamine-regulated transcript (CART), which inhibit food intake, and lesions induce hyperphagia [35].

Importantly, the hypothalamus also interacts with several peripheral hormones, such as leptin and ghrelin. The former is released by white adipose tissue, its levels increasing proportionally to fat mass. High levels of leptin can, for example, suppress food intake and stimulate metabolic processes that dissipate excessive energy stores [36]. Ghrelin, on the other hand, is produced in the stomach and its levels increase due to negative energy balance, thus stimulating food intake and energy storage [36]. In humans, high levels of ghrelin precede the initiation of a meal [37],

while in animals, ghrelin content increases during food deprivation and decreases when food passes through the stomach [38].

Although leptin and ghrelin receptors are expressed throughout the body, the arcuate nucleus (Arc) of the hypothalamus has extremely high densities. In this nucleus, leptin-regulated signaling on anorexigenic POMC/CART neurons suppresses feeding and increases metabolic rate. On pro-appetite NPY/AgRP neurons, this hormone normally leads to food intake [2]. As other important mechanisms also take part in the homeostatic system, the reader is also referred to more detailed and recent reviews on this topic [2, 25, 35].

Hedonic Drive for Ingestive Behavior

Unlike the homeostatic control, the hedonic drive for ingestive behavior is centered on the food's rewarding properties, and may act independent of the body's energy stores. It is important to point out, however, that these two systems do interact at times. For instance, hormones that indicate metabolic states (e.g., leptin, insulin) have been shown to decrease gustatory and olfactory perception and as a result influence the amount and type of food consumed by mice [39].

Berridge and Kringelbach [40] have argued that pleasure was "evolution's boldest trick," as it increases the probability that specific stimuli-related actions will re-occur, thus motivating behaviors that are essential for survival. So, not surprisingly, food and sexual partners have traditionally been viewed as the most prominent natural rewards. Whilst our ancestors seem to have benefited, in particular, from the hedonic aspects of palatable foods [26], in today's "obesogenic" environment with abundant, readily-available, highly palatable foods, the pleasure derived from eating such items may well be a liability leading to maladaptive pursuits [41].

The reward system is mainly located within the brain's mesocorticolimbic pathway [26], in which the VTA, ventral striatum, amygdala, and PFC play a fundamental role [22]. In general, this system — and in particular the shell of the ventral striatum's nucleus *accumbens* (NAc) — integrates the "wanting" and "liking" motivational aspects related to different types of stimuli in our environment, including food [42].

The first aspect is viewed as the incentive salience that is attributed to a specific food item [43], and is mediated mainly by the dopamine (DA) neurotransmitter system [44]. In fact, DA signaling plays a crucial role in translating motivation into action [45]. An increased DA activity within the brain's mesocorticolimbic pathway induces "reward-seeking" behaviors, but does not seem to directly generate the pleasurable experience related to food consumption [46]. This is generally attributed to the "liking" aspect of the reward system, which consists of the pleasure-related sensations induced by the stimulus [43]. These hedonic properties seem to only indirectly interact with the DA system via opioid receptors located on inhibitory GABAergic neurons within the shell of the NAc. Inhibition of these neurons in rodents increased the release of DA in reward-related areas of the brain and consequently the ingestion of high-fat or sugar foods, regardless of being or not in an energy-depleted state [47, 48].

Nonetheless, two main hypotheses have been put forward to explain the participation of DA in ingestive behaviors. The "gluttony hypothesis" posits that overindulgence is based on the positive correlation between DA release and pleasurable sensory experiences [49, 50]. The "reward-deficiency hypothesis," on the other hand, suggests that overindulgence is a self-medication attempt to elevate deficient DA signaling to a "pleasurable" level [17].

Both the "wanting" and "liking" aspects are also linked to the learning-related executive function discussed above [43]. The incentive salience component tends to dominate the initial appetitive phase of ingestive behaviors, the hedonic properties take part mainly in the subsequent consummatory phase, and learning occurs throughout [40]. Also, a rewarding eating experience activates the DA-mediated mesocorticolimbic circuit, which in turn enables food intake-related cues (i.e., flavor, smell, texture) to

become conditioned to the stimulus. Repeated exposure to reward-associated eating leads to a gradual enhancement of the DA response (sensitization) to conditioned stimuli, which in turn reinforces the incentive salience of that particular food item.

Interaction Between Food and Drug Addiction: General Aspects

The concept of drug addiction and its main characteristics have been under debate for some time and still remain a controversial issue. Whether it is a "lifestyle choice," a pre-set "biological vulnerability" or even both is uncertain, even if drug initiation is essentially a voluntary behavior and its continued use is triggered by inhibition of self-control areas of the brain [51].

Substance-use dependence (SUD), according to the DSM-V [12], comprises a maladaptive pattern of substance use represented by cognitive, behavioral, and physiological symptoms that lead to clinically significant impairment or distress. Its symptomatology includes: (a) tolerance, with increasing amounts being consumed to achieve the same effects or experiencing diminished effects with continued use of the same amount, (b) withdrawal symptoms, when it is no longer consumed, (c) use of larger amounts or over a longer period than intended, (d) a persistent desire or unsuccessful efforts to cut down its use, (e) increased time and effort to obtain or use it, or recover from its effects, (f) decreased social, occupational, and/or recreational activities because of its use, and (g) use despite persistent physical and/or psychological problems caused or exacerbated by the substance [12].

Within this framework, there has been a growing interest in addiction-like behaviors related to putative natural rewards, such as food. Although deemed essential for an individual's survival, they are controlled by both homeostatic and hedonic drives. As the latter is part of the brain's reward system, natural rewards have been shown to induce non-adaptive changes in this neural circuit in a manner similar to that of drugs of abuse [52–54]. However, the homeostatic component (and the evolutionary aspects) related to natural rewards may confound the precise establishment of a pathological state. As such, while the DSM-V recognizes non-substance-related disorders, such as gambling, it still does not include natural reward-related pathologies as a mental disorder [12].

Nonetheless, "food addiction" refers to the notion that specific types of food – particularly highly palatable items — can be overly consumed, regardless of physiological homeostatic needs and in a compulsive-like feeding pattern, but there is as yet no consensual definition. "Food addiction" actually seems to have a compulsive element similar to SUD, while at the same time encompasses symptoms ascribable to both BED and obesity [55]. BED constitutes a diagnostic category of Feeding and Eating Disorders within the DSM-V [12], while others argue that obesity should be viewed as a neuroadaptive disorder [56, 57].

Theron Randolph, in 1956, was possibly the first to use the term [58], and since then sporadic comparisons between drug addiction and pathological eating patterns have been made [59]. Only in the last 20 years has "food addiction" actually received systematic and focal attention. This is clearly demonstrated, for example, by the number of scientific publications per year on this topic as of 2008, compared to 1990–2008, with over 70 articles being published in 2014 [60].

Hoebel et al. [61] carried out in rodents one of the first systematic studies on the similarities between SUD and the behaviors observed towards specific food types. Rats submitted to a feeding schedule consisting of a period of caloric restriction, followed by access to a glucose solution, demonstrated a behavioral response similar to that of drug addicts, i.e., episodes of compulsive-like search and consumption, accompanied by signs of withdrawal when the sugar solution was withheld [13, 62].

Furthermore, the Yale Food Addiction Scale (YFAS) — a questionnaire based on SUD criteria [63] and some additional aspects to clinically assess impairment and/or distress due to overeating — has also provided important clinical

validation for the "diagnosis" of "food addiction" in humans [64]. Based on this instrument, prevalence rates varied between 5–10%, 15–25% and 40–60% in non-clinical, obese, and obese individuals with BED or morbidly obese bariatric patients, respectively [65–75]. Accordingly, "food addiction" symptoms were more commonly observed in a specific subset of obese individuals, having a higher correlation with a binge-type eating pattern than with obesity per se [75].

An overlap in core behavioral symptoms can be seen in terms of pathological overeating, "food addiction" and SUD, as is the case of craving [76] — an intense desire to consume a particular item from which it is exceptionally hard to refrain [77]. While the ingestion of any food item alleviates a hunger sensation, only the specific desired food relieves crave feelings for that item [77]. Food craving is thus unrelated to caloric restriction, dieting, or food deprivation [14], but self-reported craving rates are positively correlated with "food addiction" as measured in the YFAS. Craved foods are usually high in sugar and/or fat content and thus very palatable. Chocolate, pizza, ice cream, and other "junk food" items are typically craved [78], and in the YFAS they comprise the items most likely consumed in an addictive-like manner [79].

Craving sensation is not only a good behavioral example of the similarities between food and substance dependence [77], but a neurobiological one as well. Neuroimaging studies have revealed that both food and drug craving activate the same neural structures of the reward system, such as the PFC [80, 81], insula, NAc, ACC, and amygdala [82]. Self-induced craving also increased hippocampus, caudate, and insula activity [83]. Nonetheless, other neurobiological aspects — particularly those related to DA function in the NAc — also indicate an important interaction between food and drug dependence. For example, prolonged exposure to palatable foods was found to down-regulate D2 receptors in the ventral striatum [17, 32]. The opioid system has also been implicated in withdrawal of both drugs and food [84] and a higher mu-opioid receptor polymorphism is seen in BED patients.

Interaction Between Food and Drug Addiction: Animal Models and Pre-clinical Data

A considerable number of studies that have demonstrated similarities between drugs and palatable foods are based on animal research. Existing pre-clinical "food addiction" tests typically induce behavioral responses that are also seen in putative drug addition protocols, such as tolerance, continued consumption in spite of an aversive stimulus, withdrawal signs, relapse and cue-induced feeding [reviewed in 12].

While there is a widespread use of non-human primates in the study of drug addiction, there are surprisingly few reports on "food addiction" or binge-like behaviors in this animal model, compared to studies in rodents. This may be partly due to costs and housing difficulties inherently associated with non-human primates.

In 1969, Miller proposed an isolation model for binge eating, where rhesus monkeys isolated from social contact during the first year of life ate and drank approximately twice as much as control animals [85]. Later, in Richard Foltin's laboratory, baboons submitted to an intermittent candy access protocol (see below for more details), after a nine-week period consumed 75% of their total daily caloric intake in candy during their first meal of the day [86]. More recently, in our laboratory, we demonstrated that a highly palatable food (i.e., chocolate) induced a conditioned place preference response in marmoset monkeys, similar to that of drugs of abuse [87, 88].

In the rodent sugar-bingeing model, rats submitted to 12 h of restricted access to a sweet solution (sucrose, glucose, or saccharin) typically developed a binge-like eating pattern [89]. These sugar-bingeing animals repeatedly released DA [90] and had reduced D2 receptor binding in the NAc [91] — a generally accepted neurobiological correlate of drug addiction. In addition, a withdrawal-like state was seen when sucrose availability was interrupted, as indicated by increased levels of anxiety [13] and depression [92]. Administration of the opioid antagonist naloxone increased withdrawal symptoms in rats

that were glucose-fed, similar to rat models of morphine addiction [93].

In addition, satiated rodents with intermittent access to fat gradually escalated their consumption of pure vegetable shortening, resulting in a fat-bingeing eating pattern when this item was available [94–97]. Similarly to animals that are fed a sugar diet, fat-bingeing rats had higher levels of DA in the NAc [52, 53, 98]. Obesity-prone animals, with lower basal DA concentrations, overly consumed fat and increased DA content towards a more "rewarding level" [97, 99]. This may corroborate the "reward-deficiency hypothesis" that was described above [100]. Fat-bingeing rats also demonstrated signs of tolerance and overeating, yet withdrawal symptoms have not been reported so far [56, 101, 102]. Therefore, fat-bingeing may not necessarily fulfill all facets putatively related to addiction [79].

The sweet–fat protocol, on the other hand, may well be the first model to induce obesity, as well as behavioral and neurobiological correlates of addiction. It is based on an intermittent access to a "cafeteria diet" made up of foods with elevated fat and sugar content that are highly processed and commercially available, such as chocolate, cake, cheese and condensed milk [57, 103]. As it resembles more the items of our daily life, it is thought to better mimic the conditions seen in humans [104]. Rats with 2-h access to a cafeteria diet and ad libitum chow binged on these palatable foods [105] and gained significantly more weight compared to other models [13]. Geiger et al. [106] reported a down-regulation in the expression of striatal D2 receptors and a reduced mesolimbic DA transmission in this animal model. Interestingly, diet-induced obesity in rats also down-regulated D2 receptors in the ventral striatum, similar to drug addiction [107].

Based on the models discussed above, a highly relevant feature for inducing a compulsive binge-like eating pattern in animals seems to be the restricted and intermittent manner in which the palatable food is provided [108]. Under such conditions, rats have been shown to escalate food intake [91, 93], present behavioral and neurochemical indicators of withdrawal, and develop cross-sensitization with amphetamine, mimicking behaviors seen in drug-addiction protocols [109].

Under food restriction conditions, preference for palatable foods also seems to increase concurrently to changes in DA function in the NAc. For instance, rats submitted to a restricted-feeding schedule had lower basal extracellular DA levels in the NAc, which increased significantly in response to food or amphetamine [110, 111]. Restricted diets also induced a higher binding of DA to its pre-synaptic reuptake transporter (DAT) in the NAc and VTA, as well as more DAT mRNA in the VTA of rats [112]. Therefore, repeated ingestion of palatable foods under restricted access regimens induces neuroadaptations in the mesoaccumbens DA system, corroborating the notion that similar cellular changes may be involved in restrictive eating disorders and reward bingeing [40].

Lastly, cross-sensitization is another important element that seems to link drugs of abuse to pathological eating patterns. In this behavioral response, repeated exposure to one reward leads to a more robust response of another stimulus. Different studies have demonstrated that exposure to a food reward cross-sensitizes with several types of drugs of abuse. For example, sucrose-bingeing was reported to facilitate cocaine-induced sensitization [113, 114]. On the other hand, cocaine-sensitized rats chose saccharin over cocaine, and maintained this preference even with increasing doses of the drug reward [115, 116]. Sugar-bingeing rats also cross-sensitized with amphetamines and alcohol [117, 118].

Conclusion

Homeostasis depends on the integration and communication between CNS structures and peripheral mediators. Excessive and easy access to hypercaloric (and consequently palatable) foods, along with habit formation, may induce pathologies such as obesity and compulsive eating, which in turn lead to significant neural adaptations. As these mainly include changes in DA neurotransmission within reward areas of the brain, "food" and

drug addiction may have a similar neurobiological basis.

"Food addiction," binge eating, and obesity may well be maladaptive conditions to our contemporary calorie-rich and "obesogenic" environment. However, contrary to drugs of abuse, it is very difficult to completely remove sugar and fat from our diet. So a better understanding of our eating patterns may lead to more efficient treatments and the prevention of food-related pathologies.

The complex relationship that we have with food — ranging from physiological, environmental, social, emotional, contextual, and circadian aspects — does pose a significant challenge to the study of food-intake-related pathologies, as well as to the development and validation of animal models. In fact, small methodological variations in animal-based studies induce significant differences in results and conclusions. It is therefore essential to take into account both the physiology and natural habits of the animal model under investigation, as well as to approximate the experimental design as much as possible to the conditions seen in humans. For this, non-human primates may provide new and important insights on binge eating and "food addiction" at the molecular, cellular, physiological, and behavioral levels.

Taken together, animal models have contributed to the study of palatable food intake and the development of "food addiction" in humans. Both sugar and sweet-fat protocols induced tolerance, escalation, bingeing, and withdrawal-like symptoms. So far, they underscore the importance of feeding patterns (in particular intermittent access) in habit formation and pathological eating, as much as the palatable food itself. This behavior seems to mimic compulsive eaters, who tend to alternate between self-restriction and binge episodes.

Acknowledgments The writing of this chapter was supported by CNPq (478930/2012-7) and FAP-DF (193.001.026/2015). R.B.M.D. received a doctoral fellowship from CNPq and M.B. a research fellowship from CNPq (304041/2015-7).

References

1. Volkow ND, Wise RA. How can drug addiction help us understand obesity? Nat Neurosci. 2005;8:555–60.
2. Saper CB, Chou TC, Elmquist JK. The need to feed: homeostatic and hedonic control of eating. Neuron. 2002;36:199–211.
3. Sternson SM, Nicholas Betley J, Cao ZF. Neural circuits and motivational processes for hunger. Curr Opin Neurobiol. 2013;23:353–60.
4. Mustajoki P. Obesegenic food environment explains most of the obesity epidemic. Duodecim. 2015;131:1345–52.
5. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. JAMA. 2010;303:235–41.
6. Rodin J, Mancuso J, Granger J, Nelbach E. Food cravings in relation to body mass index, restraint, and estradiol levels: a repeated measures study in healthy women. Appetite. 1991;17:177–85.
7. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93:359–404.
8. Finkelstein EA, Trogdon JG, Cohen JW, Dietz W. Annual medical spending attributable to obesity: payer- and service-specific estimates. Health Aff. 2009;28:822–31.
9. Fontaine KR, Redden DT, Wang C, Westfall AO, Allison DB. Years of life lost due to obesity. JAMA. 2003;289:187–93.
10. Berridge KC, Ho CY, Richard JM, DiFeliceantonio AG. The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. Brain Res. 2010;1350:43–64.
11. Gasbarri A, Pompili A, Packard MG, Tomaz C. Habit learning and memory in mammals: behavioral and neural characteristics. Neurobiol Learn Mem. 2014;114:198–208.
12. APA – American Psychiatric Association. Diagnostic and statistical manual of mental disorders (DSM-V). 5th ed. Washington, DC: American Psychiatric Association; 2013.
13. Avena NM, Rada P, Hoebel BG. Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. Neurosci Biobehav Rev. 2008;32:20–39.
14. Davis C, Carter JC. Compulsive overeating as an addiction disorder. A review of theory and evidence. Appetite. 2009;53:1–8.
15. Gold MS, Frost-Pineda K, Jacobs WS. Overeating, binge eating, and eating disorders as addictions. Psychiatr Ann. 2003;33:117–22.
16. Jong JW, Vanderschuren LJ, Adan RA. Towards an animal model of food addiction. Obes Facts. 2012;5:180–95.
17. Volkow ND, Wang GJ, Fowler JS, Telang F. Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. Philos Trans R Soc Lond B Biol Sci. 2008;363:3191–200.

18. Wilson GT. Eating disorders, obesity and addiction. Eur Eat Disord Rev. 2010;18:341–51.
19. Benoit SC, Tracy AL. Behavioral controls of food intake. Peptides. 2008;29:139–47.
20. Woods SC. The control of food intake: behavioral versus molecular perspectives. Cell Metab. 2009;9:489–98.
21. Fulton S. Appetite and reward. Front Neuroendocrinol. 2010;31:85–103.
22. Dagher A. Functional brain imaging of appetite. Trends Endocrinol Metab. 2012;23:250–60.
23. Strubbe JH, Woods SC. The timing of meals. Psychoanal Rev. 2004;111:128–41.
24. Woods SC, Lutz TA, Geary N, Langhans W. Pancreatic signals controlling food intake, insulin, glucagon and amylin. Philos Trans R Soc B Biol Sci. 2006;361:1219–35.
25. Schneeberger M, Gomis R, Claret M. Hypothalamic and brainstem neuronal circuits controlling homeostatic energy balance. J Endocrinol. 2014;220:25–46.
26. Volkow ND, Wang GJ, Tomasi D, Baler RD. Obesity and addiction: neurobiological overlaps. Obes Rev. 2013;14:2–18.
27. Vainik U, Dagher A, Dube L, Fellows LK. Neurobehavioural correlates of body mass index and eating behaviours in adults: a systematic review. Neurosci Biobehav Rev. 2013;37:279–99.
28. Dalley JW, Everitt BJ, Robbins TW. Impulsivity, compulsivity, and top-down cognitive control. Neuron. 2011;69:680–94.
29. Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley J, Robbins TW. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. Philos Trans R Soc Lond B Biol Sci. 2008;363:3125–35.
30. Gautier JF, Chen K, Salbe AD, Bandy D, Pratley RE, Heiman M, Ravussin E, Reiman EM, Tataranni PA. Differential brain responses to satiation in obese and lean men. Diabetes. 2000;49:838–46.
31. Gearhardt AN, Yokum S, Orr PT, Stice E, Corbin WR, Brownell KD. Neural correlates of food addiction. Arch Gen Psychiatry. 2011;68:808–16.
32. Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. Trends Cogn Sci. 2011;15:37–46.
33. Morton GJ, Cummings DE, Baskins DG, Barsh GS, Schwartz MV. Central nervous system control of food intake and body weight. Nature. 2006;21:289–95.
34. Morton GJ, Meek TH, Schwartz MW. Neurobiology of food intake in health and disease. Nat Rev Neurosci. 2014;15:367–78.
35. Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature. 2000;404:661–71.
36. Zigman JM, Elmquist JK. From anorexia to obesity — the yin and yang of body weight control. Endocrinology. 2003;144:3749–56.
37. Cummings DE, Shannon MH. Roles for ghrelin in the regulation of appetite and body weight. Arch Surg. 2003;138:389–96.
38. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Letters Nature. 1999;402:656–60.
39. Shigemura N, Ohta R, Kusakabe Y, Miura H, Hino A, Koyano K, Nakashima K, Ninomiya Y. Leptin modulates behavioral responses to sweet substances by influencing peripheral taste structures. Endocrinology. 2004;145:839–47.
40. Berridge KC, Kringelbach ML. Pleasure systems in the brain. Neuron. 2015;86:646–64.
41. Egecioglu E, Skibicka KP, Hansson C, Alvarez-Crespo M, Friberg PA, Jerlhag E, Engel JA, Dickson LS. Hedonic and incentive signals for body weight control. Rev Endocr Metab Disord. 2011;12:141–51.
42. Castro DC, Berridge KC. Advances in the neurobiological bases for food 'liking' versus 'wanting'. Physiol Behav. 2014;136:22–30.
43. Berridge KC, Robinson TE. Parsing reward. Trends Neurosci. 2003;26:507–13.
44. Richard JM, Castro DC, Difeliceantonio AG, Robinson MJ, Berridge KC. Mapping brain circuits of reward and motivation: in the footsteps of Ann Kelley. Neurosci Biobehav Rev. 2013;37:1919–31.
45. Berthoud HR, Lenard NR, Shin AC. Food reward, hyperphagia, and obesity. Am J Physiol Regul Integr Comp Physiol. 2011;300:1266–77.
46. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev. 1998;28:309–69.
47. Wise RO. Dopamine, learning and motivation. Nat Rev Neurosci. 2004;5:483–94.
48. Zhang M, Gosnell BA, Kelley AE. Intake of high-fat food is selectively enhanced by mu opioid receptor stimulation within the nucleus accumbens. J Pharmacol Exp Ther. 1998;285:908–14.
49. Hajnal A, Acharya NK, Grigson PS, Covasa M, Twining RC. Obese OLETF rats exhibit increased operant performance for palatable sucrose solutions and differential sensitivity to D2 receptor antagonism. Am J Physiol Reg Integ Compar Physiol. 2007;293:1846–54.
50. Westerink BH, Teisman A, De Vries JB. Increase in dopamine release from the nucleus accumbens in response to feeding: a model to study interactions between drugs and naturally activated dopaminergic neurons in the rat brain. Naunyn Schmiedeberg's Arch Pharmacol. 1994;349:230–5.
51. Volkow ND, Morales M. The brain on drugs: from reward to addiction. Cell. 2015;162:712–25.
52. Olsen CM. Natural rewards, neuroplasticity, and non-drug addictions. Neuropharmacology. 2011;61:1109–22.
53. Pitchers K, Balfour M, Lehman M. Neuroplasticity in the mesolimbic system induced by natural reward and subsequent reward abstinence. Biol Psychiatry. 2010;67:872–9.
54. Weatherford SC, Greenberg D, Gibbs J, Smith GP. The potency of D-1 and D-2 receptor antagonists

is inversely related to the reward value of sham-fed corn oil and sucrose in rats. Pharmacol Biochem Behav. 1990;37:317–23.
55. Brownell KD, Gold MS. Food and addiction: a comprehensive handbook. Oxford: Oxford University Press; 2012.
56. Kenny PJ. Common cellular and molecular mechanisms in obesity and drug addiction. Nat Rev Neurosci. 2011;12:638–51.
57. Shafat A, Murray B, Rumsey D. Energy density in cafeteria diet induced hyperphagia in the rat. Appetite. 2009;52:34–8.
58. Randolph TG. The descriptive features of food addiction: addictive eating and drinking. Q J Stud Alcohol. 1956;17:198–224.
59. Rogers PJ, Smit HJ. Food craving and food "addiction": a critical review of the evidence from a biopsychosocial perspective. Pharmacol Biochem Behav. 2000;66:3–14.
60. Meule A. Back by popular demand: a narrative review on the history of food addiction research. Yale J Biol Med. 2015;88:295–302.
61. Hoebel BG, Hernandez L, Schwartz DH, Mark GP, Hunter GA. Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior. Theoretical and clinical implications. Ann N Y Acad Sci. 1989;575:171–91.
62. Avena NM, Rada P, Hoebel BG. Sugar and fat bingeing have notable differences in addictive-like behavior. J Nutr. 2009;139:623–8.
63. APA – American Psychiatric Association. Diagnostic and statistical manual of mental disorders (DSM-IV). 4th ed. Washington DC: American Psychiatric Association; 1994.
64. Gearhardt AN, Corbin WR, Brownell KD. Food addiction: an examination of the diagnostic criteria for dependence. J Add Med. 2009;3:1–7.
65. Burmeister JM, Hinman N, Koball A, Hoffmann DA, Carels RA. Food addiction in adults seeking weight loss treatment. Implications for psychosocial health and weight loss. Appetite. 2013;60:103–10.
66. Davis C, Curtis C, Levitan RD, Carter JC, Kaplan AS, Kennedy JL. Evidence that 'food addiction' is a valid phenotype of obesity. Appetite. 2011;57:711–7.
67. Eichen DM, Lent MR, Goldbacher E, Foster GD. Exploration of "food addiction" in overweight and obese treatment-seeking adults. Appetite. 2013;67:22–4.
68. Flint E, Cummins S, Sacker A. Associations between active commuting, body fat, and body mass index: population based, cross sectional study in the United Kingdom. BMJ. 2014;349:1–9.
69. Gearhardt AN, Corbin WR, Brownell KD. Preliminary validation of the Yale Food Addiction Scale. Appetite. 2009;52:430–6.
70. Gearhardt AN, White MA, Masheb RM, Morgan PT, Crosby RD, Grilo CM. An examination of the food addiction construct in obese patients with binge eating disorder. Int J Eat Disord. 2012;45:657–63.
71. Gearhardt AN, White MA, Masheb RM, Grilo CM. An examination of food addiction in a racially diverse sample of obese patients with binge eating disorder in primary care settings. Compr Psychiatry. 2013;54:500–5.
72. Lent MR, Eichen DM, Goldbacher E, Wadden TA, Foster GD. Relationship of food addiction to weight loss and attrition during obesity treatment. Obesity. 2014;22:52–5.
73. Mason SM, Flint AJ, Field AE, Austin SB, Rich-Edwards JW. Abuse victimization in childhood or adolescence and risk of food addiction in adult women. Obesity. 2013;21:775–81.
74. Meule A, Heckel D, Kübler A. Factor structure and item analysis of the Yale Food Addiction Scale in obese candidates for bariatric surgery. Eur Eat Disord Rev. 2012;20:419–22.
75. Davis C. From passive overeating to "food addiction": a spectrum of compulsion and severity. ISRN Obes. 2013;2013:1–20.
76. Hormes JM, Rozin P. Does 'craving' carve nature at the joints? Absence of a synonym for craving in many languages. Addict Behav. 2010;35:459–63.
77. Martin CK, Mcclernon FJ, Chellino A, Correa JB. Food cravings: a central construct in food intake behavior, weight loss, and the neurobiology of appetitive behavior. In: Handbook of behavior, food and nutrition. New York: Springer; 2011. p. 741–55.
78. Weingarten HP, Elston D. Food cravings in a college population. Appetite. 1991;17:167–75.
79. Hone-Blanchet A, Fecteau S. Overlap of food addiction and substance use disorders definitions: analysis of animal and human studies. Neuropharmacology. 2014;85:81–90.
80. Filbey FM, Schach JP, Meyrs US, Chavez RS, Hutchison KE. Marijuana craving in the brain. Proc Natl Acad Sci U S A. 2009;106:13016–21.
81. Kober H, Mende-Siedlecki P, Kross EF, Weber J, MischeL W, Hat CL, Ochsner KN. Prefrontal-striatal pathway underlies cognitive regulation of craving. Proc Natl Acad Sci U S A. 2010;107:14811–6.
82. Wang GJ, Volkow ND, Thanos PK, Fowler JS. Imaging of brain dopamine pathways: implications for understanding obesity. J Addict. 2009;3:8–18.
83. Pelchat ML, Johnson A, Chan R, Valdez J, Ragland JD. Images of desire: food-craving activation during fMRI. NeuroImage. 2004;23:1486–93.
84. Davis C, Patte K, Curtis C, Reid C. Immediate pleasures and future consequences. A neuropsychological study of binge eating and obesity. Appetite. 2010;54:208–13.

85. Miller RE, Mirsky IA, Caul WF, Sakata T. Hyperphagia and polydipsia in socially isolated rhesus monkeys. Science. 1969;165:1027–8.
86. Foltin RW, Haney M. Effects of the cannabinoid antagonist SR141716 (rimonabant) and d-amphetamine on palatable food and food pellet intake in non-human primates. Pharmacol Biochem Behav. 2007;86:766–73.
87. Duarte RBM, Patrono E, Borges AC, César AA, Tomaz C, Ventura R, Gasbarri A, Puglisi-Allegra S, Barros M. Consumption of a highly palatable food induces a lasting place-conditioning memory in marmoset monkeys. Behav Process. 2014;107:163–6.
88. Duarte RBM, Patrono E, Borges AC, Tomaz C, Ventura R, Gasbarri A, Puglisi-Allegra S, Barros M. High versus low fat/sugar food affects the behavioral, but not the cortisol response of marmoset monkeys in a conditioned-place-preference task. Physiol Behav. 2015;139:442–8.
89. Rada P, Avena NM, Hoebel BG. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience. 2005;134:737–44.
90. Cottone P, Sabino V, Steardo L, Zorrilla EP. Consummatory, anxiety-related and metabolic adaptations in female rats with alternating access to preferred food. Psychoneuroendocrinology. 2009;34:38–49.
91. Colantuoni C, Schwenker J, Mccarthy J, Rada P, Ladenheim B, Cadet JL, Schwartz GJ, Moran TH, Hoebel BG. Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. Neuroreport. 2001;12:3549–52.
92. Mathes CM, Ferrara M, Rowland NE. Cannabinoid-1 receptor antagonists reduce caloric intake by decreasing palatable diet selection in a novel dessert protocol in female rats. Am J Physiol Regul Integr Comp Physiol. 2008;295:67–75.
93. Colantuoni C, Rada P, Mccarthy J, Patten C, Avena NM, Chadeanye A, Hoebel BG. Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. Obes Res. 2002;10:478–88.
94. Corwin RL. Bingeing rats: a model of intermittent excessive behavior? Appetite. 2006;46:11–5.
95. Corwin RL, Buda-Levin A. Behavioral models of binge-type eating. Physiol Behav. 2004;82:123–30.
96. Corwin RL, Wojnicki FH, Fisher JO, Dimitriou SG, Rice HB, Young MA. Limited access to a dietary fat option affects ingestive behavior but not body composition in male rats. Physiol Behav. 1998;65:545–53.
97. Rada P, Bocarsly ME, Barson JR, Hoebel BG, Leibowitz SF. Reduced accumbens dopamine in Sprague-Dawley rats prone to overeating a fat-rich diet. Physiol Behav. 2010;101:394–400.
98. Liang NC, Hajnal A, Norgren R. Sham feeding corn oil increases accumbens dopamine in the rat. Am J Physiol Regul Integr Comp Physiol. 2006;291:1236–9.
99. de Araujo IE, Oliveira-Maia AJ, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MA, Simon SA. Food reward in the absence of taste receptor signaling. Neuron. 2008;57:930–41.
100. Volkow ND, Fowler JS, Wang GJ, Baler R, Telang F. Imaging dopamine's role in drug abuse and addiction. Neuropharmacology. 2009;56:3–8.
101. Bocarsly ME, Berner LA, Hoebel BG, Avena NM. Rats that binge eat fat-rich food do not show somatic signs or anxiety associated with opiate-like withdrawal: implications for nutrient-specific food addiction behaviors. Physiol Behav. 2011;104:865–72.
102. Kenny PJ. Reward mechanisms in obesity: new insights and future directions. Neuron. 2011;69:664–79.
103. Heyne A, Kiesselbach C, Sahún I, McDonald J, Gaiffi M, Dierssen M, Wolffgramm J. An animal model of compulsive food-taking behaviour. Addict Biol. 2009;14:373–83.
104. Finlayson G, King N, Blundell JE. Is it possible to dissociate 'liking' and 'wanting' for foods in humans? A novel experimental procedure. Physiol Behav. 2007;90:36–42.
105. Kinzig KP, Hargrave SL, Honors MA. Binge-type eating attenuates corticosterone and hypophagic responses to restraint stress. Physiol Behav. 2008;95:108–13.
106. Geiger BM, Haburcak M, Avena NM, Moyer MC. Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. Neuroscience. 2009;159:1193–9.
107. Johnson PM, Kenny PJ. Addiction-like reward dysfunction and compulsive eating in obese rats: role for dopamine D2 receptors. Nat Neurosci. 2010;13:635–41.
108. Hagan MM, Moss DE. Persistence of binge-eating patterns after a history of restriction with intermittent bouts of refeeding on palatable food in rats: implications for bulimia nervosa. Int J Eat Disord. 1997;22:411–20.
109. Avena NM, Long KA, Hoebel BG. Sugar-dependent rats show enhanced responding for sugar after abstinence: evidence of a sugar deprivation effect. Physiol Behav. 2005;84:359–62.
110. Pothos EN. The effects of extreme nutritional conditions on the neurochemistry of reward and addiction. Acta Astronaut. 2001;49:391–7.
111. Pothos EN, Hernandez L, Hoebel BG. Chronic food deprivation decreases extracellular dopamine in the nucleus accumbens: implications for a possible neurochemical link between weight loss and drug abuse. Obes Res. 1995;3:525–9.

112. Bello NT, Lucas LR, Hajnal A. Repeated sucrose access influences dopamine D2 receptor density in the striatum. Neuroreport. 2002;13:1575–8.
113. Gosnell BA. Sucrose intake enhances behavioral sensitization produced by cocaine. Brain Res. 2005;1031:194–201.
114. Zeeni N, Daher C, Fromentin G, Tome D, Darcel N, Chaumontet C. A cafeteria diet modifies the response to chronic variable stress in rats. Stress. 2013;16:211–9.
115. Lenoir M, Serre F, Cantin L, Ahmed SH. Intense sweetness surpasses cocaine reward. PLoS One. 2007;2:1–10.
116. Manian J, Morris MJ. Palatable cafeteria diet ameliorates anxiety and depression-like symptoms following and adverse early environment. Psychoneuroendocrinology. 2010;35:717–28.
117. Avena NM, Hoebel BG. Amphetamine-sensitized rats show sugar-induced hyperactivity (cross-sensitization) and sugar hyperphagia. Pharmacol Biochem Behav. 2003;74:635–9.
118. Cota D, Tschop MH, Horvath TL, Levine AS. Cannabinoids, opioids and eating behavior: the molecular face of hedonism? Brain Res Rev. 2006;51:85–107.

19 Deep Brain Stimulation: A Promising Therapeutic Approach to the Treatment of Severe Depressed Patients — Current Evidence and Intrinsic Mechanisms

Laura Perez-Caballero, Sonia Torres-Sanchez, Juan Antonio Mico, and Esther Berrocoso

Abstract

Major depressive disorder represents one of the most severe disabling disorders, affecting around 4.7% of the worldwide population. Many patients suffering this neuropsychiatric illness are treated successfully with various treatments, including antidepressant drugs and psychotherapy but also physical measures (electroconvulsive therapy, repetitive transcranial magnetic stimulation, vagal nerve stimulation). Despite the different treatment approaches available, unfortunately 30–40% of the patients fail to respond to most first-line treatments, and between 5 and 10% do not respond to conventional therapy at all. Thus, a considerable number of patients have a poor outcome and unfortunately fail to reach sustained remission. These data highlight the need to find new therapeutic approaches that especially focus on refractory patients. In this context, deep brain stimulation (DBS) emerges as an innovative physical treatment for refractory depressed patients. DBS has been successfully used for years in some neurological disorders such as Parkinson's disease. Currently, in addition to its use in treating depression, DBS is also being tested in other psychiatric illness such as obsessive–compulsive disorder. Most studies on DBS have focused on efficiency and efficacy, or improvement in the technique, and a few

L. Perez-Caballero • E. Berrocoso (✉)
Department of Psychology, Area of Psychobiology, Neuropsychopharmacology and Psychobiology Research Group, University of Cádiz, CIBER for Mental Health (CIBERSAM), Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz (INiBICA), Cádiz, Spain
e-mail: laura.perez@uca.es; esther.berrocoso@uca.es

S. Torres-Sanchez
Neuropsychopharmacology and Psychobiology Research Group, University of Cádiz, CIBER for Mental Health (CIBERSAM), Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz (INiBICA), Cádiz, Spain
e-mail: sonia.torres@uca.es

J.A. Mico
Department of Neuroscience, Pharmacology and Psychiatry, Neuropsychopharmacology and Psychobiology Research Group, CIBER for Mental Health (CIBERSAM), Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz (INiBICA), Cádiz, Spain
e-mail: juanantonio.mico@uca.es

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_19

were devoted to understanding the intrinsic mechanisms responsible. Understanding the molecular mechanisms of action of DBS is currently considered as crucial, not only in order to understand its efficacy but also to propose new antidepressant approaches. The aim of this chapter is to review the foundations, the efficacy, and the efficiency of DBS in depression, and to provide insight into the intrinsic mechanisms of action described until now. In addition, future developments will be discussed.

Keywords
Deep brain stimulation • Major depressive disorder • Ventral capsule/ventral striatum • Nucleus accumbens • Subgenual cingulate cortex • Lateral habenula • Medial forebrain bundle • Inferior thalamic peduncle

Introduction

Major depressive disorder (MDD) is a psychiatric illness with a prevalence around 4.7% among the worldwide population [1]. According to the World Health Organization this disorder ranks among the leading causes of disability [2], and it is expected to be the second most common cause of disability by 2020 [3]. Nowadays, clinical, neurochemical, neuroimaging, and postmortem evidence suggests that MDD is not a disease that affects a particular anatomical region or a single system of neurotransmission. It is postulated that MDD is a dysfunction of cortical, subcortical, and limbic system areas and their connections, and hence neurotransmitter systems and molecular mediators.

Multiple pharmacological and psychotherapeutic treatments are currently available for MDD. The first-line therapy for depression involves the use of antidepressant drugs that mainly act by inhibiting monoamine reuptake, thereby increasing monoamine levels in the synaptic cleft. But unfortunately, it has been estimated that 30–40% of MDD patients do not improve in response to this pharmacological treatment [4]. As such, in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, a randomized and multi-center clinical trial conducted in 2004 in the USA, patients diagnosed with non-psychotic MDD received sequenced treatment at four levels, and the overall cumulative remission rate was 67% after the four levels of treatment [5]. Other non-pharmacological approaches are effective for MDD, such as psychotherapy or physical approaches including electroconvulsive therapy, repetitive transcranial magnetic stimulation, or vagal nerve stimulation [6–9]. Although the majority of patients with MDD respond to the broad range of current treatments, between 5% and 10% fail to respond, having a poor outcome and failing to achieve sustained remission [4, 5, 10]. This critical issue results in an enormous economic burden, poor quality of life, personal suffering, and a high risk of suicide [3, 11, 12]. Thus, the lack of a satisfactory response in treatment-resistant depressive patients highlights the need to progress toward the discovery of effective alternative therapies.

In this context, deep brain stimulation (DBS) therapy arose as an experimental alternative for patients suffering from resistant MDD. DBS is an invasive approach involving stereotaxic surgery. Stimulation electrodes are permanently connected to a neurostimulator with the capacity to deliver electrical chronic stimulation of a targeted brain region. Several data indicate that DBS produces similar benefits to the ablation of the target area [13, 14] but with fewer side effects, because DBS is a reversible approach and the stimulation device is adjustable to attain the desired therapeutic effect. Indeed, electric current intensity, pulse width, and frequency of the stimulation applied can be appropriately modified. Thus, the parameters of stimulation are adapted for each disease, each target of stimulation, or even depending on the individual needs of each patient. The extensive experience using this technique reveals that it is a safe and well-tolerated therapy, and most of the side effects

reported are related to the surgical procedure. Originally, DBS was developed as a technique for movement disorders. The effectiveness demonstrated in several neurological disorders such as Parkinson's disease, dystonia, and essential tremor [15] led to the exploration of this approach to manage psychiatric diseases. As a result, the US Food and Drug Administration approved DBS for treatment-resistant obsessive-compulsive disorder (OCD) in 2009. DBS is currently used in research studies to treat other neuropsychiatric disorders such as MDD.

Although multiple trials have been performed using this technique, the mechanisms underlying the therapeutic effects of DBS have not yet been elucidated. However, authors have proposed several hypotheses. Indeed, some studies have suggested that DBS induces a simple local neuronal activation of the targeted area [16], while other results indicate that DBS preferentially inhibits cell bodies and only stimulates axon terminals [17]. Nevertheless, DBS seems to present a much more complex mechanism of action than a simple activation or inhibition of the target area, even being able to widely modulate brain network activity [18, 19]. In this way, understanding the inherent mechanisms of DBS could give us crucial information to discern the distinctive biological features related to treatment-resistant disorders.

With regard to DBS for treating refractory MDD, only a few clinical trials have been reported. Even so, DBS was effective in patients suffering resistant MDD, showing a promising improvement of depressive symptomatology. In this way, several brain areas have been tested as targets for DBS: subgenual cingulate cortex (SCC), nucleus accumbens (NAc), ventral capsule/ventral striatum (VC/VS), inferior thalamic peduncle (ITP), medial forebrain bundle (MFB) and lateral habenula (LHb). Despite the fact that DBS causes a significant reduction in depression rating scales when applied to most of them, there is still considerable debate about which is the best stimulation target to treat refractory depressed patients.

The aim of the present chapter is to collect evidence of clinical and preclinical data and attempt to discern potential intrinsic mechanisms of DBS in the different brain sites of stimulation tested for resistant MDD.

Ventral Capsule/Ventral Striatum and Nucleus Accumbens

Based on the efficacy of capsulotomy, a lesional therapy widely used for more than 50 years in OCD patients, the VC/VS was proposed as a brain area to be stimulated by DBS for this psychiatric disorder. The VC/VS target corresponds anatomically with the ventral limb of the internal capsule and the adjoining ventral striatum (Fig. 19.1), and DBS applied to this region has been found to be effective for intractable OCD [20, 21]. Given that an unexpected improvement in depressive symptoms was observed in patients primarily diagnosed with OCD [20, 22, 23], this area was proposed as a putative DBS target for refractory MDD patients. The first clinical intervention targeting VC/VS showed encouraging results for MDD patients, who achieved close to a 60% response rate at the last follow-up visit [24]. However, the randomized sham-controlled clinical trial using VC/VS DBS did not report significant differences between DBS and sham control patients, and the response rate obtained was only 20% in both cohorts [25].

The ventral striatum complex includes the NAc, another region that was proposed as a DBS target area for refractory depressed patients (Fig. 19.1). The NAc plays a crucial role in the reward circuitry and motivation processes [26]. Given that impairment in reward processing is related to the anhedonic symptoms of depression

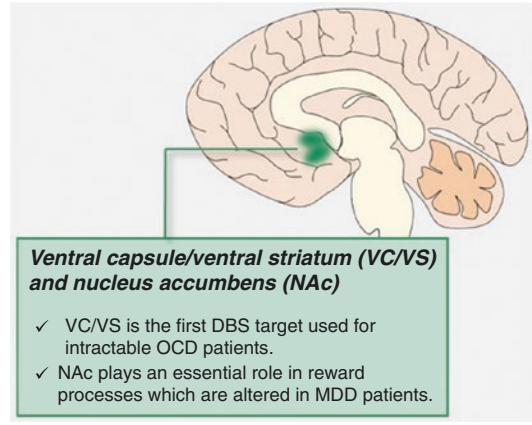


Fig. 19.1 General schematic representation of the VC/VS and NAc as targets for refractory MDD

[27, 28], the effectiveness of DBS on the NAc was evaluated in clinical studies. They reported a sustained alleviation of depressive symptomatology, reaching approximately 50% in the rate of response, accompanied by an antianhedonic and anxiolytic effect [29–31]. Additionally, functional neuroimaging in patients revealed changes in metabolic activity induced by NAc DBS. Among the changes found, the hypofunction observed in prefrontal subregions such as the SCC should be highlighted [29].

Neuropsychological assessments are routinely performed in these patients since as an invasive technique, DBS could impair attention, memory, or other cognitive processes. Interestingly, a significant improvement in cognitive performance tasks was reported after NAc DBS [32], and VC/VS DBS [33] did not produce neuropsychological decline. The safety and the encouraging response obtained in these clinical trials could promote additional controlled studies to verify the efficacy of DBS of both targets for resistant MDD.

To extend our knowledge and understanding of the mechanism of action of DBS, preclinical studies applying DBS in the core or shell portion of NAc have been performed in naïve and animal models of depression. In this way, NAc DBS in naïve animals induced an antidepressant-like effect measured in the forced swimming test (FST) [34, 35]. The FST is the most often used behavioral test to predict antidepressant-like activity in rodents. This is a classical paradigm to screen the response when the subject is faced with a problem without an obvious solution ("waiting/searching strategy") [36, 37]. Additionally, considering that one of the essential symptoms of depression is anhedonia, the antidepressant-like effect is also assessed by the portion of sucrose intake in the sucrose consumption test (SCT) [38]. Surprisingly, NAc DBS fails to produce a clear and remarkable hedonic effect [35, 39].

Furthermore, the antidepressant effect of NAc DBS has been reported in several animal models of depression. Chronic DBS to the NAc core induced an antidepressant-like effect in the Flinders sensitive line (FSL) [40], a validated genetic animal model of depression [41]. This effect was also observed in the high anxiety-related behavior (HAB) mouse model [42] and the model of depression induced by chronic adrenocorticotropic hormone (ACTH) administration [43]; both models resistant to standard antidepressant therapies [44, 45]. Additionally, chronic NAc DBS increased sucrose intake preference in animals submitted to mild randomized stressors for several weeks [46], in a rodent depression model called the chronic unpredictable mild stress (CUMS) model [47], yet the antianhedonic effect was not observed in the FSL model [40]. However, a single session of DBS applied to the NAc core or shell was not enough to attain an antidepressant response in animal models of depression including HAB mice and the CUMS [39, 42].

The anxiolytic effect observed in patients treated with NAc DBS was also evaluated in naïve animals. Unfortunately, the data available do not confirm the anxiolytic properties of DBS NAc. This therapeutic approach reduced the escape latency in the home-cage emergence test [39] but it did not increase the time spent in the open arms of the elevated plus maze (EPM) [48], which is the most widely used paradigm to detect anxiety-related behavior in rodents [49]. It should be noted that changes in locomotor activity were not reported following acute or chronic DBS of the NAc, indicating that the behavioral effects described were not due to an alteration in the spontaneous locomotor activity [34, 39, 42, 46, 50].

The possible cognitive alterations induced by DBS were also assessed using animal paradigms such as the Morris water maze (MWM). This test evaluates the spatial memory, and it is often used as a general assay of cognitive function [51, 52]. Interestingly, NAc DBS did not induce learning impairments in CUMS animals [46]. This is according to the neuropsychological data reported in the clinical studies.

To discern the molecular mechanisms underpinning the antidepressant effect of NAc DBS, several preclinical studies have been performed to ascertain potential substrates involved. As such, the modulation of neurotransmitters release, neurotrophic factors, adult neurogenesis, or the activation of intracellular signaling pathways have

been evaluated, given that they could be the main factors to achieve the satisfactory response.

The monoaminergic theory postulates a deficiency in brain serotonergic, noradrenergic, and dopaminergic neurotransmission in depression [53]. Thus, a solid basis was established to conceptualize this neurobiological disease, which was a breakthrough for the development of current pharmacological antidepressants [54, 55]. However, the involvement of monoamines in the pathophysiology of this disease appears to be insufficient to fully understand this illness. Although monoamine regulation itself does not explain the processes that cause or maintain the depressed mood, the effect of DBS on monoamine release has been evaluated. Local monoamine levels remained unaltered after acute NAc core DBS [56], but it did induce a general cortical increase of serotonin (5-HT) and dopamine (DA) levels in the prefrontal cortex and of DA and noradrenaline (NA) in the orbitofrontal cortex [57]. However, the effect of chronic DBS on monoamine levels depends on the site within the NAc that is stimulated. DBS of the NAc core did not produce either local or cortical modifications, and NAc shell stimulation enhanced local but not cortical monoamine levels [58]. Only one study evaluated the monoamine release in an animal model of depression describing a decrease in cortical monoamine levels after NAc shell DBS in Wistar–Kyoto animals [50], a rat strain with a defined depressive phenotype [59, 60].

On the other hand, MDD is associated with a dysfunction in neuronal network plasticity. A large body of evidence has demonstrated the reduction in brain-derived neurotrophic factor (BDNF) expression and adult hippocampal neurogenesis in depressed patients [61–63]. Indeed, it has been reported that antidepressant drugs can improve both processes [64, 65]. Bearing in mind the relevance of these processes, it has been evaluated how NAc DBS affects them. Stimulation of the NAc in naïve animals was not sufficient to increase hippocampal neurogenesis [66]. However, in animal models of chronic depression, DBS was able to enhance this [42], and to promote the expression of BDNF [46].

Additionally, NAc DBS potentiated cortical dendritic plasticity [50] and it modified neuronal activity in the piriform cortex and subcortical regions, such as the VTA and lateral hypothalamus [34]. Moreover, DBS in HAB animals activated the LHb, the dentate gyrus of the hippocampus, and the orbitofrontal cortex, and it decreased the activity in the prelimbic cortex [42]. Thus, DBS is able to modulate the activation pattern of several brain areas located both close to and distant from the stimulation site.

Subgenual Cingulate Cortex

The SCC is a part of the limbic cortex, located ventral to the genu of the corpus callosum (Fig. 19.2). The SCC region has been chosen as a target for DBS on the basis of previous findings, showing that this area is generally hyperactive in depressed patients, and that antidepressants and electroconvulsive therapy decreased the metabolism in this region [67–69]. Furthermore, the functional activity of the SCC region has been proposed as a predictor of the response to antidepressant treatments [70, 71]. So, the safety and efficacy of DBS in the SCC for treating resistant MDD is currently under investigation. Previous data from three independent clinical trials have demonstrated that DBS was effective in refractory MDD patients, showing an approximately

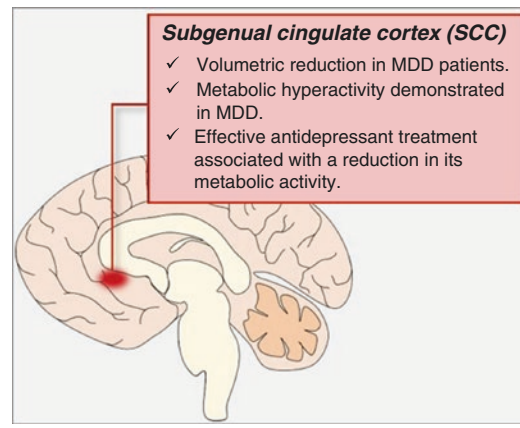


Fig. 19.2 General schematic representation of the SCC as a target for refractory MDD

60% response rate [72–74]. During the follow-up period (3–6 years) a progressive reduction in the severity of depression symptoms was reported, with only 10% of patients failing to show a decrease in symptom scores [75]. Indeed, at final follow-up, 42.9% of patients were in remission [72]. Moreover, cognition function after SCC DBS was preserved in patients, indicating the safety of this stimulation target for MDD [76, 77]. However, despite the promising results reported in these open-label trials, the last multicenter prospective randomized trial failed to demonstrate the effectiveness of SCC DBS (letter from St. Jude Medical Clinical Study Management). Thus, further clinical trials must be carried out to elucidate the efficacy of this therapy for treating refractory MDD patients.

Many studies focused on the intrinsic mechanisms of SCC DBS have been performed in animal models of depression. The ventromedial prefrontal cortex (vmPFC) is the rodent cortical region homologous to the human SCC [78]. This region compromises the infralimbic and prelimbic cortices, and there is still considerable debate as to which part of the vmPFC is the best target to apply DBS [79, 80].

Preclinical behavioral studies indicated that short- and long-term DBS applied in the vmPFC induced an antidepressant-like effect in the FST [34, 35, 79–84] and a hedonic response in the SCT in naïve animals [35, 84]. In addition, an anxiolytic effect was described after acute vmPFC DBS in the home-cage emergence test and the novelty suppressed feeding (NSF) [39, 80, 82]. The NSF measures a rodent's aversion to eating in a novel environment, and it is sensitive to chronic but not acute antidepressant treatment [85]. Therefore, the onset of action of DBS appears to be shorter than common antidepressant drugs.

Chronic DBS was able to reverse the depressive phenotype induced by the following animal models of depression: FSL, CUMS, olfactory bulbectomy (OBX), and chronic social defeat stress (CSDS) model [39, 84, 86–88]. The OBX produces a depressive behavior by disrupting the normal functioning of the limbic system [89], whereas the CSDS is based on the confrontation among conspecific animals to induce a psychosocial stress [90]. Furthermore, the anxiolytic effect of vmPFC was also demonstrated in the CUMS model of depression, increasing the time spent in the open arms in the EPM [86]. On the other hand, learning performance was not affected by vmPFC DBS in the MWM [46]. Overall, this indicates that DBS exerts an antidepressant response accompanied by an anxiolytic effect that is not associated to a cognitive impairment.

As occurred using NAc DBS, the spontaneous locomotor activity after acute or chronic DBS in the vmPFC remained unaltered [34, 39, 46, 80, 82, 86]. On the other hand, using the intracranial self-stimulation (ICSS) paradigm in FSL rats, it has been reported that the hedonic effect of DBS in the vmPFC does not depend on a direct modification of the mesolimbic dopaminergic reward system [84, 91].

The monoaminergic implication in the antidepressant-like effect induced by vmPFC DBS has been studied as a possible mechanism of action. Thus, vmPFC DBS was able to locally enhance 5-HT, NA, and DA at the site of stimulation [80, 87]. Nevertheless, the large majority of the studies available were carried out to discern the role of the serotonergic system. Despite 5-HT levels remaining unaltered in the dorsal raphe (DR) nucleus after vmPFC DBS, it has been shown to modulate the electrical activity of 5-HT neurons and enhance the glutamate concentration in this brain area [39, 80, 81, 88, 92]. This increase in the main excitatory neurotransmitter might trigger the drastic 5-HT release reported in DR projection areas, specifically in the vmPFC and hippocampus [80, 82].

But the relevance of the serotonergic system in the antidepressant-like effect of vmPFC DBS is still unclear, given that the two studies available showed opposite effects. In one of them, the antidepressant-like effect of vmPFC DBS persisted when the serononinergic neurotransmission was compromised, but in the other study this effect seemed to depend on the integrity of the 5-HT system [80, 82].

Additionally, DBS was able to potentiate neural plasticity in the DR neurons of naïve animals. Thus, an increase in excitatory presynaptic and

postsynaptic densities was found, and in CSDS animals, DBS normalized dendritic arborization [81, 88]. vmPFC DBS also promoted synaptic plasticity locally, in the hippocampus and in the basolateral amygdala, even restoring the reduction induced by the CSDS model [88, 93].

The activation of specific intracellular cascades, such as the mammalian target of rapamycin (mTOR) pathway, has a rapid impact on synaptic plasticity. This pathway promotes the synthesis of several proteins involved in the function, formation, and maturation of new spine synapses [94], and it has been closely related to the antidepressant effect of drugs with a short onset of action [95]. DBS of the vmPFC increased cortical phosphorylation of Akt and cAMP-response element binding (CREB) [87], both components linked to the activation of mTOR signaling cascade. Moreover, an inhibitor of mTOR, temsirolimus, was able to block the antidepressant-like effect induced by vmPFC DBS, indicating that the mTOR pathway is a limiting factor [87].

As mentioned above, neurotrophic factors and neurogenesis play an important role in the antidepressant response. The effect of DBS on neurogenesis is still not clear in naïve animals and opposite results have been reported using different protocols to address this issue [66, 81]. Nonetheless, chronic vmPFC DBS restored the reduction in adult hippocampal neurogenesis induced by stress in the CUMS model [86]. On the other hand, the most relevant neurotrophic factor related to affective disorders and the unique analyzed in clinical and preclinical DBS studies is BDNF. vmPFC DBS normalized the deficient BDNF levels in CUMS animals in the striatal, cortical, and hippocampal regions [46, 86, 96]. However, peripheral BDNF concentration has been evaluated in only four patients who received DBS of the SCC, and they showed a reduction in serum BDNF levels [97].

Many original studies have evaluated the activity of several brain areas after DBS through the molecular expression of neuronal activation markers. Thus, vmPFC DBS induced the activation of the neurons around the site of stimulation, as well as in other cortical regions such as piriform, entorhinal, and orbitofrontal cortices [34, 39, 80, 88]. Additionally, the activity of several distant regions directly connected to the vmPFC was increased by DBS, for instance the hippocampus, basolateral amygdala, insula, or LHb [34, 39, 80, 88]. Thus, DBS applied to this target is able to regulate the activity of several limbic areas included in the neuronal circuitry that regulates emotions.

Lateral Habenula

The LHb is located dorsally to dorsal thalamus (Fig. 19.3). The LHb receives information from cortical and limbic structures and it is directly connected to dopaminergic-, noradrenergic-, and serotonergic-releasing midbrain areas [98]. This area constitutes a target for DBS as its volume tends to be reduced in depressed patients, and some authors have hypothesized that an overactivity in this region is related to MDD [99, 100]. In this context, a patient diagnosed with resistant MDD was treated with LHb DBS. The results showed the antidepressant response of this therapy; the patient achieved sustained remission from 4 to 12 months after the beginning of the treatment. Indeed, the patient suffered decay by the accidental cessation of stimulation, corroborating the effectiveness of this DBS target [101]. Thus, these promising results have led to the

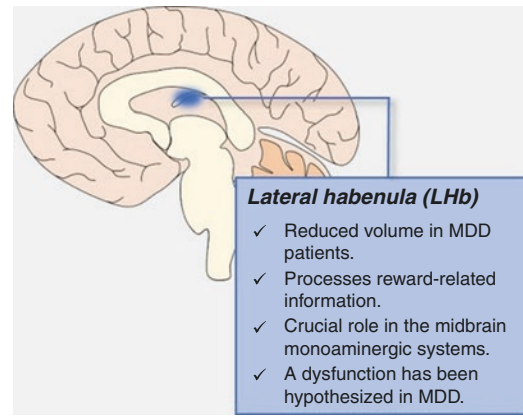


Fig. 19.3 General schematic representation of the LHb as a target for refractory MDD

intention to carry out additional clinical trials using LHb DBS for resistant MDD. In fact, a single-center study enrolling six patients is currently being conducted (ClinicalTrials.gov; identifier: NCT0198407).

The antidepressant-like effect of LHb DBS has been demonstrated in several animal models of depression. As such, DBS restored the depressive phenotype induced by CUMS, chronic ACTH administration, and in the learned helplessness (LH) model [39, 102–104]. This LH model is an experimental depression model based on exposure to repeated uncontrolled and inescapable stress, leading to helplessness [105].

Monoamine release was measured in CUMS animals after LHb DBS. Chronic exposure to unpredictable stressors provoked a reduction of 5-HT, NA and DA levels in the hippocampus and blood serum which is restored by LHb DBS [104].

An increase of BDNF was found in the blood serum of the patient treated with LHb DBS. Indeed, the BDNF levels were correlated with the improvement of depressive symptoms in this patient [106]. Furthermore, the antidepressant-like effect of LHb DBS has been preclinically linked with some molecular and cellular changes which could be crucial in the mechanism of action of DBS. In this way, LHb DBS regulates the local and cortical activation of the Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) intracellular pathway which modulates downstream signaling cascades involved, among others, in synaptic plasticity and neuronal survival [102]. New insights about cellular and molecular changes induced by LHb DBS could help in understanding the intrinsic mechanisms underpinning the antidepressant effect of DBS.

Medial Forebrain Bundle

The MFB is a fiber tract connecting the midbrain tegmentum and elements of the limbic system (Fig. 19.4) which plays an important role in the reward system [107, 108]. Clinical studies have reported the efficacy of MFB DBS as a therapeutic approach for intractable MDD patients. The pilot trial included seven patients, and DBS in the MFB induced a rapid and chronic antidepressant response, with an 86% response rate at the last follow-up [109]. Additionally, a second study demonstrated a significant alleviation of depressive symptoms in two of three patients enrolled 6 months after the beginning of DBS [110]. Finally, a third trial was approved to study the effectiveness of the MFB DBS in 12 patients diagnosed with refractory MDD (ClinicalTrials.gov; identifier: NCT01778790).

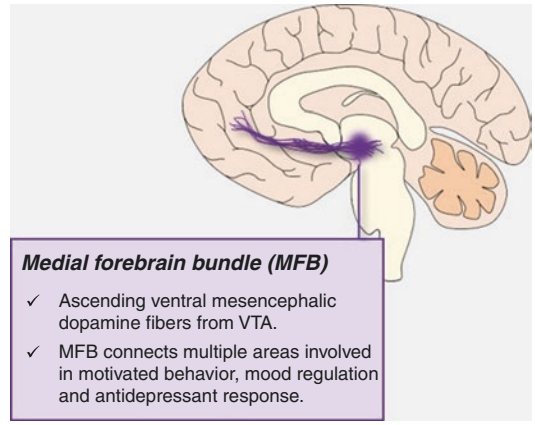


Fig. 19.4 General schematic representation of the MFB as a target for refractory MDD.

Preclinical data indicated that DBS in the MFB had an antidepressant-like effect in the FST [111] and that it reversed the anhedonic and depressive phenotype of FSL rats [91]. Surprisingly, MFB DBS induced an anxiety-like behavior assessed as an increase in the time spent in the closed arms in the EPM paradigm [91] without altering locomotor activity [111]. Only some molecular changes were evaluated after DBS is applied to the MFB. In this way, the effect of acute MFB DBS on monoamine release was assessed in the NAc and interestingly, no changes in DA or 5-HT release were reported [111]. Bearing in mind that the MFB is the fiber tract which connects the VTA with the NAc, and considering the antianhedonic effect of MFB DBS, this result was unexpected. Maybe instead of acute stimulation, chronic stimulation of the MFB

could lead to a modification of monoaminergic neurotransmission.

In addition, stimulation of the MFB altered the activity pattern of a few brain areas, such as the piriform and prelimbic cortices, the shell portion of NAc, the anterior regions of the caudate/putamen, dorsomedial thalamic nuclei, LHb, and the VTA [111, 112]. This suggests that DBS of the MFB induces changes in areas widely linked to the pathophysiology of MDD.

Inferior Thalamic Peduncle

The ITP is a bundle of fibers that connects the nonspecific thalamic system to the orbitofrontal cortex (Fig. 19.5), and DBS delivered in this region seems to be beneficial in refractory OCD patients [113]. However, only one case has been reported with regard to the efficacy of ITP stimulation in a refractory MDD patient, who achieved remission state from the first month of DBS [113–115]. Although the depressive symptomatology of this patient improved, she also suffered from bulimia and borderline personality disorder, making it difficult to extract conclusive data supporting the suitability of this target. Furthermore, up to now there are no preclinical data available that may help clarify the possible effectiveness of this therapy in this target brain area.

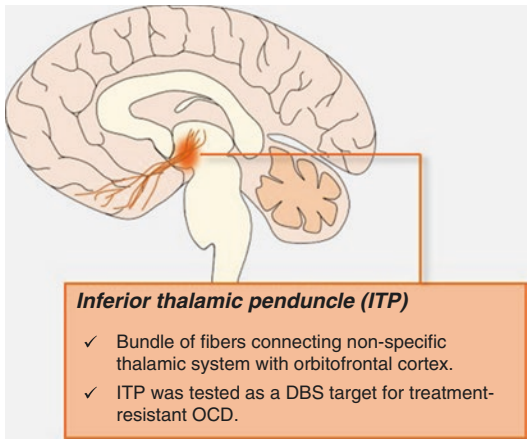


Fig. 19.5 General schematic representation of the ITP as a target for refractory MDD

Conclusions

In the last decade, the efficacy of DBS for treating refractory MDD has been evaluated, targeting several brain areas. Clinical studies that have enrolled most resistant MDD patients have been performed on the SCC and NAc regions. Indeed, they showed the most promising outcomes. Despite the data available from clinical trials, the mechanism of action underlying the antidepressant effect of DBS is still unclear. To clarify this issue, preclinical studies have evaluated the cellular and molecular changes modulated by DBS using animal models of depression. They have demonstrated that DBS regulates the release of several neurotransmitters in brain areas closely related to MDD. Moreover, DBS can enhance BDNF expression, and promote neurogenesis and neuronal plasticity. Overall, this suggests that DBS presents a complex mechanism of action involving many components, which could contribute to the initiation of remarkable neuronal network reorganization.

The evidence obtained through the studies performed to date indicates that DBS could be a safe alternative for the treatment of refractory MDD. But unfortunately, the multicenter trials fail to demonstrate a substantial improvement of the depressive symptomatology in all patients. For this reason, the search for indicators that will help to identify patients who can satisfactorily respond to DBS is mandatory. The detection of alterations in activity patterns through neuroimaging, or changes in peripheral proteins expression, could be used as clinical biomarkers to predict DBS response. Moreover, further studies will be necessary to identify the best target of stimulation in order to attain the maximum therapeutic response in each patient.

Acknowledgements This study was supported by grants from the "Instituto de Salud Carlos III (Madrid, Spain)", co-financed by the "Fondo Europeo de Desarrollo Regional" (FEDER) (PI13/02659); the "Ministerio de Economía y Competitividad", co-financed by FEDER (SAF2015-68647-R (MINECO/FEDER)); the "Centro de Investigación Biomédica en Red de Salud Mental-CIBERSAM", Spain (G18); the "Consejería de Economía, Innovación, Ciencia y Empleo de la Junta de Andalucía", Spain (CTS-510 and CTS-7748); 2015 NARSAD Young

Investigator Grant from the Brain Behavior Research Foundation; and FPI (2011-145) fellowship. The authors declare no conflict of interest.

References

1. Ferrari AJ, Somerville AJ, Baxter AJ, Norman R, Patten SB, Vos T, et al. Global variation in the prevalence and incidence of major depressive disorder: a systematic review of the epidemiological literature. Psychol Med. 2013;43(3):471–81.
2. World Health Organization. World report on disability. Geneva: WHO; 2011.
3. Murray CJ, Lopez AD. Evidence-based health policy – lessons from the Global Burden of Disease Study. Science. 1996;274(5288):740–3.
4. Frank E, Prien RF, Jarrett RB, Keller MB, Kupfer DJ, Lavori PW, et al. Conceptualization and rationale for consensus definitions of terms in major depressive disorder. Remission, recovery, relapse, and recurrence. Arch Gen Psychiatry. 1991;48(9):851–5.
5. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry. 2006;163(11):1905–17.
6. Cuijpers P, Clignet F, van Meijel B, van Straten A, Li J, Andersson G. Psychological treatment of depression in inpatients: a systematic review and meta-analysis. Clin Psychol Rev. 2011;31(3):353–60.
7. O'Reardon JP, Solvason HB, Janicak PG, Sampson S, Isenberg KE, Nahas Z, et al. Efficacy and safety of transcranial magnetic stimulation in the acute treatment of major depression: a multisite randomized controlled trial. Biol Psychiatry. 2007;62(11):1208–16.
8. Rush AJ, Marangell LB, Sackeim HA, George MS, Brannan SK, Davis SM, et al. Vagus nerve stimulation for treatment-resistant depression: a randomized, controlled acute phase trial. Biol Psychiatry. 2005;58(5):347–54.
9. UK ECT Review Group. Efficacy and safety of electroconvulsive therapy in depressive disorders: a systematic review and meta-analysis. Lancet. 2003;361(9360):799–808.
10. Fava M. Diagnosis and definition of treatment-resistant depression. Biol Psychiatry. 2003;53(8):649–59.
11. Hawton K, Casanas ICC, Haw C, Saunders K. Risk factors for suicide in individuals with depression: a systematic review. J Affect Disord. 2013;147(1–3):17–28.
12. Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the World Health Surveys. Lancet. 2007;370(9590):851–8.
13. Benabid AL, Pollak P, Gervason C, Hoffmann D, Gao DM, Hommel M, et al. Long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus. Lancet. 1991;337(8738):403–6.
14. Hardesty DE, Sackeim HA. Deep brain stimulation in movement and psychiatric disorders. Biol Psychiatry. 2007;61(7):831–5.
15. Kalia SK, Sankar T, Lozano AM. Deep brain stimulation for Parkinson's disease and other movement disorders. Curr Opin Neurol. 2013;26(4):374–80.
16. McIntyre CC, Savasta M, Kerkerian-Le Goff L, Vitek JL. Uncovering the mechanism(s) of action of deep brain stimulation: activation, inhibition, or both. Clin Neurophysiol. 2004;115(6):1239–48.
17. Nowak LG, Bullier J. Axons, but not cell bodies, are activated by electrical stimulation in cortical gray matter. II. Evidence from selective inactivation of cell bodies and axon initial segments. Exp Brain Res. 1998;118(4):489–500.
18. Kringelbach ML, Green AL, Aziz TZ. Balancing the brain: resting state networks and deep brain stimulation. Front Integr Neurosci. 2011;5:8.
19. McIntyre CC, Hahn PJ. Network perspectives on the mechanisms of deep brain stimulation. Neurobiol Dis. 2010;38(3):329–37.
20. Greenberg BD, Gabriels LA, Malone DA, Jr Rezai AR, Friehs GM, Okun MS, et al. Deep brain stimulation of the ventral internal capsule/ventral striatum for obsessive-compulsive disorder: worldwide experience. Mol Psychiatry. 2010;15(1):64–79.
21. Nuttin B, Cosyns P, Demeulemeester H, Gybels J, Meyerson B. Electrical stimulation in anterior limbs of internal capsules in patients with obsessive-compulsive disorder. Lancet. 1999;354(9189):1526.
22. Aouizerate B, Cuny E, Martin-Guehl C, Guehl D, Amieva H, Benazzouz A, et al. Deep brain stimulation of the ventral caudate nucleus in the treatment of obsessive-compulsive disorder and major depression. Case report. J Neurosurg. 2004;101(4):682–6.
23. Greenberg BD, Malone DA, Friehs GM, Rezai AR, Kubu CS, Malloy PF, et al. Three-year outcomes in deep brain stimulation for highly resistant obsessive-compulsive disorder. Neuropsychopharmacology. 2006;31(11):2384–93.
24. Malone DA, Jr Dougherty DD, Rezai AR, Carpenter LL, Friehs GM, Eskandar EN, et al. Deep brain stimulation of the ventral capsule/ventral striatum for treatment-resistant depression. Biol Psychiatry. 2009;65(4):267–75.
25. Dougherty DD, Rezai AR, Carpenter LL, Howland RH, Bhati MT, O'Reardon JP, et al. A randomized sham-controlled trial of deep brain stimulation of the ventral capsule/ventral striatum for chronic treatment-resistant depression. Biol Psychiatry. 2015;78(4):240–8.
26. Sesack SR, Grace AA. Cortico-basal ganglia reward network: microcircuitry. Neuropsychopharmacology. 2010;35(1):27–47.

27. Gorwood P. Neurobiological mechanisms of anhedonia. Dialogues Clin Neurosci. 2008;10(3):291–9.
28. Tremblay LK, Naranjo CA, Graham SJ, Herrmann N, Mayberg HS, Hevenor S, et al. Functional neuroanatomical substrates of altered reward processing in major depressive disorder revealed by a dopaminergic probe. Arch Gen Psychiatry. 2005;62(11):1228–36.
29. Bewernick BH, Hurlemann R, Matusch A, Kayser S, Grubert C, Hadrysiewicz B, et al. Nucleus accumbens deep brain stimulation decreases ratings of depression and anxiety in treatment-resistant depression. Biol Psychiatry. 2010;67(2):110–6.
30. Bewernick BH, Kayser S, Sturm V, Schlaepfer TE. Long-term effects of nucleus accumbens deep brain stimulation in treatment-resistant depression: evidence for sustained efficacy. Neuropsychopharmacology. 2012;37(9):1975–85.
31. Schlaepfer TE, Cohen MX, Frick C, Kosel M, Brodesser D, Axmacher N, et al. Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression. Neuropsychopharmacology. 2008;33(2):368–77.
32. Grubert C, Hurlemann R, Bewernick BH, Kayser S, Hadrysiewicz B, Axmacher N, et al. Neuropsychological safety of nucleus accumbens deep brain stimulation for major depression: effects of 12-month stimulation. World J Biol Psychiatry. 2011;12(7):516–27.
33. Kubu CS, Malone DA, Chelune G, Malloy P, Rezai AR, Frazier T, et al. Neuropsychological outcome after deep brain stimulation in the ventral capsule/ventral striatum for highly refractory obsessive-compulsive disorder or major depression. Stereotact Funct Neurosurg. 2013;91(6):374–8.
34. Hamani C, Amorim BO, Wheeler AL, Diwan M, Driesslein K, Covolan L, et al. Deep brain stimulation in rats: different targets induce similar antidepressant-like effects but influence different circuits. Neurobiol Dis. 2014;71:205–14.
35. Lim LW, Janssen ML, Kocabicak E, Temel Y. The antidepressant effects of ventromedial prefrontal cortex stimulation is associated with neural activation in the medial part of the subthalamic nucleus. Behav Brain Res. 2015;279:17–21.
36. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci. 2002;23(5):238–45.
37. Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl). 1995;121(1):66–72.
38. Papp M, Willner P, Muscat R. An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. Psychopharmacology (Berl). 1991;104(2):255–9.
39. Lim LW, Prickaerts J, Huguet G, Kadar E, Hartung H, Sharp T, et al. Electrical stimulation alleviates depressive-like behaviors of rats: investigation of brain targets and potential mechanisms. Transl Psychiatry. 2015;5:e535.
40. Rummel J, Voget M, Hadar R, Ewing S, Sohr R, Klein J, et al. Testing different paradigms to optimize antidepressant deep brain stimulation in different rat models of depression. J Psychiatr Res. 2016;81:36–45.
41. Overstreet DH, Wegener G. The Flinders sensitive line rat model of depression – 25 years and still producing. Pharmacol Rev. 2013;65(1):143–55.
42. Schmuckermair C, Gaburro S, Sah A, Landgraf R, Sartori SB, Singewald N. Behavioral and neurobiological effects of deep brain stimulation in a mouse model of high anxiety- and depression-like behavior. Neuropsychopharmacology. 2013;38(7):1234–44.
43. Kim Y, McGee S, Czeczor JK, Walker AJ, Kale RP, Kouzani AZ, et al. Nucleus accumbens deep-brain stimulation efficacy in ACTH-pretreated rats: alterations in mitochondrial function relate to antidepressant-like effects. Transl Psychiatry. 2016;6(6):e842.
44. Iijima M, Ito A, Kurosu S, Chaki S. Pharmacological characterization of repeated corticosterone injection-induced depression model in rats. Brain Res. 2010;1359:75–80.
45. Landgraf R, Kessler MS, Bunck M, Murgatroyd C, Spengler D, Zimbelmann M, et al. Candidate genes of anxiety-related behavior in HAB/LAB rats and mice: focus on vasopressin and glyoxalase-I. Neurosci Biobehav Rev. 2007;31(1):89–102.
46. Gersner R, Toth E, Isserles M, Zangen A. Site-specific antidepressant effects of repeated subconvulsive electrical stimulation: potential role of brain-derived neurotrophic factor. Biol Psychiatry. 2010;67(2):125–32.
47. Willner P, Muscat R, Papp M. Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neurosci Biobehav Rev. 1992;16(4):525–34.
48. van Dijk A, Klanker M, van Oorschot N, Post R, Hamelink R, Feenstra MG, et al. Deep brain stimulation affects conditioned and unconditioned anxiety in different brain areas. Transl Psychiatry. 2013;3:e289.
49. Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. Naunyn Schmiedebergs Arch Pharmacol. 1984;327(1):1–5.
50. Falowski SM, Sharan A, Reyes BA, Sikkema C, Szot P, Van Bockstaele EJ. An evaluation of neuroplasticity and behavior after deep brain stimulation of the nucleus accumbens in an animal model of depression. Neurosurgery. 2011;69(6):1281–90.

51. Brandeis R, Brandys Y, Yehuda S. The use of the Morris water maze in the study of memory and learning. Int J Neurosci. 1989;48(1–2):29–69.
52. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods. 1984;11(1):47–60.
53. Schildkraut JJ. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry. 1965;122(5):509–22.
54. Heninger GR, Delgado PL, Charney DS. The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. Pharmacopsychiatry. 1996;29(1):2–11.
55. Ruhe HG, Mason NS, Schene AH. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Mol Psychiatry. 2007;12(4):331–59.
56. van Dijk A, Mason O, Klompmakers AA, Feenstra MG, Denys D. Unilateral deep brain stimulation in the nucleus accumbens core does not affect local monoamine release. J Neurosci Methods. 2011;202(2):113–8.
57. van Dijk A, Klompmakers AA, Feenstra MG, Denys D. Deep brain stimulation of the accumbens increases dopamine, serotonin, and noradrenaline in the prefrontal cortex. J Neurochem. 2012;123(6):897–903.
58. Sesia T, Bulthuis V, Tan S, Lim LW, Vlamings R, Blokland A, et al. Deep brain stimulation of the nucleus accumbens shell increases impulsive behavior and tissue levels of dopamine and serotonin. Exp Neurol. 2010;225(2):302–9.
59. Lahmame A, Armario A. Differential responsiveness of inbred strains of rats to antidepressants in the forced swimming test: are Wistar Kyoto rats an animal model of subsensitivity to antidepressants? Psychopharmacology (Berl). 1996;123(2):191–8.
60. Will CC, Aird F, Redei EE. Selectively bred Wistar–Kyoto rats: an animal model of depression and hyper-responsiveness to antidepressants. Mol Psychiatry. 2003;8(11):925–32.
61. Kempermann G, Kronenberg G. Depressed new neurons – adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. Biol Psychiatry. 2003;54(5):499–503.
62. Neto FL, Borges G, Torres-Sanchez S, Mico JA, Berrocoso E. Neurotrophins role in depression neurobiology: a review of basic and clinical evidence. Curr Neuropharmacol. 2011;9(4):530–52.
63. Samuels BA, Hen R. Neurogenesis and affective disorders. Eur J Neurosci. 2011;33(6):1152–9.
64. D'Sa C, Duman RS. Antidepressants and neuroplasticity. Bipolar Disord. 2002;4(3):183–94.
65. Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, et al. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry. 2003;54(1):70–5.
66. Winter C, Bregman T, Voget M, Raymond R, Hadar R, Nobrega JN, et al. Acute high frequency stimulation of the prefrontal cortex or nucleus accumbens does not increase hippocampal neurogenesis in rats. J Psychiatr Res. 2015;68:27–9.
67. Goldapple K, Segal Z, Garson C, Lau M, Bieling P, Kennedy S, et al. Modulation of cortical–limbic pathways in major depression: treatment-specific effects of cognitive behavior therapy. Arch Gen Psychiatry. 2004;61(1):34–41.
68. Mayberg HS, Liotti M, Brannan SK, McGinnis S, Mahurin RK, Jerabek PA, et al. Reciprocal limbic–cortical function and negative mood: converging PET findings in depression and normal sadness. Am J Psychiatry. 1999;156(5):675–82.
69. Seminowicz DA, Mayberg HS, McIntosh AR, Goldapple K, Kennedy S, Segal Z, et al. Limbic–frontal circuitry in major depression: a path modeling metanalysis. Neuroimage. 2004;22(1):409–18.
70. Kennedy SH, Konarski JZ, Segal ZV, Lau MA, Bieling PJ, McIntyre RS, et al. Differences in brain glucose metabolism between responders to CBT and venlafaxine in a 16-week randomized controlled trial. Am J Psychiatry. 2007;164(5):778–88.
71. Mayberg HS, Brannan SK, Tekell JL, Silva JA, Mahurin RK, McGinnis S, et al. Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. Biol Psychiatry. 2000;48(8):830–43.
72. Kennedy SH, Giacobbe P, Rizvi SJ, Placenza FM, Nishikawa Y, Mayberg HS, et al. Deep brain stimulation for treatment-resistant depression: follow-up after 3 to 6 years. Am J Psychiatry. 2011;168(5):502–10.
73. Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, et al. Deep brain stimulation for treatment-resistant depression. Neuron. 2005;45(5):651–60.
74. Puigdemont D, Perez-Egea R, Portella MJ, Molet J, de Diego-Adelino J, Gironell A, et al. Deep brain stimulation of the subcallosal cingulate gyrus: further evidence in treatment-resistant major depression. Int J Neuropsychopharmacol. 2012;15(1):121–33.
75. Holtzheimer PE, Kelley ME, Gross RE, Filkowski MM, Garlow SJ, Barrocas A, et al. Subcallosal cingulate deep brain stimulation for treatment-resistant unipolar and bipolar depression. Arch Gen Psychiatry. 2012;69(2):150–8.
76. McNeely HE, Mayberg HS, Lozano AM, Kennedy SH. Neuropsychological impact of Cg25 deep brain stimulation for treatment-resistant depression: preliminary results over 12 months. J Nerv Ment Dis. 2008;196(5):405–10.
77. Serra-Blasco M, de Vita S, Rodriguez MR, de Diego-Adelino J, Puigdemont D, Martin-Blanco A, et al. Cognitive functioning after deep brain stimulation in subcallosal cingulate gyrus for treatment-resistant depression: an exploratory study. Psychiatry Res. 2015;225(3):341–6.

78. Vertes RP. Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse. 2004;51(1):32–58.
79. Hamani C, Diwan M, Isabella S, Lozano AM, Nobrega JN. Effects of different stimulation parameters on the antidepressant-like response of medial prefrontal cortex deep brain stimulation in rats. J Psychiatr Res. 2010;44(11):683–7.
80. Jimenez-Sanchez L, Castane A, Perez-Caballero L, Grifoll-Escoda M, Lopez-Gil X, Campa L, et al. Activation of AMPA receptors mediates the antidepressant action of deep brain stimulation of the infralimbic prefrontal cortex. Cereb Cortex. 2016;26(6):2778–89.
81. Etievant A, Oosterhof C, Betry C, Abrial E, Novo-Perez M, Rovera R, et al. Astroglial control of the antidepressant-like effects of prefrontal cortex deep brain stimulation. EBioMedicine. 2015;2(8):898–908.
82. Hamani C, Diwan M, Macedo CE, Brandao ML, Shumake J, Gonzalez-Lima F, et al. Antidepressant-like effects of medial prefrontal cortex deep brain stimulation in rats. Biol Psychiatry. 2010;67(2):117–24.
83. Perez-Caballero L, Perez-Egea R, Romero-Grimaldi C, Puigdemont D, Molet J, Caso JR, et al. Early responses to deep brain stimulation in depression are modulated by anti-inflammatory drugs. Mol Psychiatry. 2014;19(5):607–14.
84. Rea E, Rummel J, Schmidt TT, Hadar R, Heinz A, Mathe AA, et al. Anti-anhedonic effect of deep brain stimulation of the prefrontal cortex and the dopaminergic reward system in a genetic rat model of depression: an intracranial self-stimulation paradigm study. Brain Stimul. 2014;7(1):21–8.
85. Bodnoff SR, Suranyi-Cadotte B, Aitken DH, Quirion R, Meaney MJ. The effects of chronic antidepressant treatment in an animal model of anxiety. Psychopharmacology (Berl). 1988;95(3):298–302.
86. Bambico FR, Bregman T, Diwan M, Li J, Darvish-Ghane S, Li Z, et al. Neuroplasticity-dependent and -independent mechanisms of chronic deep brain stimulation in stressed rats. Transl Psychiatry. 2015;5:e674.
87. Jimenez-Sanchez L, Linge R, Campa L, Valdizan EM, Pazos A, Diaz A, et al. Behavioral, neurochemical and molecular changes after acute deep brain stimulation of the infralimbic prefrontal cortex. Neuropharmacology. 2016;108:91–102.
88. Veerakumar A, Challis C, Gupta P, Da J, Upadhyay A, Beck SG, et al. Antidepressant-like effects of cortical deep brain stimulation coincide with pro-neuroplastic adaptations of serotonin systems. Biol Psychiatry. 2014;76(3):203–12.
89. Song C, Leonard BE. The olfactory bulbectomised rat as a model of depression. Neurosci Biobehav Rev. 2005;29(4–5):627–47.
90. Golden SA, Covington 3rd HE, Berton O, Russo SJ. A standardized protocol for repeated social defeat stress in mice. Nat Protoc. 2011;6(8):1183–91.
91. Edemann-Callesen H, Voget M, Empl L, Vogel M, Wieske F, Rummel J, et al. Medial forebrain bundle deep brain stimulation has symptom-specific antidepressant effects in rats and as opposed to ventromedial prefrontal cortex stimulation interacts with the reward system. Brain Stimul. 2015;8(4):714–23.
92. Srejic LR, Hamani C, Hutchison WD. High-frequency stimulation of the medial prefrontal cortex decreases cellular firing in the dorsal raphe. Eur J Neurosci. 2015;41(9):1219–26.
93. Chakravarty MM, Hamani C, Martinez-Canabal A, Ellegood J, Laliberte C, Nobrega JN, et al. Deep brain stimulation of the ventromedial prefrontal cortex causes reorganization of neuronal processes and vasculature. Neuroimage. 2016;125:422–7.
94. Hoeffer CA, Klann E. mTOR signaling: at the crossroads of plasticity, memory and disease. Trends Neurosci. 2010;33(2):67–75.
95. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959–64.
96. Hamani C, Machado DC, Hipolide DC, Dubiela FP, Suchecki D, Macedo CE, et al. Deep brain stimulation reverses anhedonic-like behavior in a chronic model of depression: role of serotonin and brain derived neurotrophic factor. Biol Psychiatry. 2012;71(1):30–5.
97. Ramasubbu R, Vecchiarelli HA, Hill MN, Kiss ZH. Brain-derived neurotrophic factor and subcallosal deep brain stimulation for refractory depression. World J Biol Psychiatry. 2015;16(2):135–8.
98. Geisler S, Trimble M. The lateral habenula: no longer neglected. CNS Spectr. 2008;13(6):484–9.
99. Ranft K, Dobrowolny H, Krell D, Bielau H, Bogerts B, Bernstein HG. Evidence for structural abnormalities of the human habenular complex in affective disorders but not in schizophrenia. Psychol Med. 2010;40(4):557–67.
100. Sartorius A, Henn FA. Deep brain stimulation of the lateral habenula in treatment resistant major depression. Med Hypotheses. 2007;69(6):1305–8.
101. Sartorius A, Kiening KL, Kirsch P, von Gall CC, Haberkorn U, Unterberg AW, et al. Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient. Biol Psychiatry. 2010;67(2):e9–e11.
102. Kim Y, Morath B, Hu C, Byrne LK, Sutor SL, Frye MA, et al. Antidepressant actions of lateral habenula deep brain stimulation differentially correlate with CaMKII/GSK3/AMPK signaling locally and in the infralimbic cortex. Behav Brain Res. 2016;306:170–7.
103. Li B, Piriz J, Mirrione M, Chung C, Proulx CD, Schulz D, et al. Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. Nature. 2011;470(7335):535–9.
104. Meng H, Wang Y, Huang M, Lin W, Wang S, Zhang B. Chronic deep brain stimulation of the lateral

habenula nucleus in a rat model of depression. Brain Res. 2011;1422:32–8.
105. Seligman ME, Rosellini RA, Kozak MJ. Learned helplessness in the rat: time course, immunization, and reversibility. J Comp Physiol Psychol. 1975;88(2):542–7.
106. Hoyer C, Kranaster L, Sartorius A, Hellweg R, Gass P. Long-term course of brain-derived neurotrophic factor serum levels in a patient treated with deep brain stimulation of the lateral habenula. Neuropsychobiology. 2012;65(3):147–52.
107. Coenen VA, Panksepp J, Hurwitz TA, Urbach H, Madler B. Human medial forebrain bundle (MFB) and anterior thalamic radiation (ATR): imaging of two major subcortical pathways and the dynamic balance of opposite affects in understanding depression. J Neuropsychiatry Clin Neurosci. 2012;24(2):223–36.
108. Coenen VA, Schlaepfer TE, Maedler B, Panksepp J. Cross-species affective functions of the medial forebrain bundle-implications for the treatment of affective pain and depression in humans. Neurosci Biobehav Rev. 2011;35(9):1971–81.
109. Schlaepfer TE, Bewernick BH, Kayser S, Madler B, Coenen VA. Rapid effects of deep brain stimulation for treatment-resistant major depression. Biol Psychiatry. 2013;73(12):1204–12.
110. Fenoy AJ, Schulz P, Selvaraj S, Burrows C, Spiker D, Cao B, et al. Deep brain stimulation of the medial forebrain bundle: distinctive responses in resistant depression. J Affect Disord. 2016;203:143–51.
111. Bregman T, Reznikov R, Diwan M, Raymond R, Butson CR, Nobrega JN, et al. Antidepressant-like effects of medial forebrain bundle deep brain stimulation in rats are not associated with accumbens dopamine release. Brain Stimul. 2015;8(44):708–13.
112. Furlanetti LL, Dobrossy MD, Aranda IA, Coenen VA. Feasibility and safety of continuous and chronic bilateral deep brain stimulation of the medial forebrain bundle in the naive Sprague-Dawley rat. Behav Neurol. 2015;2015:256196.
113. Jimenez F, Velasco F, Salin-Pascual R, Hernandez JA, Velasco M, Criales JL, et al. A patient with a resistant major depression disorder treated with deep brain stimulation in the inferior thalamic peduncle. Neurosurgery. 2005;57(3):585–93.
114. Jimenez F, Nicolini H, Lozano AM, Piedimonte F, Salin R, Velasco F. Electrical stimulation of the inferior thalamic peduncle in the treatment of major depression and obsessive compulsive disorders. World Neurosurg. 2013;80(3–4):S30. e17-25
115. Jimenez F, Velasco F, Salin-Pascual R, Velasco M, Nicolini H, Velasco AL, et al. Neuromodulation of the inferior thalamic peduncle for major depression and obsessive compulsive disorder. Acta Neurochir Suppl. 2007;97(2):393–8.

Eye Movements: Parameters, Mechanisms, and Active Vision

20

Jens R. Helmert and Sebastian Pannasch

Abstract

Human eye movements are essential for visual perception, as the physiological structure of the eyes limits high acuity and colorful vision to a small fraction of the retina. Measuring the dynamic interplay of fixations (i.e., the eyes are stable relative to an object of interest) and saccades (i.e., the eyes are directed to a new target) makes possible fundamental insights into the organization of vision. A complex interaction of several types of eye movements is required when performing different tasks, such as orienting in space, identifying objects, or interacting with persons. Here, we discuss the characteristics of fixations and saccades in the context of active vision, with particular focus on the relationship between the two parameters. Analyzing the duration of fixations and the amplitude of saccades during everyday activities can reveal insights into the processing of visual information, allowing an understanding of what details of the environment receive attention. In addition, by considering fixations and saccades in combination, it can be determined how such details were processed within the context of ongoing activities.

Keywords

Eye fixations • Saccades • Attention • Cognitive mechanisms • Active vision

J.R. Helmert
Department of Psychology, Technische Universität Dresden, Helmholtzstr. 10, Dresden 01062, Saxony, Germany

S. Pannasch (✉)
Department of Psychology, Technische Universität Dresden, Helmholtzstr. 10, Dresden 01062, Saxony, Germany

Department of Psychology, Engineering Psychology and Applied Cognitive Research, Technische Universität Dresden, Helmholtzstr. 10, Dresden 01062, Saxony, Germany
e-mail: sebastian.pannasch@tu-dresden.de

Introduction

In this chapter, we point to the fundamental basics of humans' probably most important sense — sight. For instance, when asking people which modality they would miss most if lost, the majority is likely to indicate vision [1, 2]. In addition, if people describe objects they primarily use adjectives that refer to visual (60%) or tactual (32%) modalities [3]. For visual processing of

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_20

the environment, the important role of eye movements has been repeatedly emphasized in the literature (e.g., [4, 5]). The main reason for this importance is based on the fact that the allocation of visual attention mostly corresponds to the direction of the eyes (e.g., [6]). Processing visual information is governed by a multitude of neural structures, both cortical and subcortical. Therefore, measuring eye movements is not only a complex study object in itself, it also delivers diagnostic information on different levels. To guide the reader to the potentials of the methods described here for current psychiatry, we refer to examples from schizophrenia research, where applicable.

Natural sampling of information from the environment during visual perception occurs via "active vision" [5, 7]. Because of the uneven distribution of light-sensitive receptors across the retina, the highest visual acuity is limited to the small foveal area (about 2 degrees of arc). Outside the high-resolution foveal area — in parafoveal and peripheral regions — vision becomes blurred and the perception of color is reduced. Given the constraints on visual acuity, eye movements are mandatory to perceive the environment. Saccades — fast ballistic movements — rotate the foveal region of the eyes from one point to another. The relatively stable periods in-between are called fixations. The intake of visual information occurs within fixations but is largely suppressed during saccades [8]. In most everyday situations, such as reading text or inspecting an image, oculomotor activity can be described as interplay between fixations and saccades.

Following a rather crude classification, three main areas of eye movement research can be identified: (i) analysis of eye movements in order to understand facets of reading, (ii) efforts to investigate gaze behavior during free visual exploration of natural stimuli, and (iii) work that comprises the examination of visual search processes in relation to eye movement strategies (see [9]). This classification provides a general overview but is an over-simplification. For instance, these categories do not consider the long history of eye movement research in clinical settings (for a recent overview in this particular field, see [10]). A closer examination of eye movement research reveals that significant work has been done to combine insights from different research areas, contributing to a more general understanding of common processes during, for example, reading and scene viewing [11]. In recent years, much interdisciplinary work has connected eye movement analyses with research questions from other disciplines. For instance, in the development and design of attention-sensitive interfaces, eye movement registration and analysis have become integral parts (e.g. [12–14]).

Eye movements are often considered "the window to the soul" [15], which can provide access to ongoing information processing. Combining video-based eye tracking technology — the means for non-invasive, extremely accurate, and fast measurements of different activities — with other measurements provides even more advantages. For instance, parallel recording with brain imaging enriches the explanatory power of eye movement data [16, 17]. Furthermore, it allows for a better understanding of brain activity since details about the inspected visual information can be extracted and assigned to the respective brain signals [18]. Human eye movements are a common output of a variety of psychophysiological mechanisms located well beyond the low-level oculomotor nuclei of the brain stem. Some of these mechanisms are hierarchically (or heterarchically; see [19]) organized. This vertical dimension of cognitive organization is an interesting object of scientific investigation in itself, for instance, to examine the correspondence between parameters of eye movements and the relative dominance of one (or several) such mechanisms. The results are expected to provide a better understanding of eye movement behavior and their control mechanisms. Such understanding will enable us to use eye movements as a powerful diagnostic instrument for the real-time measurement of different forms of cognitive activities or their impairments. As a result, eye tracking will become of greater importance in the development of future applications, as well as part of future applications themselves.

Parameters of Eye Movements

Understanding vision and visual perception is of long-lasting interest and dates back to classical antiquity. For instance, Plato (427–327 B.C.) developed the extramission theory to explain the process of vision. He imagined that vision occurs when light comes out of the eye and hits objects outside. Objects then release "flame particles" representing different colors [20]. Since these early attempts, many important discoveries have been made about vision in general and the function and purpose of individual parameters (for a review, see [21]). Much of the knowledge about eye movements has been uncovered in laboratory experiments by investigating various eye movement parameters. Nowadays, the interest in eye movement research has shifted to understanding the interaction between parameters and the meaning as a whole in the process of active vision (e.g. [5]).

The neural systems controlling eye movements are interesting because they form a network in the whole brain. Analysis of eye movements therefore provides amenable access to mechanisms in the active brain. Two different perspectives are possible when considering eye movements. Eye movements can be understood as the result of a highly complex and very precise motor system, but also as part of a sensory system that is instead concerned about where the eyes are directed to in space, technically referred to as *gaze*. For the study of gaze behavior, a functional distinction should be made between the various types of eye movements. Some eye movements are dedicated to *gaze-holding* and others are responsible for *gaze-shifting*.

Gaze-holding eye movements produce a stable image on the retina, which is important for perceiving and processing of information, similar to taking a picture with a camera. However, unlike a camera, the eyes can be held stable even if the head or body is moving. As soon as any form of head or body movements occurs, our visual system compensates for these movements. Gaze-holding movements are driven by the balance organs in the inner ear (the vestibular system). Accordingly they are named the vestibulo-ocular reflexes (e.g., [22, 23]). In the case of retinal image motion, for instance while looking out of the window of a moving train, another gaze-holding mechanism becomes activated, referred to as the optokinetic response ([24], see also [25]). Fixating on a moving object in front of a stationary or dynamic background requires a different class of gaze-holding movements to keep the object stable at the foveal region. Therefore the eyes need to be in motion but must also be stable with respect to the object. Such movements are called dynamic fixation or smooth pursuit movements (see, e.g., [26, 27, 28]). Smooth pursuit movements have also been of interest to psychiatric research from the very beginning onwards, as can be seen from the seminal work of Diefendorf and Dodge [29]. Among other tests, "ocular pursuit-reactions" were identified as a promising candidate for the investigation of schizophrenia (or dementia praecox as was the scientific term at that time). A meta-analytic review [30] reported maintenance gain, total saccade rate, and leading saccades to be the most promising specific measures in smooth pursuit research in the context of schizophrenia.

Gaze-shifting eye movements are necessary to redirect the small high-resolution foveal region to the respective points of interest. These saccadic movements are executed whenever a new object needs to be fixated. On average, about three saccades a second are performed. Further coordination is required because we have two eyes which need to be adjusted so that the image of an object falls on exactly the same parts of the two retinae. For distant objects, the two eyes must move in a conjunct manner. If an object comes closer, the eyes must converge to line up in a disjunct manner. This conjunct and disjunct behavior is summarized as vergence movements.

Furthermore, during visual fixations, our eyes make tiny movements (microsaccades, tremor, and drift) of which we are not aware (see, e.g., [31]). The existence of these movements was already mentioned about 150 years ago [32] and they were assumed to support the perception of fine spatial details [33, 34]. Early investigations disproved this hypothesis by reporting effects of visual fading if fixational eye movements are eliminated (e.g., [35]). The term microsaccade

was introduced by Zuber and Stark [36], designating fixational movements within a range of 2 to 12 min arc. Around this time, it was hypothesized that microsaccades simply serve to compensate for errors produced by the slow drifts [37], but there was strong experimental evidence against this argumentation. It was reported that microsaccades can be voluntarily suppressed without indications of visual fading (e.g., [38]). Moreover, it was found that microsaccades disappear during the performance of high-acuity tasks, such as threading a needle [39]. These controversial findings provided the start of a long-lasting debate about the purpose of microsaccades (for overviews, see [40, 41, 42]).

Recent experimental findings have shown that fixational eye movements are important for preventing visual fading [43]. It also has been reported that microsaccades enhance the discrimination of fine spatial details [44] and briefly-presented stimuli [45]. Moreover, it was proposed that microsaccadic activity provides an index for covert attention shifts [46, 47]. Although recent reports emphasize the importance of microsaccades for visual perception [41, 43], the contribution of microsaccades in the process of active vision still remains unclear [48], and their significance during natural viewing is still debated [31]. Recently, microsaccades came into the focus of psychiatric research, too [49]. The authors investigated differences in free viewing ocular behaviour between healthy subjects and schizophrenic patients. Results showed no differences in terms of microsaccades, but overall scanning behaviour heavily depended from image content. However, based on the idea of a common mechanism of saccade generation [50], it will be a future task to broaden our understanding on the relationship between saccades and microsaccades in the context of schizophrenia research.

Microsaccades, clearly a facet of gaze-holding movements, can be taken as an example to illustrate that the distinction is somewhat artificial, motivated by the researchers' interest in classification of phenomena. Both saccades, falling into the gaze-shifting category, and microsaccades can be drawn onto a single continuum of a main sequence, a term which has been adopted from astronomy [51]. Here, the relationship between the peak velocity and the amplitude of the saccadic movement can be mapped. It has been shown that the linearity of the relationship continues from the smallest microsaccades to wide saccades measured in everyday activities such as free inspection of a natural scene [52], providing some evidence for the hypothesis that both kinds of saccades are generated by the very same instances. Therefore, whether gaze is being held or being shifted might be a matter of perspective, or of scale. The distinction, however, is useful to examine underlying processes, and especially to pinpoint the qualitative and functional differences. In the following sections, we will take a closer look at fixations, saccades, and finally at the dynamic interplay of both in active vision.

Characteristics of Fixations

When investigating fixations, different aspects can be considered. First, the duration can be measured, indicating how long the eyes are stabilized with regard to a particular region or object in the environment. Accordingly, the duration of fixations provides a temporal characteristic and can also indicate where our eyes are attracted. The spatial distribution of fixations contains information about which object is fixated on first and where the eye is going with the next saccade. Another important issue is related to the information that is processed within a single fixation. What is the amount of information that can be perceived and processed? One can think about this question in terms of a particular window that might have a certain shape and size. Given a window size of approximately 2 degrees of visual angle, how can two successive fixations that are spatially separated by only 1 degree be resolved? Should these fixations be considered as only one fixation within a region? What are the mechanisms that control for all these different features? Which brain structures are involved in the temporal and spatial control of fixations? Since some of the mechanisms are known already, different models for fixational control have been suggested.

The early beginnings of eye movement research revealed that fixations vary with regard to their durations [53–55]. Fixation durations can vary from less than 100 ms to several seconds, but the vast majority of fixations are terminated after about 200 and 250 ms [56]. The variation in the duration of fixations has been attributed to different reasons. Evidence has been found that task type and difficulty influences the fixation duration. For instance, in silent reading, the mean fixation durations are shorter (225–250 ms) than in oral reading (275–325 ms; [9]). This difference could be related to the motor component when reading aloud. However, the observation of shorter fixation durations in visual search (180–275 ms) compared to longer fixations in scene perception (260–330 ms) indicates that the nature of the task clearly influences the length of fixations [9]. Furthermore, fixation durations can even be different within the same task. It has been found that inspecting the same visual stimuli under different instructions leads to significant changes in fixation durations [57, 58]. This has been interpreted as evidence for a relationship between fixation duration and the level of information processing, according to Craik and Lockhart [59].

The approaches discussed so far assume a direct connection between the duration of a fixation and the ongoing information processing. These direct control theories are supported by results from the stimulus onset delay paradigm (e.g., [60, 61, 62]). In this paradigm, the stimulus is removed during a saccade and reappears within the next fixation with specified delays. An increase in the fixation duration by the amount of the onset delay provides evidence that fixations are under direct control (e.g., [60]). Changes in the quality of available information can also influence the duration of fixations [63–65]. For example, Mannan and colleagues [64] reported longer fixations for low-pass-filtered than for unfiltered scenes. A prolongation of fixations has also been found when the amount of either foveal or peripheral information was limited by a gaze-contingent mask [66].

In contrast to the direct control assumption, it has also been argued that fixations might be governed indirectly by other factors. These indirect control theories propose that (i) the stimulus processing within a fixation is too slow to have an immediate effect (delayed control), (ii) the global parameters, such as the task, stimulus properties, etc., determine the length of fixations (global parameter control; e.g., [67, 68]), and (iii) there is an internal timing mechanism keeping the eyes moving at a constant rate. Recently, a mixed control model for fixation durations has been suggested [69, 70]. Applying the scene-onset delay paradigm to scene perception resulted in a prolongation of a certain proportion of fixations (supporting direct control) while other fixations remained unaffected by the scene-onset manipulation (supporting indirect control). These findings have resulted in a recent computational model for the control of fixations that accounts for variations in fixation durations in scene viewing [71]. The timing signals (i.e., fixation durations) of the model are based on continuous-time random walks. Furthermore, the level of visual and cognitive processing can modulate the onset of a saccade and thereby determine the length of a fixation.

One critical limitation of the discussed theories is the missing link to brain structures and their ongoing activity. A lot of information about structures and their functional contribution to the control of fixations have been accumulated during recent decades. However, these findings are mostly excluded from theories developed to explain the control and duration of fixations.

The spatial distribution of fixations across an image or in relation to the environment represents further important information for understanding the nature of visual sampling and processing. The locations of fixations reveal the strong interrelation between fixations and saccades. The spatial distribution of fixations can be examined with regard to the regions and objects, i.e., considering the location of the eyes for a certain period of time. Similarly, it can be explored why a saccade was performed towards a particular location in a scene. Regardless of which approach is taken (direct or indirect control), the eyes remain within a particular region until the feature extraction and information processing is

completed. One of the first contributions to this topic was the feature integration theory [72, 73]. The approach was introduced to explain serial and parallel mechanisms in visual search. The key concept is based on the extraction of primary features, such as color, orientation, and shape, which are represented in separate feature maps. These feature maps are integrated in a saliency map that is accessible and used to direct attention to the most conspicuous areas.

The concept of the saliency map has become an essential part of computational models of focal visual attention, and thereby for the explanation of eye movement behavior (e.g., [74, 75, 76]). These attempts provide promising results and a first approximation for modelling the spatial distribution of fixations during the inspection of naturalistic stimuli. However, the essential limitation of the saliency approach is due to its exclusive focus on primary physical features of a scene. If the spatial distribution of fixations could be sufficiently explained by the analysis of such simple features, it could be concluded that visual attention is exclusively controlled in a bottom-up manner. Recent evidence revealed that this is not the case; rather, the deployment of visual attention is based on bottom-up as well as top-down influences [77, 78]. Moreover, it has been found that task-demands can override saliency features [78–80]. Thus, it seems that top-down mechanisms (e.g., instructions) dominate gaze behavior during visual tasks (e.g., [54, 80]) and in the performance of visually-guided actions (e.g. [81, 82]). A fairly new and promising approach that tries to overcome the problems of the traditional saliency approach has been suggested by Hwang, Wang, and Pomplun [83]. The authors conducted experiments that combined several interdisciplinary methods in novel ways to examine semantic guidance within a visual scene. This method integrates bottom-up and top-down saliency information, thereby allowing predictions about eye gaze behavior that are presumably closer to the processing mechanisms of the visual system.

In psychiatric research, effort has been taken to investigate fixation distributions over different kinds of stimuli, in order to compare schizophrenic patient groups to healthy subjects (e.g., [84, 85]). Phillips and David [84] were interested in where deluded schizophrenic patients would direct their visual attention to when inspecting images of faces, both familiar and unfamiliar. They showed that schizophrenic patients actively avoided informative regions by mostly fixating areas outside the faces; moreover, in conditions when two faces were presented, fixation durations of deluded patients were prolonged as compared to the non-deluded and the healthy control subjects. Sprenger and colleagues [85] showed photographs of everyday situations to schizophrenic patients. In comparison to healthy control subjects, they found fewer fixation clusters, longer fixation durations as well as deviant attentional landscapes and scan paths.

Characteristics of Saccades

Saccades are necessary to direct the fovea from one point to another. In most visual activities, we perform about three saccades a second [86]. During a saccade, the processing of visual information is suppressed because the image is rapidly moving across the retina [8]. The period where information encoding is suppressed starts before the actual saccade and outlasts the saccadic eye movement by about 50–60 ms [87–89]. In contrast to visual perception, cognitive processing seems not to be interrupted during saccades [90]. Saccades are of high velocities to minimize the periods in which we are nearly blind.

Different types of saccades are documented in the literature. Saccades can be elicited by the onset or change of a visual stimulus, designated as exogenous, reflexive, or visually-guided saccades. Moving the eyes to a target which is recalled from memory requires the performance of an endogenous, voluntary, or memory-guided saccade. These saccades do not necessarily rely on a visual stimulus. During natural viewing, we either perform visually or memory-guided saccades. Another form of saccade, the so-called antisaccade, is often used in neurophysiological research for diagnostic purposes (e.g., [91, 92]). In the antisaccade task, the eyes have to move

away from a visual target appearing on the screen. The accurate performance of an antisaccade requires inhibiting a reflexive saccade to the onset location, together with a voluntarily move of the eye in the opposite direction. The antisaccade task requires cognitive control, evidenced by the fact that observers often have difficulties in suppressing the reflexive saccades in the direction of the target. Programming and performing a correct antisaccade is more delayed than visually-guided saccades.

A network of several brain structures is involved in the planning and execution of saccades. Knowledge about the contribution of particular brain structures has been gathered by the investigation of different saccade parameters. In the following, we will discuss commonly analyzed parameters of saccades before briefly reviewing those brain regions which have been identified to significantly contribute to saccadic activity. Saccadic eye movements bring the fovea the regions of interest, which can vary with regard to the distances in between, requiring the *saccade amplitudes* to be of different lengths. In everyday tasks, saccade amplitudes vary from a few degrees up to 130 degrees of arc, with an average saccade size of about 18–20 degrees [93, 94]. As a result of the variation in saccade length, there are also differences in *saccade durations.* During reading, saccade durations are on average 20 to 30 ms but they can last up to about 100 ms. The parameter *saccadic peak velocity* describes the maximum speed that can be achieved within a saccade (up to 900 degrees/s), almost linearly related to the saccade amplitude. For the detection of saccades, when processing the raw data of an eye-tracking device, the *saccade acceleration* represents another important parameter (to differentiate between other eye movements, a minimum of 150 deg./s−2 is often applied; see, e.g., [95]).

Another feature is the *saccade trajectory*. Saccades are rarely straight (e.g., [54]) and most of them show a tendency to curve towards the horizontal meridian [96]. Moreover, other objects within the visual scene have been found to influence the magnitude and direction of the curvature observed. The presentation of a distractor has been found to curve a saccade towards a distractor (e.g., [97]) but also away from a distractor (e.g., [98]). The direction of the curvature, i.e., towards or away from a distractor, appears to depend upon the overall neural activity distribution produced by the target and the nearby distractor. According to the population coding theory proposed by Tipper, Howard, and Houghton [99], possible target objects are represented by large neuronal populations that encode a movement vector aimed at the target. If the target and distractor are nearby, their population codes will be combined into one distribution, resulting in a vector which represents an intermediate location between the objects [100].

An often-used parameter, which is mainly of importance for laboratory experiments, is the *saccade latency*. The latency of a saccade describes the time interval between the appearance of a target and the execution of a saccade towards the target. For healthy adults, saccade latencies are reported within a range of 200 to 250 ms. Saccade latency also seems to be related to cognitive development in children, as the latencies of visually-guided saccades in children are longer than in adults [101]. Obviously, the latency shortens progressively with age. Laboratory experiments have identified a subpopulation of saccades with very short latencies at around 100 ms [102]. The existence and function of these so-called express saccades has been debated, e.g., see [103]. The latency period is necessary to complete several processes, such as attentional disengagement from the actual fixation position, a shift of visual attention to the new target location, and the computation of saccade metrics. Each of these processes involves activation of different cortical and subcortical areas (see below).

The saccade latency represents a cognitive–physiological parameter, and has been extensively studied with different paradigms. The manipulation of information at the fixation location has been found to substantially influence saccade latency. In its simplest form, this manipulation involves a disappearing fixation target before the onset of the next target. The resulting saccade latency is significantly shortened, a phenomenon

which is known as the gap effect [104]. In contrast, an increase in the saccadic latency has been found when two stimuli are shown at the same time, one of which is the target, the other a distractor [105, 106]. This "remote distractor effect" has the strongest influence on the saccade latency when the distractor appears at the fixation location [107]. We will elaborate more on the saccade latency when discussing the relationship between fixations and saccades.

In schizophrenia research, saccadic latency is a prominent parameter [108–110]. Manoach and colleagues [109] investigated microstructural integrity of brain structures related to volitional saccades, i.e., anterior cingulate cortex, frontal eye fields, and right hemisphere parietal cortex, using diffusion tensor imaging. Their results suggest that slower volitional saccades in schizophrenic medicated patients are associated with reduced integrity. The relationship between latency and peak velocity in pro- and antisaccades was investigated, both in groups of healthy subjects and in schizophrenia patients [110], and revealed for both groups that antisaccades had lower peak velocities than prosaccades, and that peak velocities of antisaccades were independent of latencies. For prosaccades with long latencies, however, schizophrenia patients showed significant decrease of peak velocities. The authors explained this effect with a possible decay of the transient visual signal at the saccade target, or a reduction of target-related neural activity in the saccade system. Latencies of saccades are also task-dependent. Schwab and colleagues [108] studied schizophrenia patients and their first-degree relatives as compared to healthy subjects in a low and high demand visual task. Their results showed smaller differences between the tasks for the patients, as compared to the other two groups, possibly reflecting a specific oculomotor attentional dysfunction.

Due to the fact that we perform about three saccades a second, a high degree of accuracy regarding the landing position of saccades is required. If saccades often failed to land at the desired target, our visual perception would certainly be impaired. During a saccade, no visual feedback is available for online control due to saccadic suppression and the short durations of saccades. The accuracy of saccades is achieved by an *adaptive gain control* mechanism correcting for the tendency of saccades to either undershoot or overshoot their targets. Such adjustments are related to the distance to the target. Especially for saccadic amplitudes larger than 10 degrees, a tendency for undershooting is known. This undershooting is assumed to reduce programming costs for the follow-up corrective saccade because it requires less programming effort to make the follow-up saccade in the same direction. The dynamic aspects of the gain control are usually investigated by displacing the target during the saccade to a certain extent. After several repetitions, saccades begin to compensate for this displacement and the displacement is included in the saccade execution. This modification of the saccade amplitude represents a strong indicator for an adaptive control mechanism (e.g., [111]). This paradigm of visuomotor adaptation has recently been investigated with schizophrenic patients showing neurological soft signs [112]. In healthy subjects, one would expect to see adaptations of the visual system within 30–80 trials (see, e.g., [113]). In contrast to previous results [114], Picard and colleagues [112] did not find differences in terms of adaptation speed. Both groups of healthy subjects and patients took about 70 cycles to adapt to the displacement. However, adaption rate was higher for healthy subjects than for the schizophrenic patients.

In recent decades, numerous brain regions have been identified which contribute to the programming and generation of saccades, including structures of the brainstem as well as cortical areas (e.g., [115]). When executing saccadic eye movements, the ocular motoneurons receive input from vertical and horizontal saccadic burst generators — two sets of nuclei of the reticular formation [116]. Based on instructions from higher-level structures, the saccadic burst generators produce commands that are necessary for the generation of saccades with the desired metrics.

The two structures playing a key role for sending commands to the saccadic burst generators are the superior colliculus (SC) and the frontal eye fields (FEF, [115]). Within the SC, visual,

auditory, and somatosensory signals from different brain structures are integrated. In addition, cognitive signals are also important for the information integration, such as attention, motivation, and context [117]. The SC controls the direction of the eyes, but is also important for the orientation of the whole body. The control of the orientation of the whole body takes place mainly in the cerebellum. Therefore, commands from the SC to the saccadic burst generators are also adaptively modulated by signals from the cerebellum [118]. These signals provide input when performing saccades in order to calibrate the system in terms of a long-term adaptation of the saccadic gain, allowing for an online correction of each saccade according to the variability of the rest of the saccade-generating circuitry [114]. Additional inhibitory input is sent to the SC from the basal ganglia, a set of subcortical nuclei. Since most of the cortical input to the SC is excitatory in nature, this inhibitory information is essential to prevent excessive demands for motor outputs [119].

In addition to the subcortical connections, there are various cortical areas involved in the generation of saccades, such as the posterior parietal cortex, the parietal eye field, the dorsolateral prefrontal cortex, the anterior cingulate cortex, the pre-supplementary, supplementary, and frontal eye fields (pre-SEF, SEF, and FEF respectively; [120]). Each of these structures has at least one particular function in the generation of saccades. The FEF is involved in triggering intentional saccades, while the parietal eye field contributes to reflexive saccades. The initiation of motor programs for saccades requires activity in the SEF. The learning of these programs is associated with the pre-SEF, while the dorsolateral prefrontal cortex is involved in saccade inhibition, prediction, and spatial working memory [121].

Much research in recent decades has been dedicated to understanding the functionality and the interconnectivity of various saccade-related brain regions. These efforts have resulted in numerous computational models describing various subparts of the saccade-related network, but some models also try to explain saccade control in general. These models are helpful for interpreting empirical findings. An extensive overview of state-of-the-art models for saccade control was published in [117].

Active Vision: The Relationship between Fixations and Saccades, and Beyond

In 1935, Guy T. Buswell published his influential book, *How People Look at Pictures: A Study of The Psychology of Perception in Art*. This work is important to the history of eyetracking because it represents the first systematic investigation of eye movements during the exploration of complex scenes. Buswell recorded eye movements of over 200 subjects while they viewed 55 photographs of objects ranging from paintings and statuary pieces to tapestries, patterns, architecture, and interior design. One of the most important discoveries by Buswell was the fact that observers exhibit two forms of eye movement behavior. In some cases, viewing patterns were characterized by a general survey of the overall image; fixations were distributed over the main features of the picture. In other cases, observers made long fixations over smaller sub-regions of the image. Apart from the fact that each observer exhibited an idiosyncratic viewing behavior, Buswell noticed a majority of quick, global fixations early in the viewing, transitioning to longer fixations (and smaller saccades) as the viewing time increased [53, 122].

Buswell also concluded that the "mental set" obtained from experimental instructions significantly influenced how people looked at pictures, a finding that was later confirmed by the work of the Russian eye-movement pioneer Alfred L. Yarbus. In his book *Eye Movements and Vision* [54] Yarbus demonstrated how viewing instructions directly influenced eye movement behavior. In his often cited experiment, Yarbus recorded eye movements of one observer inspecting I. E. Repin's painting, "They Did Not Expect Him" (1884; see, e.g., [123]). The experiment started with a 3-min period of free viewing followed by another six inspections, each with a different instruction, such as "Estimate the ages of

the people" and "Remember the clothes worn by the people". The results from this experiment verified the earlier observations by Buswell (1935), providing striking evidence for top-down influences on eye movements when exploring visual scenes. A recent replication of the classical Yarbus experiment with 17 subjects and shorter inspection times confirmed the earlier findings, but showed less dramatic influences of the different instructions [124].

While early work on eye movements analyzed the interplay between fixations and saccades during natural viewing, in subsequent decades it became of greater interest to investigate particular fixation and saccade characteristics in isolation. The chosen approach can be described in terms of *passive vision*, which eliminates as many confounding variables as possible. To give an example here, a typical experimental paradigm requires the participant to perform single saccades from a start point to a target position. With such a procedure, it is possible to precisely analyze mechanisms of saccadic control. However, the subjects' task represents a rather artificial fixate-and-jump cycle which never happens in natural gaze behavior.

Although this approach can be criticized for its unnatural and artificial procedure, most of the existing knowledge about eye movements originates from this form of research. One of the reasons for the use of such an artificial approach was the lack of methodical and technical alternatives for applying more natural paradigms to the investigation of eye movements. Furthermore, particular characteristics of saccades can hardly be explored in the process of natural viewing. For instance, examining adaptive gain control mechanisms when programming and executing saccades requires the repeated performance of single saccades under exactly the same conditions (e.g., [125]). The paradigm of the antisaccade task is essentially based on the performance of saccades, which is in clear contradiction to natural gaze behavior [92]. Nevertheless, the paradigm provides important insights in specific gaze-control mechanisms and represents a helpful diagnostic tool [91]. Moreover, to understand gist processing — the question of how much information about a complex scene can be processed within a certain amount of time — also requires a deviation from the way in which we naturally perceive our environment [126]. In experiments investigating gist processing, a scene is typically shown for a few milliseconds only.

Within the last decade, however, the active vision approach [5, 7] has gained increasing interest (for recent examples see, e.g., [4, 82, 127]). Apart from the issues discussed above, there are further arguments emphasizing the importance of the active vision approach in the investigation of eye movements. First, understanding the complex structure of eye-movement behavior, which is one of the central goals of this field of research, can only be achieved with the analysis of natural viewing sequences. Second, the existing knowledge gained from investigations using laboratory settings and paradigms needs to be validated in the context of natural viewing settings. Finally, there is a growing interest in using eye movements in various fields of application beyond answering pure research questions (e.g., [128, 129]). Therefore, a comprehensive understanding of eye movements can only be based on the active vision approach.

When considering sequences of eye movements, saccades and fixations are clearly related in several aspects. The fact that vision consists of the alternate performance of saccades and fixations has several implications for the analysis of eye movement behavior. First, there is a temporal interrelation: the longer the fixations, the fewer saccades are performed within the same amount of time. In contrast, increasing the frequency of saccades also increases the number of fixations but decreases the duration of fixations. Second, there is a spatial interrelation: when inspecting a particular scene, one can analyze the spatial distribution of fixations to understand which regions were of interest. However, saccades need to be programmed and executed in order to bring the eye from the original to the new target location. Therefore, the spatial distribution of fixations is at the same time reflected in the amplitude of saccades. Third, one should keep in mind that saccade latency is also part of the fixation duration. Accordingly, an increase in saccade latency

automatically implies prolonged fixations. In contrast, not every increase in fixation duration necessarily implicates longer saccade latencies.

As mentioned at the beginning of this section, a characteristic relationship between fixations and saccades has already been reported by early work in eye-movement research [53, 130]. In addition to the characteristic changes over time, another relation between the two parameters is of interest: larger saccades always bring the eyes further away from the original location. Assuming that the processing during fixations before and after a saccade is somehow related implies a higher semantic relationship between consecutive fixations for short saccades, as well as large saccades. In fact, the saccade length can help identify coarse-to-fine strategies [68, 131], and can be used for understanding the interplay between global and local processing mechanisms [132–134]. Recent progress in the development of advanced tools allows for comparing the sequences of saccades and fixations [135–137]. This is also of importance for clinical research, since the analysis of visual scanpaths has been a prominent topic for instance in schizophrenic patients [138–140].

Understanding the neurocognitive mechanisms of (active) vision has been a challenge for centuries. Research during past decades dealt with rather simple and artificial tasks to exclude interfering artifacts. As a result, the parameters and mechanisms of interest were mostly investigated in isolation, such as the study of the relationship between overt and covert attention shifts. This relationship is often examined by presenting a cue which directs covert attention to a particular region. A single saccade to this region can be executed after a variable temporal delay. In most experimental paradigms, such a trial consists of a single fixation followed by a saccade. Such a cycle clearly is an oversimplification of normal visual exploration behavior. Real-life processing of visual information is based on sequences of fixations and saccades, allowing many degrees of freedom. The interest in studying gaze behavior in more naturalistic settings has increased recently (e.g., [4, 141]), which is also due to the advances of modern eye trackers (e.g., [142]). These technological achievements make it possible to examine, for example, gaze strategies in driving [143], in operating theatres [144, 145], while making tea in a kitchen [146], or during shopping in a supermarket [147]. Such types of work demonstrate that the analysis of gaze behavior can be applied to real-life applications [148, 149]. This, however, raises the question whether the knowledge about visual information processing gathered from simple and artificial paradigms is sufficient for successful application. Bridging the gap in the knowledge between findings from controlled laboratory settings and real-life behavior requires understanding the mechanisms of active vision.

References

1. Schifferstein H. The perceived importance of sensory modalities in product usage: a study of self-reports. Acta Psychol (Amst). 2006;121(1):41–64.
2. Fenko A, Otten JJ, HNJ S. Describing product experience in different languages: the role of sensory modalities. J Pragmat. 2010;42(12):3314–27.
3. Stadtlander LM, Murdoch LD. Frequency of occurrence and rankings for touch-related adjectives. Behav Res Methods Instrum Comput. 2000;32(4):579–87.
4. Hayhoe MM, Ballard DH. Eye movements in natural behavior. Trends Cogn Sci. 2005;9(4):188–94.
5. Findlay JM. Active vision: visual activity in everday life. Curr Biol. 1998;8(18):R640–R2.
6. Henderson JM. Regarding scenes. Curr Dir Psychol Sci. 2007;16(4):219–22.
7. Aloimonos J, Weiss I, Bandyopadhyay A. Active vision. Int J Comput Vis. 1987;1(4):333–56.
8. Matin E. Saccadic suppression: a review and an analysis. Psychol Bull. 1974;81(12):899–917.
9. Rayner K. Eye movements and attention in reading, scene perception, and visual search. Q J Exp Psychol. 2009;62(8):1457–506.
10. Klein C, Ettinger U. A hundred years of eye movement research in psychiatry. Brain Cogn. 2008;68(3):215–8. Epub 2008/10/07
11. Andrews TJ, Coppola DM. Idiosyncratic characteristics of saccadic eye movements when viewing different visual environments. Vision Res. 1999;39(17):2947–53.
12. Vertegaal R, Shell JS, Chen D, Mamuji A. Designing for augmented attention: towards a framework for attentive user interfaces. Comput Hum Behav. 2006;22(4):771–89.
13. Vertegaal R, Velichkovsky BM, Van der Veer G. Catching the eye: management of joint attention

in cooperative work. ACM SIGCHI Bull. 1997;29(4):87–99.
14. Tamura Y, Sugi M, Arai T, Ota J. Attentive deskwork support system. J Adv Comput Intell Intell Informatics. 2010;14(7):758–69.
15. Wundt W. Grundriss der Psychologie [Outlines of psychology]. Leipzig: Wilhelm Engelmann; 1897.
16. Bonhage CE, Mueller JL, Friederici AD, Fiebach CJ. Combined eye tracking and fMRI reveals neural basis of linguistic predictions during sentence comprehension. Cortex. 2015;68:33–47.
17. Kafkas A, Montaldi D. Striatal and midbrain connectivity with the hippocampus selectively boosts memory for contextual novelty. Hippocampus. 2015;25(11):1262–73.
18. Richlan F, Gagl B, Hawelka S, Braun M, Schurz M, Kronbichler M, et al. Fixation-related fMRI analysis in the domain of reading research: using self-paced eye movements as markers for hemodynamic brain responses during visual letter string processing. Cereb Cortex. 2014;24(10):2647–56.
19. Velichkovsky BM. Heterarchy of cognition: the depths and the highs of a framework for memory research. Memory. 2002;10(5/6):405–19.
20. Ackerman JS. Leonardo's eye. J Warburg Courtauld Inst. 1978;41:108–46.
21. Wade NJ, Tatler BW. The moving tablet of the eye: the origins of modern eye movement research. Oxford: Oxford University Press; 2005.
22. Roy JE, Cullen KE. Selective processing of vestibular reafference during self-generated head motion. J Neurosci. 2001;21(6):2131–42.
23. Duchowski AT, Medlin E, Cournia N, Murphy H, Gramopadhye A, Nair S, et al. 3-D eye movement analysis. Behav Res Methods Instrum Comput. 2002;34(4):573–91.
24. Chokron S, Brickman AM, Wei T, Buchsbaum MS. Hemispheric asymmetry for selective attention. Cogn Brain Res. 2000;9:85–90.
25. Büttner-Ennever JA, AKE H. Anatomical substrates of oculomotor control. Curr Opin Neurobiol. 1997;7(6):872–9.
26. Barnes GR. Cognitive processes involved in smooth pursuit eye movements. Brain Cogn. 2008;68(3):309–26. Epub 2008/10/14.
27. Thier P, Ilg UJ. The neural basis of smooth-pursuit eye movements. Curr Opin Neurobiol. 2005;15(6):645–52.
28. Spering M, Montagnini A. Do we track what we see? Common versus independent processing for motion perception and smooth pursuit eye movements: a review. Vision Res. 2011;51(8):836–52. Epub 2010/10/23.
29. Diefendorf AR, Dodge R. An experimental study of the ocular reactions of the insane from photographic records. Brain. 1908;31:451–89.
30. O'Driscoll GA, Callahan BL. Smooth pursuit in schizophrenia: a meta-analytic review of research since 1993. Brain Cogn. 2008;68(3):359–70.
31. Collewijn H, Kowler E. The significance of microsaccades for vision and oculomotor control. J Vis. 2008;8(14):1–21.
32. von Helmholtz H. Handbuch der physiologischen Optik. Hamburg: Voss; 1866.
33. Hering E. Über die Grenzen der Sehschärfe. Berichte der Königlichen Sächsischen Gesellschaft der Wissenschaften Mathematisch-Physische Klasse. 1899;20:16–24.
34. Averill HI, Weymouth FW. Visual perception and the retinal mosaic, II. The influence of eye movements on the displacement threshold. J Comp Psychol. 1925;5:147–76.
35. Riggs LA, Ratliff F. The effects of counteracting the normal movements of the eye. J Opt Soc Am. 1952;42:782–3.
36. Zuber BL, Stark L. Microsaccades and velocity–amplitude relationship for saccadic eye movements. Science. 1965;150(3702):1459–60.
37. Cornsweet TN. Determination of the stimuli for involuntary drifts and saccadic eye movements. J Opt Soc Am. 1956;46(11):987–93.
38. Steinman RM, Cunitz RJ, Timberla GT, Herman M. Voluntary control of microsaccades during maintained monocular fixation. Science. 1967;155(3769):1577–9.
39. Bridgeman B, Palca J. The role of microsaccades in high acuity observational tasks. Vision Res. 1980;20(9):813–7.
40. Engbert R. Microsaccades: a microcosm for research on oculomotor control, attention, and visual perception. Prog Brain Res. 2006;154:177–92.
41. Martinez-Conde S. Fixational eye movements in normal and pathological vision. Prog Brain Res. 2006;154:151–76.
42. Rolfs M. Microsaccades: small steps on a long way. Vision Res. 2009;49:2415–41.
43. Martinez-Conde S, Macknik SL, Troncoso XG, Dyar TA. Microsaccades counteract visual fading during fixation. Neuron. 2006;49(2):297–305.
44. Rucci M, Iovin R, Poletti M, Santini F. Miniature eye movements enhance fine spatial detail. Nature. 2007;447(7146):851–4.
45. Rucci M, Desbordes G. Contributions of fixational eye movements to the discrimination of briefly presented stimuli. J Vis. 2003;3(11):852–64.
46. Laubrock J, Engbert R, Kliegl R. Microsaccade dynamics during covert attention. Vision Res. 2005;45(6):721–30.
47. Laubrock J, Engbert R, Rolfs M, Kliegl R. Microsaccades are an index of covert attention: commentary on Horowitz, Fine, Fencsik, Yurgenson, and Wolfe (2007). Psychol Sci. 2007;18(4):364–6.
48. Steinman RM. Gaze control under natural conditions. In: Chalupa LM, Werner JS, editors. The visual neurosciences. Cambridge: MIT Press; 2003. p. 1339–56.
49. Egana JI, Devia C, Mayol R, Parrini J, Orellana G, Ruiz A, et al. Small saccades and image complexity

during free viewing of natural images in schizophrenia. Front Psych. 2013;4:37.
50. Martinez-Conde S, Otero-Millan J, Macknik SL. The impact of microsaccades on vision: towards a unified theory of saccadic function. Nat Rev Neurosci. 2013;14(2):83–96.
51. Bahill AT, Clark MR, Stark L. The main sequence, a tool for studying human eye movements. Math Biosci. 1975;24(3–4):191–204.
52. Otero-Millan J, Troncoso XG, Macknik SL, Serrano-Pedraza I, Martinez-Conde S. Saccades and microsaccades during visual fixation, exploration, and search: foundations for a common saccadic generator. J Vis. 2008;8(14):21.1–18.
53. Buswell GT. How people look at pictures. Chicago: University of Chicago Press; 1935.
54. Yarbus AL. Eye movements and vision. New York: Plenum Press; 1967.
55. Brown AC. The relation between the movements of the eyes and the movements of the head. London: Henry Frowde; 1895.
56. Rayner K. Eye movements in reading and information processing: 20 years of research. Psychol Bull. 1998;124(3):372–422.
57. Velichkovsky BM, Sprenger A, Pomplun M. Auf dem Weg zur Blickmaus: Die Beeinflussung der Fixationsdauer durch kognitive und kommunikative Aufgaben. In: Liskowsky R, Velichkovsky BM, Wünschmann W, editors. Usability engineering. Stuttgart: Teubner; 1997.
58. Velichkovsky BM. Levels of processing: validating the concept. In: Naveh-Benjamin M, Moscovitch M, Roediger III HL, editors. Perspectives on human memory and cognitive aging: essays in honour of fergus IM craik. Philadelphia: Psychology Press; 2001. p. 48–71.
59. FIM C, Lockhart RS. Levels of processing: a framework of memory research. J Verbal Learn Verbal Behav. 1972;11:671–84.
60. Morrison RE. Manipulation of stimulus onset delay in reading: evidence for parallel programming of saccades. J Exp Psychol Hum Percept Perform. 1984;10(5):667–82.
61. Rayner K, Pollatsek A. Eye-movement control during reading: evidence for direct control. Q J Exp Psychol A Hum Exp Psycol. 1981;33:351–73.
62. Vaughan J. Control of fixation duration in visual search and memory search: another look. J Exp Psychol Hum Percept Perform. 1982;8(5):709–23.
63. Loftus GR. Picture perception: effects of luminance on available information and information-extraction rate. J Exp Psychol Gen. 1985;114(3):342–56.
64. Mannan SK, Ruddock KH, Wooding DS. Automatic control of saccadic eye movements made in visual inspection of briefly presented 2-D images. Spat Vis. 1995;9(3):363–86.
65. Parkhurst DJ, Culurciello E, Niebur E. Evaluating variable resolution displays with visual search: task performance and eye movements. In: Duchowski AT, editor. Proceedings of the symposium on eye tracking research and applications. New York: ACM Press; 2000. p. 105–9.
66. van Diepen PMJ, d'Ydewalle G. Early peripheral and foveal processing in fixations during scene perception. Vis Cogn. 2003;10(1):79–100.
67. Henderson JM, Weeks PA, Hollingworth A. The effects of semantic consistency on eye movements during complex scene viewing. J Exp Psychol Hum Percept Perform. 1999;25(1):210–28.
68. Over EAB HITC, BNS V, Erkelens CJ. Coarse-to-fine eye movement strategy in visual search. Vision Res. 2007;47(17):2272–80.
69. Henderson JM, Smith TJ. How are eye fixation durations controlled during scene viewing? Further evidence from a scene onset delay paradigm. Vis Cogn. 2009;17(6–7):1055–82.
70. Henderson JM, Pierce GL. Eye movements during scene viewing: evidence for mixed control of fixation durations. Psychon Bull Rev. 2008;15(3):566–73.
71. Nuthmann A, Smith TJ, Engbert R, Henderson JM. CRISP: a computational model of fixation durations in scene viewing. Psychol Rev. 2010;117(2):382–405.
72. Treisman A, Gelade G. A feature-integration theory of attention. Cogn Psychol. 1980;12(1):97–136.
73. Treisman A. How the deployment of attention determines what we see. Vis Cogn. 2006;14(4–8):411–43.
74. Itti L, Koch C. Computational modeling of visual attention. Nat Rev Neurosci. 2001;2(3):194–203.
75. Parkhurst DJ, Law K, Niebur E. Modeling the role of salience in the allocation of overt visual attention. Vision Res. 2002;42(1):107–23.
76. Torralba A, Oliva A, Castelhano MS, Henderson JM. Contextual guidance of eye movements and attention in real-world scenes: the role of global features in object search. Psychol Rev. 2006;113(4):766–86.
77. Underwood G, Foulsham T, Humphrey K. Saliency and scan patterns in the inspection of real-world scenes: eye movements during encoding and recognition. Vis Cogn. 2009;17(6–7):812–34.
78. Foulsham T, Underwood G. What can saliency models predict about eye movements? Spatial and sequential aspects of fixations during encoding and recognition. J Vis. 2008;8(2):6.1–17.
79. Tatler BW. The central fixation bias in scene viewing: selecting an optimal viewing position independently of motor biases and image feature distributions. J Vis. 2007;7(14):4.1–17.
80. Einhäuser W, Rutishauser U, Koch C. Task-demands can immediately reverse the effects of sensory-driven saliency in complex visual stimuli. J Vis. 2008;8(2):2.1–19.
81. Hayhoe MM. Vision using routines: a functional account of vision. Vis Cogn. 2000;7(1–3):43–64.
82. Land MF. Vision, eye movements, and natural behavior. Vis Neurosci. 2009;26(1):51–62.

83. Hwang AD, Wang HC, Pomplun M. Semantic guidance of eye movements in real-world scenes. Vision Res. 2011;51(10):1192–205.
84. Phillips ML, David AS. Visual scan paths are abnormal in deluded schizophrenics. Neuropsychologia. 1997;35(1):99–105.
85. Sprenger A, Friedrich M, Nagel M, Schmidt CS, Moritz S, Lencer R. Advanced analysis of free visual exploration patterns in schizophrenia. Front Psych. 2013;4:737.
86. Land MF. Motion and vision: why animals move their eyes. J Comp Physiol A Sens Neural Behav Physiol. 1999;185(4):341–52.
87. Latour PL. Visual threshold during eye movements. Vision Res. 1962;2(3):261–2.
88. Diamond MR, Ross J, Morrone MC. Extraretinal control of saccadic suppression. J Neurosci. 2000;20(9):3449–55.
89. Loschky LC, Wolverton GS. How late can you update gaze-contingent multiresolutional displays without detection? ACM Trans Multimed Comput Commun Appl. 2007;3(4):1–10.
90. Irwin DE, Gordon RD. Eye movements, attention and trans-saccadic memory. Vis Cogn. 1998;5(1–2):127–55.
91. Smyrnis N. Metric issues in the study of eye movements in psychiatry. Brain Cogn. 2008;68(3):341–58. Epub 2008/10/10
92. Everling S, Fischer B. The antisaccade: a review of basic research and clinical studies. Neuropsychologia. 1998;36(9):885–99.
93. Land MF. The coordination of rotations of the eyes, head and trunk in saccadic turns produced in natural situations. Exp Brain Res. 2004;159(2):151–60.
94. Land MF, Mennie N, Rusted J. The roles of vision and eye movements in the control of activities of daily living. Perception. 1999;28(11):1311–28.
95. Haarmeier T. Sakkadische Augenbewegungen in der neurologischen Diagnostik. Neurophysiol Labor. 2010;32(3):146–52.
96. Viviani P, Berthoz A, Tracey D. The curvature of oblique saccades. Vision Res. 1977;17(5):661–4.
97. Godijn R, Theeuwes J. Programming of endogenous and exogenous saccades: evidence for a competitive integration model. J Exp Psychol Hum Percept Perform. 2002;28(5):1039–54.
98. Sheliga BM, Riggio L, Rizzolatti G. Spatial attention and eye movements. Exp Brain Res. 1995;105(2):261–75.
99. Tipper SP, Howard DV, Houghton G. Behavioral consequences of selection from population codes. In: Monsell S, Driver J, editors. Attention and performance. Cambridge: MIT Press; 2000.
100. Port NL, Wurtz RH. Sequential activity of simultaneously recorded neurons in the superior colliculus during curved saccades. J Neurophysiol. 2003;90:1887–903.
101. Fukushima J, Hatta T, Fukushima K. Development of voluntary control of saccadic eye movements. I: age-related changes in normal children. Brain Dev. 2000;22:173–80.
102. Fischer B, Ramsberger B. Human express saccades: extremely short reaction times of goal directed eye movements. Exp Brain Res. 1984;57:191–5.
103. Kingstone A, Klein RM. What are human express saccades? Percept Psychophys. 1993;54:260–73.
104. Saslow MG. Latency for saccadic eye movement. J Opt Soc Am. 1967;57:1030–3.
105. Lévy-Schoen A. Determination and latency of oculo-motor response to simultaneous and successive stimuli according to their relative eccentricity. Annee Psychol. 1969;69(2):373–92.
106. Findlay JM, Walker R. A model of saccade generation based on parallel processing and competitive inhibition. Behav Brain Sci. 1999;22(4):661–721.
107. Walker R, Deubel H, Schneider WX, Findlay JM. Effect of remote distractors on saccade programming: evidence for an extended fixation zone. J Neurophysiol. 1997;78:1108–19.
108. Schwab S, Jost M, Altorfer A. Impaired top-down modulation of saccadic latencies in patients with schizophrenia but not in first-degree relatives. Front Behav Neurosci. 2015;9(44):1–7.
109. Manoach DS, Ketwaroo GA, Polli FE, Thakkar KN, JJS B, Goff DC, et al. Reduced microstructural integrity of the white matter underlying anterior cingulate cortex is associated with increased saccadic latency in schizophrenia. Neuroimage. 2007;37(2):599–610.
110. Ramchandran RS, Manoach DS, Cherkasova MV, Lindgren KA, Goff DC, JJS B. The relationship of saccadic peak velocity to latency: evidence for a new prosaccadic abnormality in schizophrenia. Exp Brain Res. 2004;159(1):99–107.
111. Deubel H. Adaptivity of gain and direction in oblique saccades. In: O'Regan JK, Levy-Schoen A, editors. Eye movements: from physiology to cognition. New York: Elsevier/North-Holland; 1987. p. 181–90.
112. Picard H, Le Seac'h A, Amado I, Gaillard R, Krebs MO, Beauvillain C. Impaired saccadic adaptation in schizophrenic patients with high neurological soft sign scores. Psychiatry Res. 2012;199(1):12–8.
113. Hopp J, Fuchs AE. The characteristics and neuronal substrate of saccadic eye movement plasticity. Prog Neurobiol. 2004;72(1):27–53.
114. Optican L, Robinson D. Cerebellar dependent adaptive control of primate saccadic system. J Neurophysiol. 1980;44:1058–76.
115. Moschovakis AK. The superior colliculus and eye movement control. Curr Opin Neurobiol. 1996;6(6):811–6.
116. Scudder CA, CRS K, Fuchs AF. The brainstem burst generator for saccadic eye movements A modern synthesis. Exp Brain Res. 2002;142(4):439–62.
117. Girard B, Berthoz A. From brainstem to cortex: computational models of saccade generation circuitry. Prog Neurobiol. 2005;77:215–51.

118. Quaia C, Lefèvre P, Optican LM. Model of the control of saccades by superior colliculus and cerebellum. J Neurophysiol. 1999;82(2):999–1018.
119. Hikosaka O, Takikawa Y, Kawagoe R. Role of the basal ganglia in the control of purposive saccadic eye movements. Physiol Rev. 2000;80(3):953–78.
120. Platt M, Lau B, Glimcher PW. Situating the superior colliculus within the gaze control network. In: Hall WC, Moschovakis A, editors. The superior colliculus new approaches for studying sensorimotor integration. Boca Raton: CRC Press; 2004. p. 1–34.
121. Pierrot-Deseilligny C, Müri RM, Ploner CJ, Gaymard B, Rivaud-Péchoux S. Cortical control of ocular saccades in humans: a model for motricity. Prog Brain Res. 2003;142:3–17.
122. Babcock JS, Lipps M, Pelz JB. How people look at pictures before, during, and after scene capture: Buswell revisited. In: Rogowitz BE, Pappas TN, editors. Human vision and electronic imaging V, SPIE Proceedings, 46622002. Bellingham: SPIE; 2000. p. 34–47.
123. Tatler BW, Wade NJ, Kwan H, Findlay JM, Velichkovsky BM. Yarbus, eye movements, and vision. Iperception. 2010;1(1):7–27.
124. De Angelus M, Pelz JB. Top-down control of eye movements: yarbus revisited. Vis Cogn. 2009;17(6/7):790–811.
125. Wallman J, Fuchs AF. Saccadic gain modification: visual error drives motor adaptation. J Neurophysiol. 1998;80(5):2405–16.
126. Greene MR, Oliva A. The briefest of glances: the time course of natural scene understanding: research article. Psychol Sci. 2009;20(4):464–72.
127. 't Hart BM, Vockeroth J, Schumann F, Bartl K, Schneider E, Konig P, et al. Gaze allocation in natural stimuli: comparing free exploration to head-fixed viewing conditions. Vis Cogn. 2009;17(6–7):1132–1158.
128. Pannasch S, Helmert JR, Velichkovsky BM. Eye tracking and usability research: an introduction to the special issue. J Eye Mov Res. 2008;2(4):1–4.
129. Bernhaupt R, Palanque P, Winckler M, Navarre D. Usability study of multi-modal interfaces using eye-tracking. Lect Notes Comput Sci. 2007;4663:412–24.
130. Yarbus AL. The motions of the eye in the process of changing points of fixation. Biofizika. 1956;2(1):76–8.
131. Hegdé J. Time course of visual perception: coarse-to-fine processing and beyond. Prog Neurobiol. 2008;84(4):405–39.
132. Pieters R, Wedel M. Goal control of attention to advertising: the Yarbus implication. J Consum Res. 2007;34(2):224–33.
133. Wedel M, Pieters R, Liechty J. Attention switching during scene perception: how goals influence the time course of eye movements across advertisements. J Exp Psychol Appl. 2008;14(2):129–38.
134. Pannasch S, Helmert JR, Roth K, Herbold A-K, Walter H. Visual fixation durations and saccadic amplitudes: shifting relationship in a variety of conditions. J Eye Mov Res. 2008;2(2):1–19.
135. Anderson NC, Anderson F, Kingstone A, Bischof WF. A comparison of scanpath comparison methods. Behav Res Methods. 2015;47(4):1377–92.
136. Cristino F, Mathot S, Theeuwes J, Gilchrist ID. ScanMatch: a novel method for comparing fixation sequences. Behav Res Methods. 2010;42(3):692–700.
137. Mathôt S, Cristino F, Gilchrist ID, Theeuwes J. A simple way to estimate similarity between pairs of eye movement sequences. J Eye Mov Res. 2012;5(1):1–15.
138. Beedie SA, Benson PJ, Giegling I, Rujescu D, St Clair DM. Smooth pursuit and visual scanpaths: independence of two candidate oculomotor risk markers for schizophrenia. World J Biol Psychiatry Off J World Fed Soc Biol Psychiatry. 2012;13(3):200–10.
139. Loughland C. Visual scanpath dysfunction in first-degree relatives of schizophrenia probands: evidence for a vulnerability marker? Schizophr Res. 2004;67(1):11–21.
140. St Clair DM. Atypical scanpaths in schizophrenia: evidence of a trait- or state-dependent phenomenon? J Psychiatry Neurosci. 2011;36(3):150.
141. Tatler BW, Hayhoe MM, Land MF, Ballard DH. Eye guidance in natural vision: Reinterpreting salience. J Vis. 2011;11(5):1–23. Epub 2011/05/31
142. Pelz JB, Canosa R, Babcock J, Kucharczyk D, Silver A, Konno D. Portable eyetracking: a study of natural eye movements. In: Rogowitz BE, Pappas TN, editors. Human vision and electronic imaging, Proceedings of the San Jose, CA: SPIE; 2000. p. 566–82.
143. Land MF, Lee DN. Where we look when we steer. Nature. 1994;369:742–4.
144. Grundgeiger T, Wurmb T, Happel O. Eye tracking in anesthesiology: literature review, methodological issues, and research topics. Proc Hum Fact Ergon Soc Ann Meet. 2015;59(1):493–7.
145. Atkins MS, Tien G, RSA K, Meneghetti A, Zheng B. What do surgeons see: capturing and synchronizing eye gaze for surgery applications. Surg Innov. 2013;20(3):241–8.
146. Tatler BW. Characterising the visual buffer: real-world evidence for overwriting early in each fixation. Perception. 2001;30(8):993–1006.
147. Gidlof K, Wallin A, Dewhurst R, Holmqvist K. Using eye tracking to trace a cognitive process: gaze behaviour during decision making in a natural environment. J Eye Mov Res. 2012;6(1(3)):1–14.
148. Fedota J, Parasuraman R. Neuroergonomics and human error. Theor Issues Ergon Sci. 2010;11(5):402–21.
149. Parasuraman R. Neuroergonomics: research and practice. Theor Issues Ergon Sci. 2003;4(1):5–20.

Eye Movement Behavior Analyses for Studying Cognitive Performance and Conversion to Pathologies

21

Gerardo Fernández, Paola Buedo, David Orozco, and Osvaldo Agamennoni

Abstract

The interest of linking eye movement and reading has shown an exponential growth in the last decades. Additionally, the technology for tracking gaze position has advanced considerably and has become widely available for its use. Reading is a feedback process that requires the integration of different cognitive systems, and is an ideal field for exploring the relationships between eye movements and top-down processes. Several of the associated cognitive processes such as working memory and semantic memory are known to be relevant when reading sentences. For example, during reading high-predictable sentences the upcoming word predictability facilitates word processing in healthy readers. In the present chapter, we show the effect of contextual word predictability on the eye-movement behavior in patients with mild cognitive impairment due to dementia and to neuropsychiatric pathologies (i.e., Alzheimer's disease and schizophrenia respectively) in comparison with control groups of similar ages and education. The differences in the pattern are clearly presented. Our results show that it is possible to develop a new, objective, noninvasive, and economical technique to evaluate mild cognitive impairments that could help in the early and proper diagnosis of such pathologies.

Keywords

Cognition • Eye movements; Alzheimer: schizophrenia • Early diagnosis

G. Fernández
Instituto de Investigaciones en Ing. Eléctrica (IIIE) (UNS – CONICET), Bahía Blanca, Argentina
e-mail: gerardofernandez480@gmail.com

P. Buedo
Laboratory of Cognitive Neurosciences Development, Institute of Electric Engineering Research, National University of the South (Universidad Nacional del Sur), Argentina National Scientific and Technological Research (IIIE-CONICET-UNS), Bahía Blanca, Argentina
e-mail: paolabuedo@gmail.com

D. Orozco
Clínica Privada Bahiense, Bahía Blanca, Argentina
e-mail: davidorozco@live.com

O. Agamennoni (✉)
Universidad Nacional del Sur – Comision de Investigaciones Científicas, Departamento de Ing. Eléctrica y de Computadoras, Avda Alem 1253, Bahia Blanca, Buenos Aires 8000, Argentina
e-mail: oagamen@uns.edu.ar

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_21

Introduction

Cognition is defined as a mental process whereby we acquire knowledge that allows us to develop an activity in a given domain. This mental process includes awareness, perception, judgment, reasoning, and decision-making. Eyes are the most important organ that we have for perceiving our surroundings, and that is why our eyes are continuously moving between different points of saliency of our domain of interest, trying to get the relevant information needed to carry on our activities in the environment. The interest in linking eye movement and cognition goes back to the works of Buswell (1935) [1] and Yarbus (1967) [2]. Eye movement analysis is a research area that has shown exponential growth in recent decades. Furthermore, the technology for tracking gaze position has advanced considerably and, more importantly, has become widely available.

Reading is a cognitive activity that has received considerable attention from researchers evaluating human cognitive performance. Reading requires the integration of several central cognitive subsystems, from attention and oculomotor control to word identification and language comprehension. Eye movements show a reproducible pattern during normal reading. Each eye movement ends up in a fixation point, which allows the brain to process the incoming information and program the following saccade. Different neuropsychiatric pathologies produce abnormalities in eye movements and disturbances in reading, each of them having a particular pattern that can be registered and measured.

Eye movements can be classified into three groups:

- Movement for maintaining the image on the fovea (area of the retina with acuity vision), compensating head or object movements.
- Movements for shifting the eyes, when the attention changes from one object to another. There are subtypes of shifting movements: saccades (looking for a new center of visual attention), monitoring, and vergence (simultaneous movement of both eyes in opposite directions to obtain or maintain single binocular vision). These last movements are slower than saccades and are responsible for carrying the image of interest to both foveae, allowing stereoscopic vision).
- Movements of binocular fixation that also prevent fading of the image. These movements have three variations: tremor, drift, and microsaccade.

Saccades are rapid big eye movements particularly important from the cognitive point of view. Cognitive processes have direct influence on such movements. Each saccade has its direction. People usually read from left to right, and most saccadic eye movements are oriented accordingly. These normal reading movements are called forward saccades. Reading movements going from right to left are called regressions. The saccade movement alternates with a fixation made when the eyes are directed to a particular target [3].

In the present chapter, we show the effect of contextual word predictability on the eye movement behavior in patients with mild cognitive impairment due to dementia and to neuropsychiatric pathologies (i.e., Alzheimer's disease (AD) and Schizophrenia (SZ) respectively). Well-characterized processing patterns concerning fixation duration and its relationship with syntactic, semantic, and morphological properties of the sentences/words used as stimulus are shown, and the possibilities to distinguish between healthy people and patients in earlier stages of both pathologies are clearly understood.

In section 2, we describe some cognitive functions related to predictive capacities of the brain and its implications with working memory and eye movement behavior. In Section 3, we present a description of the eye movements study procedure. In section 4, we summarize our work in eye movement evaluation during reading in patients with mild AD and their respective matching controls. In section 5, we present the results obtained with patients of SZ

and its comparison with controls. In section 6, we introduce a number of conclusions and implications for diagnosing pathologies.

Eye Movement During Reading: Memory and Prediction

The visual system carries out a number of complex tasks, and one of the most important is the eye movement. Eyes are moved toward the direction that the brain predicts to find relevant information. Predict means to anticipate an upcoming event in a known context, and to do that, present and past information is needed. Then, to perform an accurate prediction, all the relevant past information of our environment needs to be available in our memory (working, semantic, and retrieval). Reading is a common and a well-defined activity for the great majority of people, which makes it possible to test the predictive system of the brain. Reading is an ideal field for exploring the relationships between eye movements and memory processes. For example, when we are reading a book, all the time we are applying our learned grammatical structure. It is when we find some strange grammatical expression that we need to re-analyze the sentence.

Healthy readers move their eyes on the average every quarter of a second. During the time that the eyes are fixated, new information is brought into the processing system. The average fixation duration is between 150–250 milliseconds (ms), the range is from 100 ms to over 700 ms [3]. The distance that the eyes move in each saccade (or short rapid eye movement) is between one and 20 characters, with an average between seven and nine characters. The primary function of the saccade is to bring a new region of text into the foveal vision. Saccade execution takes about 20–50 ms. Information uptake for processing is largely restricted to fixations. Reading studies have relied on different measures of fixation durations [3–5].

Reading saccades and fixations give us a considerable amount of information that can be correlated with word properties. When reading, the syntactic, semantic, and morphological properties of the word affect how long the fixation lasts. Fixation duration increases with word length and decreases with the frequency and predictability of the word [3, 4]. Researchers agree that information about the length, orthography, and phonology of the upcoming word is available during fixations on previous words. Indeed, some of this information is necessary for programming saccades. At the same time, there is strong evidence that the so-called high-level lexical properties (such as word frequency or predictability) of parafoveal words also influence fixation durations before the eyes reach these words (see, for example, [4, 6, 7, 8]). Cloze predictability [9] — i.e., the proportion of subjects that fill in a particular word as the most probable next word in a sentence from a previous context — is an important factor during fluent reading. Recent findings [7, 8] suggest that the buildup of predictions is indeed a rapid process.

High-predictability sentences and proverbs are read faster than the sentences of low predictability. The contextual predictability facilitates the reading process [10]. When a healthy reader advances in reading "Pinocchio's nose..." he or she can predict that the incoming word is "grows. [...]". And that is indeed what healthy readers do: use the information they have stored in their memory to "complete" the incoming information. Data from eye movements during language processing provide evidence not only that context-directs expectations or predictions about the upcoming stimuli "on the fly", but also that these predictions are highly specific and sufficiently detailed to guide behavior. Thus, healthy readers skip high-predictability words more often than low-predictability ones.

Study Procedure

In the following, the most relevant aspects of our study procedure will be presented. The investigation adhered to the principles of the Declaration of Helsinki, and was approved by the Institutional

Bioethics Committee of the Municipal Acute Care Hospital (Hospital Municipal de Agudos, Bahía Blanca, Buenos Aires, Argentina). All patients and their care givers, and all control subjects, signed an informed consent prior to their inclusion in the study.

Apparatus and Eye Movement Data

Single sentences were presented on the center line of a 20-inch LCD Monitor (1024 × 768 pixels resolution; font: regular; New Courier; 12 point, 0.21 in height). Participants sat at a distance of 60 cm from the monitor. Eye movements were recorded with an Eye Link 1000 Desktop Mount (SR Research) eye tracker, with a sampling rate of 1000 Hz and eye position resolution of 20 arcsec. All recordings and calibration were binocular. Only right eye data were used for the analyses. Figure 21.1 shows the eye-movement registering process of a control group person.

After validation of calibration, a trial began with the appearance of a fixation point on the position where the first letter of the sentence was to be presented. As soon as both eyes were detected within the fixation spot, the sentence was presented. After reading it, participants looked at a dot in the lower right corner of the screen; when the gaze was detected on the final spot, the trial ended. To assess whether subjects comprehended the texts, they were presented with a three alternative multiple-choice question about the sentence in progress in 20% of the sentence trials. Participants answered the questions by moving a mouse and choosing the response with a mouse click. An example of the eye movements recorded during reading of two sentences, showing eye fixations of both controls and AD, is shown in Fig. 21.2.

Fig. 21.1 Eye-movement registering process. The monitor can be seen, where the sentences are displayed, and the eye tracker (above the monitor), which consists of a 1,000 frames per second video camera and an infrared illuminator to increase pupil contrast and facilitate its detection

Sentence Corpus

The sentence corpus was composed of regular sentences and proverbs (Fernández et al., 2013a [10] for a Corpus description). Sentences comprise a well-balanced number of content and function words, and had similar grammatical structure. We used the Spanish Lexical Léxesp corpus [11] for assigning a frequency to each word of the sentence corpus. Word predictability was measured in an independent experiment with 18 researchers of the Electrical Engineering and Computer Science Department of Universidad Nacional del Sur. We used an incremental cloze task procedure in which participants had to guess the next word given only the prior words of the sentence [10].

Linear Mixed-Effect Models (LMMS)

LMM are linear models in which the linear predictor is contained in addition to the usual fixed effects. The LMM makes it possible to account for the correlation within profiles and to consider the profiles as a random sample from a common population distribution, which is, generally, more realistic. We used the lmer program of the lme4 package (version 0.999999–2) [12] for estimating fixed and random coefficients. We chose log gaze duration as the dependent variable because this measure includes refixations on a word, and refixations usually reflect a lexical-processing difficulty for word N [3, 4, 13]. Fixed effects in LMM terminology correspond to regression coefficients in standard linear

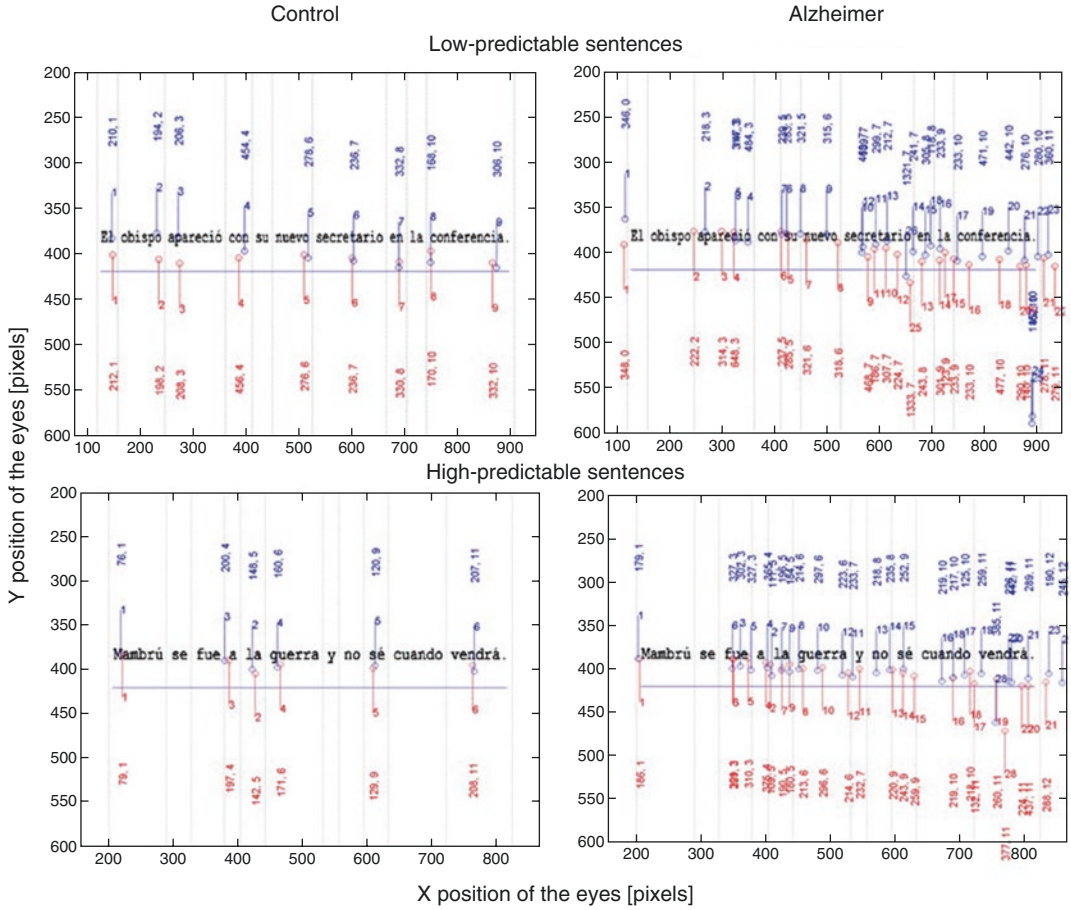


Fig. 21.2 Eye-movement recording observed during reading low- and highly predictable sentences by a control subject (*left*) and an AD patient (*right*). Fixation points for *right* (*red*) and *left* (*blue*) eyes are included in the graphs. The down and right movements signaled the end of reading; numbering linked to points indicates fixation sequences; fixation durations of each eye are listed with their corresponding colors. The number following fixation duration (after the comma) indexes the word number in the sentence. The English translation of the Spanish sentences "*el Obispo apareció con su nuevo secretario en la conferencia*", and "*Mambrú se fue a la guerra y no sé cuando vendrá*" are: "the bishop appeared with his new secretary in the conference" and "Mambrú went to war and I don't know when he will come back", respectively

regression models. They can also estimate slopes or differences between conditions. A number of fixed effects were entered into the model: logit predictabilities (i.e., the average predictability measured from the cloze task transformed using a logit function [10]), log frequencies and 1/length of word N − 1, word N, and word N + 1. Using the reciprocal of word length (i.e., 1/length), renders the multiplicative interaction of frequency and length or predictability and length as a ratio or relative frequency and predictability measure (i.e., normalized on word length). Regression coefficients (bs) standard errors (SEs) and *t*-values (t = b/SE) are reported for the LMMs. In this work, we only present a summarized graphical view of the outcomes. The interested reader can find a complete description of the LMMs results in [14]. Since there is no clear definition of "degree of freedom" for LMMs, precise *p*-values cannot be reported. In general, however, given the large number of observations, subjects, and items entering our analysis and the comparatively small number of fixed and random effects estimated, the *t*-distribution is equivalent to the normal distribution for all practical purposes (i.e., the contribution of the

degrees of freedom to the test statistics is negligible). Our criterion for referring to an effect as significant is $t = b/SE > \pm 1.96$.

Eye Movements During Reading in Patients with Mild AD

Early diagnosis of AD is still difficult. People with early to moderate AD usually show impairment in learning and a deterioration of episodic memory, symptoms that are typically used for diagnosis of the pathology. However, the subtle alterations in movement coordination and planning that may also be present while performing fine motor tasks such as writing or reading at the very beginning of the disease are harder to detect and go commonly unnoticed [15, 16]. Therefore, it is difficult to get an early diagnosis of this disease. Evaluation of eye movements might provide considerable insight into the integrity of control circuits in AD.

Our hypothesis was that in AD patients, an increase in average cloze predictability of the incoming word would probably not facilitate reading for impairments in top-down processing. To test this hypothesis, we evaluated the eye movements in control and AD patients during the reading of sentences with either high or low average word predictability. We investigated whether an increase in the average predictability of the upcoming word (N + 1) affected gaze duration (i.e., the sum of consecutive forward fixations on a word) in both groups. Our results [14] showed that while high predictable sentence and word predictability exerted its influence on gaze duration in healthy subjects, such predictability did not modify word processing during reading in mild AD patients.

Participants

Twenty patients (12 females and eight males; mean age 69 years, SD = 7.3 years) with the diagnosis of mild cognitive impairment probably due to AD were recruited at the Hospital Municipal of Bahía Blanca (Buenos Aires, Argentina). The clinical criteria to diagnose AD at its early stages remain under debate [17]. In the present work, diagnosis was based on the criteria for dementia outlined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [18]. The control group consisted of 40 elderly adults (29 females and 11 males; mean age 71 years old; SD = 6.1), with no known neurological and psychiatric disease according to their medical records, and no evidence of cognitive decline or impairment in daily activities. A one-way ANOVA showed no significant differences between the ages of AD and control individuals. The mean scores of controls and AD patients in the Mini-Mental State Examination (MMSE) [19] were 27.8 (SD = 1.0) and 24.2 (SD = 0.8) respectively, the latter suggesting early mental impairment. A one-way ANOVA showed significant differences between MMSE in AD patients and controls ($p < 0.001$). The mean score of AD patients in the Addenbrooke's Cognitive Examination — Revised (ACE-R) [20] was 84.4 (SD = 1.1). The mean school education trajectories in AD patients and controls were 15.2 (SD = 1.3) years and 15.1 (SD = 1.0) years respectively. A one-way ANOVA showed no significant differences between education of AD and control individuals.

Results

As shown in Fig. 21.3, the log mean gaze duration was significantly longer in AD patients than in controls, in both kinds of sentences. It is noteworthy that no significant decrease in gaze duration was observed for AD patients when reading highly predictable sentences ($t = -1.40$). This implies that, while controls, i.e., healthy subjects, were able to use the context sentences for predicting words, significantly reducing gaze duration, patients with mild AD had already lost this ability. Additionally, the significance of effect of word N − 1 on gaze duration was present neither in controls nor in AD patients when reading low and high-predictability sentences.

Next, we evaluated the effect on log gaze duration of the frequency of word N − 1, N, and N + 1 (See Fig. 21.4). Gaze duration decreased significantly with an increase in the frequency of word N − 1 when considering averaging over all predictors ($t = -5.87$), probably due to a partial processing of the word N in the previous fixation

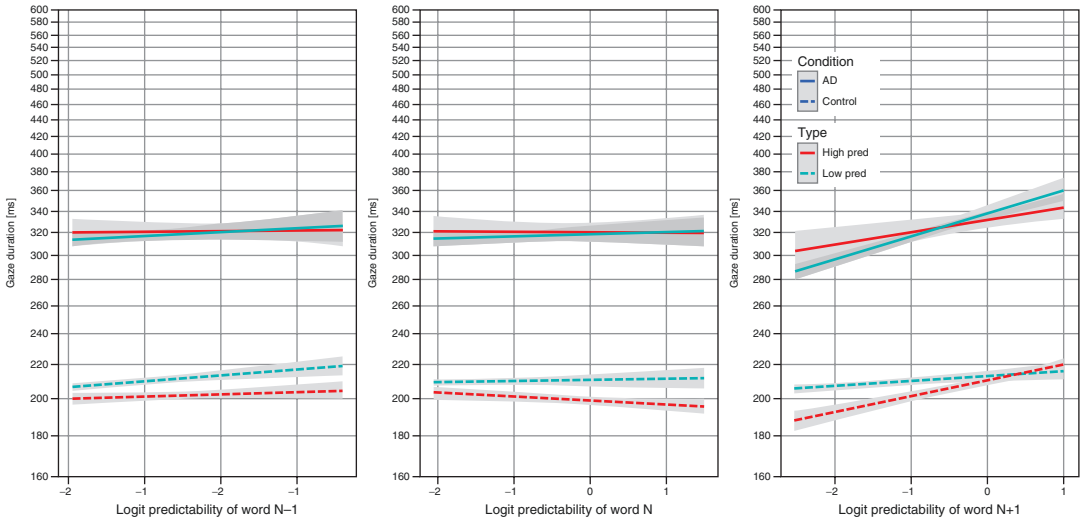


Fig. 21.3 Effects of the predictability of word N − 1 (*left*), word N (*center*), and word N + 1 (*right*) on gaze durations on word N, broken down by low-predictable sentences and high-predictable sentences, for controls and for AD. *Panels* reflect regression of gaze durations on word N on respective logits of predictability. *Shaded areas* are 95% confidence intervals. The log mean gaze duration was significantly longer in AD patients than in controls, both for sentences of low or high predictability. While controls, i.e., healthy subjects, were able to use the context sentences for predicting words, significantly reducing gaze duration, patients with mild AD had already lost this ability

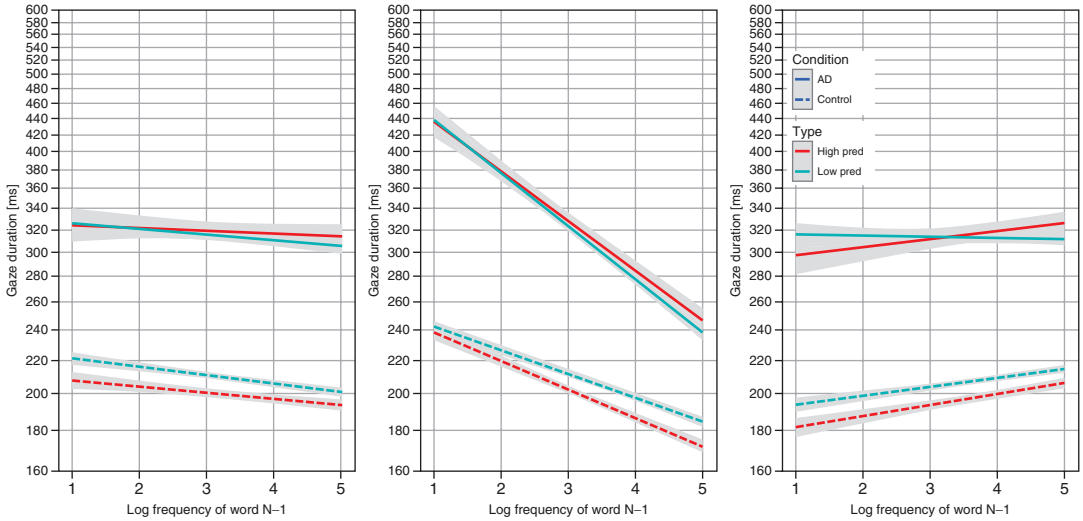


Fig. 21.4 Effects of the frequency of word N − 1 (*left*), word N (*center*), and word N + 1 (*right*) on gaze durations on word N, broken down by low-predictable sentences and high-predictable sentences, for controls and for AD. *Panels* reflect regression of gaze durations on word N on respective log of frequency. *Shaded areas* are 95% confidence intervals

(i.e., spillover). Similarly, gaze duration significantly decreased with an increased frequency of word N when considering averaging over all predictors ($t = -5.87$), This suggests that more frequent words require less processing, thus leading to shorter gaze durations, and that the ability to recognize these words is unaffected in AD patients, at least at this early stage of their

disease. The increased frequency of word N + 1 was not significant when considering averaging over all predictors ($t = -0.11$).

Eye Movements During Reading in Patients with Schizophrenia.

Little is known about the effect of schizophrenia on eye movement behavior during reading sentences with different contextual predictability (e.g., proverbs vs regular sentences). Some previous studies evidenced schizophrenia-related reading difficulties [21–24]. Abnormalities in both language and oculomotor control are well documented in individuals with schizophrenia [24–28]. However, fewer studies have examined the capacities through which linguistic material is processed when reading proverbs. The same example of the eye movements recorded during reading of two sentences, showing eye fixations of both controls and AD in Fig. 21.2 is repeated for control and SZ patients in Fig. 21.5. At first glance, it can be appreciated that the main difference between SZ and control is in regular (low predictable) sentences.

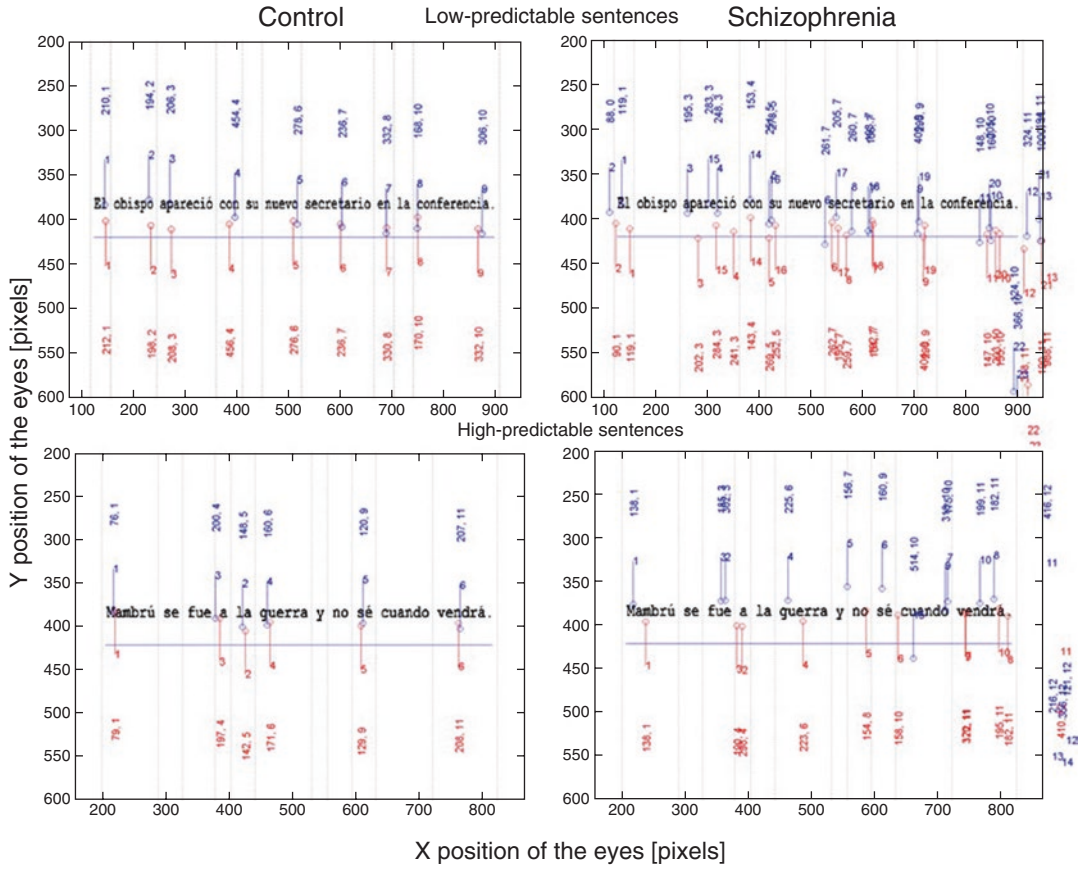


Fig. 21.5 Eye movements recording observed during reading low- and highly predictable sentences by a control subject (*left*) and an SZ patient (*right*). Fixation points for *right* (*red*) and *left* (*blue*) eyes are included in the graphs. The down and right movements signaled the end of reading; numbering linked to points indicates fixation sequences; fixation durations of each eye are listed with their corresponding colors. The number following fixation duration (after the comma) indexes the word number in the sentence. The same sentences as Fig. 21.2 are shown, to demonstrate the differences between AD and SZ in comparison with control. See the caption of Fig. 21.2 for sentence translation

In our earlier work [29], we evaluated the eye movement behavior in 40 healthy individuals and in 18 SZ during reading of our corpus of sentences, and analysed whether the fixation duration on the current word (word N) were influenced by the contextual predictability of the past word (word N − 1) and of the upcoming word (word N + 1). Our hypothesis was that in SZ patients a predictable-semantic context would enhance their reading performance and thereby reduce the difference between SZ and controls. To test this hypothesis, we evaluated the eye movements in control and SZ patients during reading of proverbs and regular sentences, and investigated whether an increase in the average predictability of the words affected gaze duration (i.e., the sum of consecutive forward fixations on a word) in the two groups. Our results showed that proverbs exerted its influence on gaze duration in SZ, suggesting that when a cue is present in the reading material SZ are capable of enhancing their working memory performance.

Participants

Eighteen outpatients (nine male, nine female) who met criteria for schizophrenia according to the *Diagnostic and Statistical Manual of Mental Disorders* [18] were tested. Diagnosis of schizophrenia was confirmed through the patient version of the Structured Clinical Interview for DSM–IV (SCID) Axis I disorders [30] and through chart review. Patients were clinically stable, with no change in medication dose for at least 4 weeks prior to testing. Current symptoms were rated using the Brief Psychiatric Rating Scale (BPRS) [31], with an average total score of 38.05 (±10.83). Inclusion criteria included estimated verbal IQ greater than 80 (based on the Vocabulary subtest of the Wechsler Adult Intelligence Scale–Revised [WAIS–R] [32]. Exclusion criteria included history of neurological impairment (other than schizophrenia), current substance abuse or history of substance dependence within 4 weeks prior to testing, current use of drugs that affect saccade velocities (e.g., benzodiazepines, chloral hydrate), and visual deficiencies (e.g., uncorrected deficits in visual acuity). Forty non-psychiatric controls (20 male, 20 female) were tested. Controls were matched to patients on gender, language background, age, and parental socioeconomic status (SES) based on parental occupation, ranked on an ordinal scale from 1 (*major professional*) to 9 (*unemployed*) using the Hollingshead Occupational Scale [33]. A one-way ANOVA showed no significant differences between controls and SZ in age (50.1 vs 48.7 years respectively) or parental SES (4.5 vs 4.7).

Results

As we expected, there was no reliable difference between controls and SZ readers for proverbs. The proverbs are known to the SZ patients, and therefore serve as a cue that allows for more efficient reading. The impact of current word predictability (word N) on the gaze duration exhibits an interesting pattern (See Fig. 21.6, central panel). Again, gaze duration differences between groups decreased with proverbs. The familiarity with proverbs allows these SZ readers to read efficiently with fixation durations similar to the ones of control readers: SZ appear to allocate more processing resources to the integration of known words as they are introduced in text.

With regard to the predictability of the upcoming word (word N + 1), the pattern is similar to the observed when processing current words, although upcoming word predictability exhibited a less pronounced trend on gaze duration (see the right-hand panel in Fig. 21.6). Our results reveal that effects tied to properties of the upcoming word may exert an influence on word processing, indicating whether an accurate representation of the sentence has already been achieved by relying on memory retrieval for the prediction of the upcoming word. The gaze duration in SZ during the reading of proverbs might reflect an effective use of memory and interpretation of words. Hence, external cues may neutralize SZ' deficiencies when the task is facilitated.

In summary, the main finding of our work shows that differences between controls and

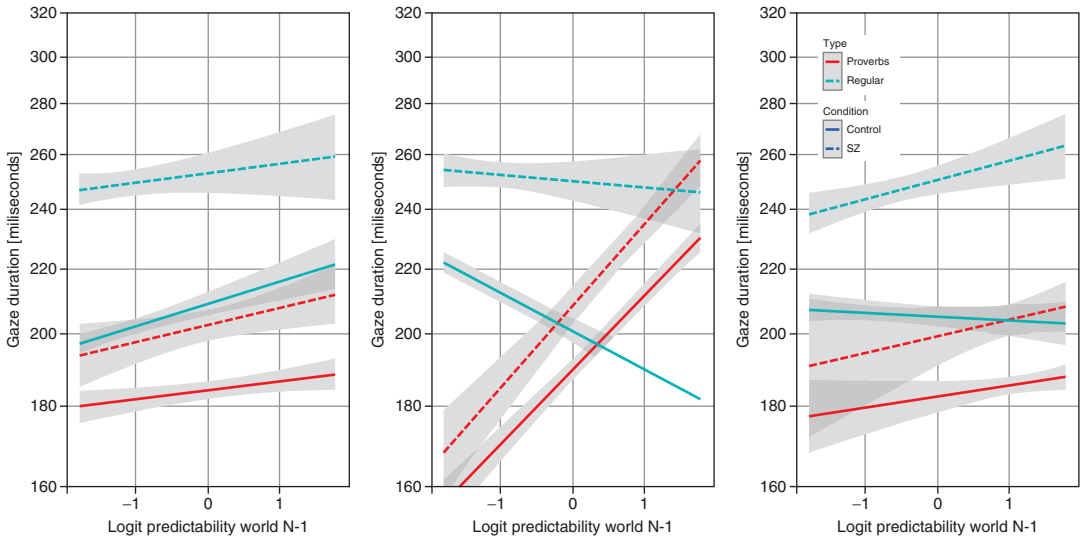


Fig. 21.6 Effect of the predictability of word N − 1 (*left*), word N (*center*) and word N + 1 (*right*) on gaze durations on word N, broken down by low-predictable sentences and high-predictable sentences for controls and SZ. *Panels* reflect regression of gaze durations on word N on respective logits of predictability. *Shaded areas* are 95% confidence intervals

SZ are only for regular sentences regarding to the gaze duration. This suggests that SZ might compensate for top-down processing and working memory deficiencies by using stored information due to familiarity with the proverbs. To our knowledge, this is the first study using word-based properties embedded in regular sentences and proverbs for analyzing working memory and semantic memory performance in SZ patients.

Conclusions

Our study provides a test-bed for initial research on cognitive impairments linked to semantic, working, and retrieval memory deficiencies. We were able to prove that deficits in the capacity for processing complex information are linked to memory-guided eye movements. Early detection and monitoring opportunities in AD patients will be improved by this test. Furthermore, the results obtained with this novel methodology could become in time a simple marker for early disease detection. In relation to SZ, the results of our study could assist in the clinical identification of specific groups of patients, and in the development of remedial procedures for use in rehabilitation programs.

The study procedure has a number of important issues to remark. First, it is an objective technique that makes it possible to achieve a precise numeric evaluation of different aspect of cognition impairment, making it possible to develop longitudinal studies. Detecting progressive cognitive impairment provides additional evidence that the individual has mild cognitive impairment (MCI) resulting from a neurodegenerative disease such as AD [34]. Second, it is a non-invasive technique (a high-speed camera is recording eye movements during silent reading) and patients are relaxed during the study. Thus, the results are not perturbed by stress. Third, the technology needed to develop the study is not expensive. It would be possible to implement population screening for early detection and diagnosis of pathologies that today are, in general, detected in later stages of their evolution.

Acknowledgements This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica from Argentina under grant PICT 2013 0403, the Universidad Nacional del Sur under grant PGI 24/K048, and CIC.

References

1. Buswell GT. The psychology of seeing. New York: Philosophical Library; 1935.
2. Yarbus AL. Eye movements and vision. New York: Plenum Press; 1967.
3. Rayner K. Eye movements in reading and information processing: 20 years of research. Psychol Bull. 1998;124(3):372–422.
4. Kliegl R, Nuthmann A, Engbert R. Tracking the mind during reading: the influence of past, present and future words on fixation durations. J Exp Psychol Gen. 2006;135:12–35.
5. Rayner K. Eye movements in reading: models and data. J Eye Mov Res. 2009;2(5):1–10.
6. Kliegl R. Toward a perceptual-span theory of distributed processing in reading: a reply to Rayner, Pollatsek, Drieghe, Slattery, and Reichle. J Exp Psychol. 2007;136:530–7.
7. Kennedy A, Pynte J. Parafoveal-on-foveal effects in normal reading. Vision Res. 2005;45(2):153–68.
8. Fernández G, Laubrock J, Mandolesi P, Colombo O, Agamennoni O. Registering eye movements during reading in Alzheimer's disease: difficulties in predicting upcoming words. J Clin Exp Neuropsychol. 2014;36:302–16.
9. Taylor W. Cloze procedure: a new tool for measuring readability. Journalism Q. 1953;30:415–33.
10. Fernández G, Shalom D, Kliegl R, Sigman M. Eye movements during reading proverbs and regular sentences: the incoming word predictability effect. Lang Cognit Process. 2013; doi:10.1080/01690065.2012.760745.
11. Sebastián-Gallés N, Martí M, Cuetos F, Carreiras M. LEXESP: Léxico informatizado del español. Barcelona: Ediciones de la Universidad de Barcelona; 1998.
12. Bates D, Maechler M. lme4: linear mixed-effect models using S4 classes R package version 0.995–2. Vienna: R Foundation for Statistical Computing; 2013.
13. Kennedy A, Pynte J, Murray W, Paul S. Frequency and predictability effects in the Dundee corpus. Q J Exp Psychol. 2013;66(3):601–18. doi:10.1080/17470218.2012.676054.
14. Fernández G, Manes F, Rotstein N, Colombo O, Mandolesi P, Politi L, Agamennoni O. Lack of contextual word predictability during reading in patients with mild Alzheimer disease. Neuropsychologia. 2014;62:143–51.
15. Frank RA, Galasko D, Hampel H, Hardy J, de Leon MJ, Mehta PD, Rogers J, Siemers E, Trojanowski JQ, National Institute on Aging Biological Markers Working Group. Biological markers for therapeutic trials in Alzheimer's disease. Proceedings of the biological markers working group; NIA initiative on neuroimaging in Alzheimer's disease. Neurobiol Aging. 2003;24(4):521–36.
16. Taler V, Phillips N. Language performance in Alzheimer's disease and mild cognitive impairment: a comparative review. J Clin Exp Neuropsychol. 2008;30(5):501–11.
17. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS–ADRDA work group under the auspices of department of health and human services task force on Alzheimer's disease. Neurology. 1984;34(7):939–44.
18. American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-IV. 4th ed. Washington, DC: American Psychiatric Association; 1994.
19. Folstein M, Folstein S, McHugh P. Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12:189–98.
20. Mioshi E, Dawson K, Mitchell J, Arnold R, Hodges JR. The Addenbrooke's Cognitive Examination Revised (ACE-R): a brief cognitive test battery for dementia screening. Int J Geriatr Psychiatry. 2006;21(11):1078–85.
21. Hayes RL, O'Grady BM. Do people with schizophrenia comprehend what they read? Schizophr Bull. 2003;29:499–507. http://dx.doi.org/10.1093/oxfordjournals.schbul.a007022
22. Revheim N, et al. Reading impairment and visual processing deficits in schizophrenia. Schizophr Res. 2006;87:238–45. http://dx.doi.org/10.1016/j.schres.2006.06.022
23. Arnott W, Sali L, Copland D. Impaired reading comprehension in schizophrenia: evidence for underlying phonological processing deficits. Psychiatry Res. 2011;187:6–10. http://dx.doi.org/10.1016/j.psychres.2010.11.025.
24. Whitford V, et al. Reading impairments in schizophrenia relate to individual differences in phonological processing and oculomotor control: evidence from a gaze contingentmovingwindowparadigm. J.Exp. Psychol Gen. 2013;152(1):57–75.
25. Kuperman V, Dambacher M, Nuthmann A, Kliegl R. The effect of word position on eye movements in sentence and paragraph reading. Q J Exp Psychol. 2010;63(9):1838–57.doi:10.1080/17470211003602412.
26. Kuperberg GR. Language in schizophrenia (part2): what can psycho-linguistics bring to the study of schizophrenia and viceversa? Lang. Linguist. Compass. 2010;4:590–604. http://dx.doi.org/10.1111/j.1749-818X.2010.00217.x.
27. Levy DL, et al. The genetic basis of thought disorder and language and communication disturbances in schizophrenia. J Neurolinguistics. 2010;23:176–92. http://dx.doi.org/10.1016/j.jneuroling.2009.08.003.
28. Li X, Branch CA, DeLisi LE. Language pathway abnormalities in schizophrenia: are view of fMRI and other imaging studies. Curr Opin Psychiatry. 2009;22:131–9. http://dx.doi.org/10.1097/YCO.0b013e328324bc43
29. Fernández G, Sapognikoff M, Guinjoan S, Orozco D, Agamennoni O. Word processing during reading sentences in patients with schizophrenia: evidences from

the eyetracking technique. Compr Psychiatry. 2016;68:193–200.
30. First MB, Spitzer RL, Gibbon M, Williams JBW. Structured clinical interview for DSM–IV axis I disorders. NewYork: NewYork State Psychiatric Institute; 1996.
31. Overall JE, Gorham DR. The brief psychiatric rating scale. Psychol Rep. 1962;10:799–812.
32. Wechsler D. Manual for the Wechsler adult intelligence scale—revised. New York: Psychological Corporation; 1981.
33. Hollingshead AB. Four-factor index of social status. Unpublished manuscript: Yale University; 1975.
34. Alberta MS, DeKosky ST, Dickson D, Duboise B, Feldman H, Fox N, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging–Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):270–9. doi:10.1016/j.jalz.2011.03.008.

Psychotropic Medication Use in the Elderly

22

Alicia del Carmen Panini, Mauricio Roberto Teves, Emiliano Giraudo, Marisa Hilda Garraza, and Claudia Patricia Calderón

Abstract

Aging is associated with progressive decrease in the function of multiple organs, presence of comorbidities, polypharmacy, and functional and social problems that may lead to inappropriate use of drugs. Consequently, the elderly are very vulnerable to display adverse reactions, interactions, hospitalizations, mortality, and poor adherence to treatment. The most serious side-effects in the elderly are caused by antiplatelet agents, diuretics, anticoagulants, and non-steroidal anti-inflammatory drugs; and within psychotropic drugs, benzodiazepines, antidepressants, and antipsychotic medications are of high risk. Anxiolytics produce excessive sedation, decreased alertness, confusion, ataxia, falls and hip fractures, increased mortality, and impairment of dementia symptoms. Antidepressants may lead to lack of response to treatment and anticholinergic effects. Antipsychotics may produce limited benefit and stroke. A deeply rooted cultural problem in the West is to see old age as synonymous of illness, the elderly being inevitably favorite targets of medicalization, particularly with psychotropic drugs. Problems of everyday life such as sadness or anxiety have become diseases that can be treated with anxiolytics or antidepressants. Psychotropic drugs relieve symptoms that produce certain diseases; if they are used for another purpose, this is considered inappropriate use. Health professionals should contribute by: reducing excessive and unnecessary consumption of psychotropic drugs, promoting their de-prescription,

A. del Carmen Panini • M.R. Teves • E. Giraudo
M.H. Garraza • C.P. Calderón (✉)
Pharmacology – Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, Chacabuco and Pedernera, 5700 San Luis, Argentina
e-mail: farmacialahuen@yahoo.com.ar; maurote55@yahoo.com.ar; emigiraudo@yahoo.com.ar; mhgarraza@gmail.com; cpcalderon2000@gmail.com; ccal@unsl.edu.ar

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_22

encouraging patients to face their difficulties without drugs, and searching for other solutions such as adoption of hygienic–sanitary measures, reducing risks to patient health, and reducing health costs. For these reasons, our objective is to describe some aspects related to the use of psychotropic drugs in the elderly, suggesting possible measures to improve it.

Keywords
Elderly • Psychotropic drugs • Polypharmacy • Adverse reactions • Interactions • Medicalization

Introduction

Older people are the main users of drugs and often suffer physiological and pathological changes related to age or to illness that affect the pharmacokinetics and pharmacodynamics of such drugs, such as impaired renal and hepatic function. Moreover, they have with more frequently multiple comorbidities, impaired functional capacity and cognitive decline. For these reasons, older people have a higher probability of presenting problems with drug use than younger adults, especially adverse drug reactions [1–3].

Two predominant aspects of geriatric health care are the high level of medication prescription and the rate of prescription of potentially inappropriate medications. Drugs with anticholinergic effects and benzodiazepines are included as inappropriate medications, and their use is related to cognitive and physical impairments in older adults [4–7]. Moreover, anticholinergic and sedative drug use is a predictor of the length of hospital stay [8, 9]. The elderly group presents a particular risk related to the consumption of psychotropic drugs because of their great vulnerability to drug interactions, consumption of various drugs, and the danger of abuse and dependence, which is associated most often with women [10]. The features and problems encountered in old age are summarized in Fig. 22.1.

High levels of potentially inappropriate prescribing of psychotropic medicines, in particular antipsychotics and sedatives, continue to be highlighted in Australia, especially in the oldest patients [11–15].

Older adults are especially vulnerable because they often have multiple disease states and require the use of more drugs, to which must be added factors such as the specific physiological changes of aging, genetic constitution, and diet, which can alter the response to drugs, increasing the predisposition to various adverse effects and drug interactions [16].

A study conducted in Italy shows that in nursing home residents, the high consumption of psychotropic drugs is of particular concern [17]. Meanwhile, another study in Sweden showed that the prevalence of medication use and polypharmacy among older adult women has increased dramatically; in particular, the use of analgesics increased significantly, whereas antipsychotics decreased [18].

Guidelines worldwide have cautioned against the use of antipsychotics as first-line agents to treat neuropsychiatric symptoms of dementia. Vasudev et al. [19] investigated the changes over time in the dispensing of antipsychotics and other psychotropic drugs among older adults with dementia living in long-term care facilities. They found that their use declined slightly over the study period, but atypical antipsychotics continued to be used at a high rate. The use of benzodiazepines declined, and the use of sedative and non-sedative antidepressants increased. Psychotropic polypharmacy continues to be highly prevalent in these patient samples.

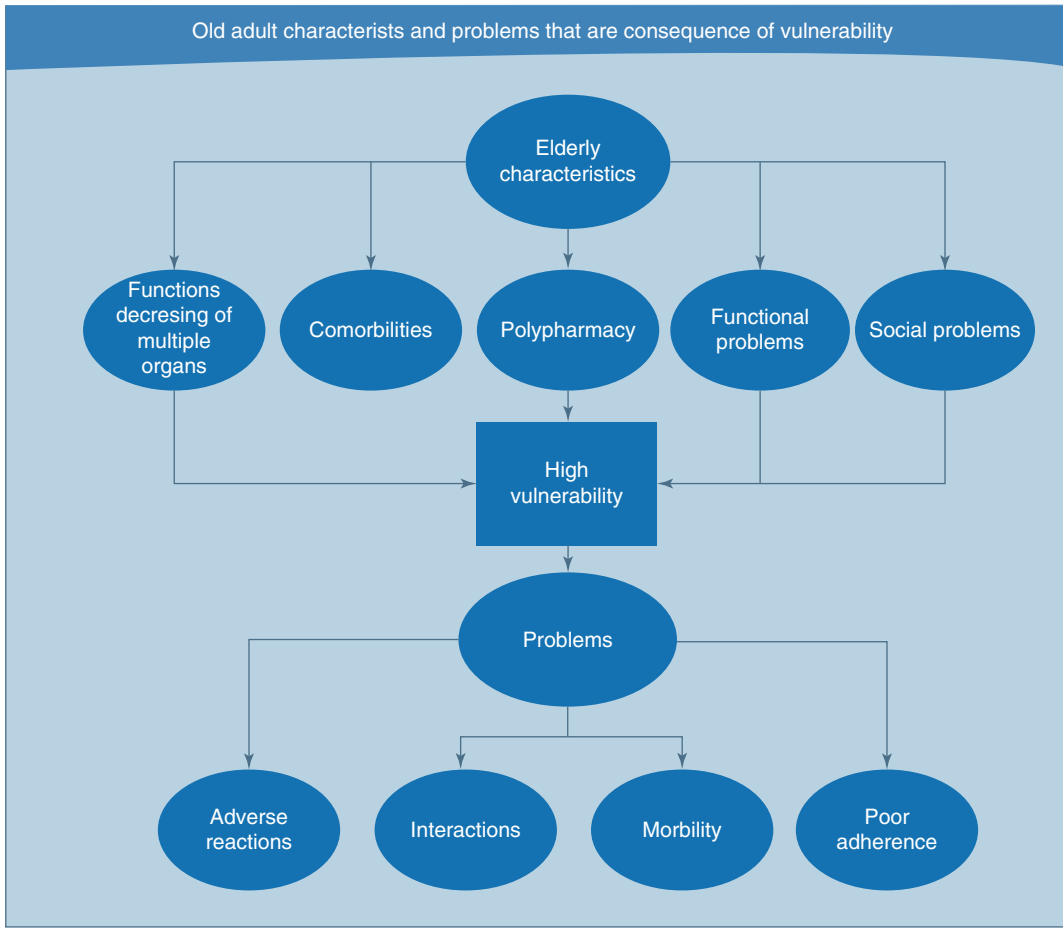


Fig. 22.1 Features and problems encountered in old age

Several clinical practice guidelines were published by French regulatory agencies between 2006 and 2009 to improve psychotropic drug use in older patients. Practice guidelines for psychotropic drug prescription were partially respected in older inpatients [20].

In Argentina, the most consumed psychoactive drugs are anxiolytics, antidepressants, and antipsychotics, which accounted for 75.3% of drugs for the central nervous system and 11.5% of all drugs dispensed in pharmacies in 2011; lorazepam, bromazepam, alprazolam, diazepam, clonazepam, and carbamazepine were the most dispensed [21]. On the other hand, in Buenos Aires City, 15.5% of the population currently consume some type of psychotropic drug, and 29.4% have consumed once in their life. The highest use is in women and the elderly population (12.2% of benzodiazepines and 3% of antidepressants). Finally, 25% of those who consume are considered to be highly dependent [22]. The reasons for increased consumption of psychotropic drugs are shown in Fig. 22.2.

The drugs used in the elderly are very complex, especially for those who use different types of psychotropic drugs. For these reasons, our objective is to describe some aspects related to the use of psychotropic drugs in the elderly, and to suggest possible measures to improve the situation.

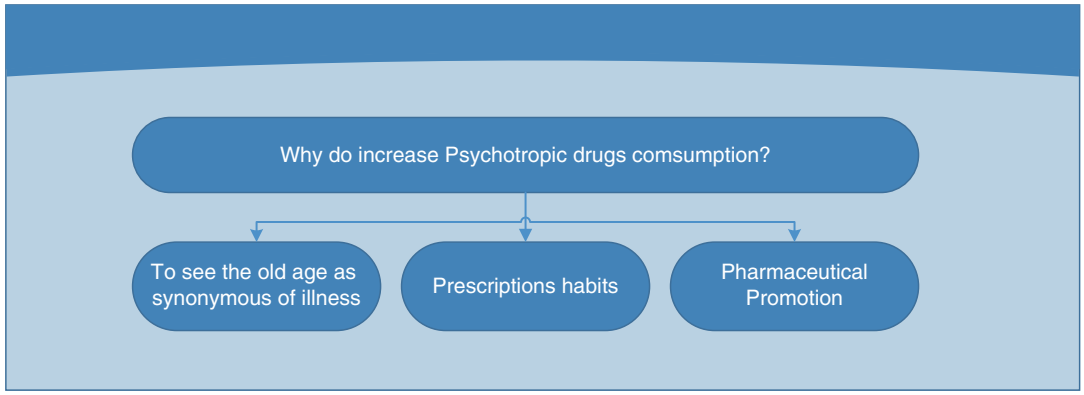


Fig. 22.2 The reason for increased consumption of psychotropic drugs

Psychotropic Drugs

A psychotropic medication is considered as one that has profound and beneficial effects on behavior, mood, and cognition, but often does not change the process of the underlying disease [23].

Psychotropic drugs are used therapeutically because they can affect the behavior and the subjective state of the person; however, there are other substances with a psychotropic action, such as alcohol, nicotine, cocaine, and heroin, which are characterized by social or addictive uses, and do not have any recognized therapeutic applications in Western medicine. Finally, painkillers and other drugs also have effects on subjective state and behavior, but are not considered as psychotropic drugs because their principal use is not related with their psychoactive effects [24].

Psychotropic is used to describe any natural or synthetic compound that is capable of influencing the psychic functions by its action on the central nervous system, and psychotropic drugs, as a pharmaceutical product composed of psychotropic substances, used with the objective of treating a mental or neurological condition [25, 21]. Psychoactive drugs are substances that are primarily used to treat three types of alterations: schizophrenia and other psychoses, depression and mania, and the conditions produced by anxiety or excessive anxiety.

The drugs for anxiety which are most known and used are benzodiazepines, which increase the actions of the neurotransmitter γ-aminobutyric acid (GABA) involved in anxiety and in the action of many anti-anxiety drugs [26].

Antidepressants can be selective inhibitors of serotonin reuptake, tricyclic or inhibitors of monoamine oxidase. Antidepressants temporarily increase neurotransmitters such as serotonin and norepinephrine, either by inhibiting their degradation, or by blocking their reuptake [27].

The first antipsychotics, known as first generation or typical, act by blocking dopamine D2 receptors. The atypical antipsychotics present some cognitive benefits, probably derived from their action on the cortical prefrontal function mediated by receptors that are not dopaminergic [28].

Clasification of Psycothropic Drugs

Psychotropic drugs can be classified from the following points of view: pharmacological, clinical, clinical–chemical, legal, industrial, and chemical–structural. De Wet [29] classified them as psycholeptics, psychoanaleptics, and generators of psychotic disorders. Later, Velasco and Alvarez [30] classified them as psycholeptics or depressants, psychoanaleptics or stimulants of the psychic activity, and psychodysleptics or hallucinogens [21].

Psychotropic drugs belong to the N group of the Anatomical–Therapeutic–Chemical classification system [31], and are specifically included within the N05 and N06 groups, corresponding to

the psycholeptics and psychoanaleptics respectively. In some countries, the opioid analgesics (N02a) are included, despite the fact that they are mainly used for pain treatment.

Psycholeptics have depressant effects on mental activity, and are commonly used to treat psychosis (antipsychotics) and anxiety disorders (anxiolytics). They include antipsychotics, anxiolytics, hypnotics, and sedatives.

In contrast, psychoanaleptics are stimulants of the central nervous system and mental activity, and are commonly used to treat depression and attention-deficit hyperactivity disorder. This group includes antidepressants, nootropics, drugs against dementia, and psychostimulants.

Medicalization, Conflicts of Interest, and Inappropiate Use of Psychodrugs

The way of understanding mental illness has changed. Now, some problems of adaptation to certain situations of everyday life, such as stress, insomnia, and depression, have become diseases to be treated with drugs. Drug use has been mainly extended to achieve personal well-being and social performance [32]. If psychotropic drugs are prescribed not for diseases, but for another kind of suffering, they are useless or act as placebos in most cases. This banal and excessive utilization is called medicalization of mental health, which is mainly caused by aggressive promotion by the pharmaceutical industry. The current globalization of health has transformed patients into real consumers of all types of health services. Of concern are the change of perception of health and disease, the increase in the misuse of the psychotropic drugs, and the adverse reactions that are produced by the inclusion of the elderly as target of medicalization [33].

A deeply rooted cultural problem in the West is to see old age as synonymous of illness, leading inevitably to the elderly becoming favorite targets of medicalization, particularly with psychotropic drugs. In this way, problems of everyday life such as sadness or anxiety have become diseases that can be treated with drugs such as anxiolytics or antidepressants.

The withdrawal reactions to second-generation antidepressant drugs (selective serotonin reuptake inhibitors, SSRIs) have started to be called discontinuation syndromes [34], a denomination that conveys a more harmless impression than the withdrawal reactions from other drugs such as benzodiazepines — despite the fact that discontinuation syndromes of SSRIs are nevertheless withdrawal reactions, and that benzodiazepines continue to be more effective and with fewer side-effects that the SSRIs [35]. Thus, SSRIs have replaced benzodiazepines in the treatment of anxiety disorders [36]. This change has probably been one of the more important successes of pharmaceutical promotion in psychiatry, with the full endorsement of guidelines and professional societies [37]. In this way, conflicts of interest have led us to "buy" the idea that SSRIs are better than anxiolytics for anxiety [38].

Aging and Comorbidities

Aging is associated with progressive decrease in the functions of multiple organs, presence of comorbidities, functional and social problems that lead to polypharmacy, and accidental or inappropriate use of drugs. Consequently, the elderly are very vulnerable to presenting adverse reactions, interactions, hospitalizations, mortality, and poor adherence to treatment.

At present, the elderly are the most rapidly growing population group in Western countries; and aging seldom comes alone, it is often accompanied by chronic diseases, comorbidities, disability, frailty, and social isolation. Multiple diseases inevitably lead to the use of multiple drugs, a condition known as polypharmacy [39–41].

The process of aging involves a continuum of changes in biological, functional, psychological, and social parameters that vary depending on genetic factors, age-related vulnerability, and differences in organ function and reserves [40–43].

Multimorbidity in the elderly has been estimated to range from 55 to 98% [44], and is highest in the very old, in women, and in individuals

belonging to low socioeconomic classes [44, 45]. Nobili et al. considered that over the last 20–30 years, problems related to aging, multimorbidity, and polypharmacy have become a prominent issue in global healthcare [43].

Adverse Drug Reactions

The most serious side-effects in the older age group are caused by antiplatelet agents, diuretics, non-steroidal anti-inflammatory drugs, and anticoagulants, among others. The prevalence of adverse reactions to drugs has risen over recent decades in older people [46–49].

Impaired physical functioning, delirium, and the increased risk of falls, cardiovascular events, and hospital admissions have been reported [50–55].

The 84% of war veterans hospitalized for hip fracture had been taking at least one medicine that could increase the risk of falls or fracture, such as antihypertensive drugs (63%), antidepressants (29%), benzodiazepines (26%), and opioids (19%) [3, 56].

Among the side-effects of benzodiazepines, we can mention sedation and the impairments in attention, memory, and psychomotricity. In addition, with the continued use that is still being observed, side-effects such as confusion, irritation, and agitation have increased significantly due to lower tolerance in the elderly. There is also the possibility of occurrence of depressive states induced by benzodiazepines. It is very important to note that although benzodiazepines are very safe and effective in the treatment of anxiety in the short term, if their use is extended by more than 6 months can lead to tolerance and dependence [57].

The oral atypical neuroleptics, such as risperidone, quetiapine or olanzapine bring an increased risk of hospitalization with acute kidney injury within the 90 days after they were prescribed in older patients. Likewise, hypotension, acute urinary retention, pneumonia, myocardial infarction, and ventricular arrhythmia are adverse effects that can lead to kidney injury and increase the risk of mortality [58].

The high usage rate of anxiolytics that has been found in patients with hip fractures may indicate that this is a risk factor for hip fractures related to falls in elderly patients [59].

Jacquin-Piques et al. [60] compared the type and number of psychotropic drugs prescribed in elderly nursing home residents with dementia with those in community-living patients. A significant association between living in a nursing home and the more frequent prescription of anxiolytics, hypnotics, and antipsychotics, with a greater number of psychotropic drug classes prescribed was found, whatever the severity of the dementia. Psychotropic drugs are frequently prescribed in nursing homes, despite the contrary was expected, because nursing home residents may have more and better psychosocial interventions than patients living at home.

An association between the incidence of diabetes mellitus and the use of antipsychotic therapy has been proven. The use of atypical antipsychotics has a stronger association than the use of typical antipsychotics. From all the antipsychotics, clozapine followed by olanzapine appear to be the atypical neuroleptics most closely related to metabolic syndrome and diabetes. This metabolic dysregulation appears to be multifactorial in origin, and the result of pharmacological, environmental, and genetic interactions [61].

Typical antipsychotics are associated with a higher incidence of extra-pyramidal dysfunctions such as tremor, parkinsonism, akathisia, neuroleptic malignant syndrome and tardive dyskinesia. They produce also a higher incidence of central anticholinergic effects such as poor attention, impaired memory, and behavioral problems. Furthermore, they can induce urinary retention, nausea, constipation, diarrhea, cholestasis, increase of transaminase enzyme activity, weight gain, and diabetes mellitus. In the case of patients suffering epilepsy, these drugs may facilitate convulsions. The main cardiovascular effects are the orthostatic hypotension that can increase the risk of falls, and possible arrhythmias.

Among the atypical antipsychotics, clozapine is associated with sedation, confusion, and agranulocytosis, with resultant requirement of regular

monitoring of blood counts. Risperidone induce extrapyramidal symptoms at low doses in older patients, particularly those with pre-existing dementia. The most frequent adverse effects of olanzapine are weight gain, hypotension, constipation, somnolence, and dizziness. The common side-effects of quetiapine include sedation, headache, and orthostatic hypotension [62].

Falls cause significant morbidity and mortality in old age, but happen at all stages of life. For this reason, two meta-analyses were performed to determine the relationship between psychopharmacological treatments and the risk of falls in people over 60 years. In both studies, the existence of an increased risk of falls associated with the use of antipsychotics, antidepressants, anxiolytics, sedatives, and benzodiazepines was found [63, 64]. Also, in this latest study, new associations between non-steroidal anti-inflammatory drugs, opioids, and risk of falls were identified. Among these psychotropic drugs, short- and long-acting benzodiazepines, analogues Z (zolpidem, zoplicone), antipsychotics, and antidepressants, alone or in combination with antihypertensive, anti-arrhythmic, and other cardiovascular medicines, have also been shown to facilitate falls [65–67].

A longitudinal study examined the dispensing of psychotropic medications in Australia from 2000 to 2011. A 58.2% increase in the dispensing of psychotropic drugs was found. The major increases were in antidepressants (95.3%), atypical antipsychotics (217.7%), and ADHD medications (72.9%). Valproate and lamotrigine also increased markedly. The anxiolytics and lithium remained unchanged, while sedatives and typical antipsychotics decreased by 26.4% and 61.2% respectively. Antidepressants accounted for 66.9% of total psychotropics, far greater than anxiolytics (11.4%), antipsychotics (7.3%), mood stabilizers (5.8%), sedatives (5.5%), or ADHD medications (3.0%) [68].

Psychotropic drugs, benzodiazepines, tricyclic antidepressants, and antipsychotic medications are frequently prescribed and are of high risk in older adults. Anxiolytics produce excessive sedation, decreased alertness, confusion, ataxia, falls, and hip fractures. Some symptoms of dementia, such as memory problems, may be worsened. Additionally, an inappropriate use of these drugs may increase mortality. Lack of response to treatment and anticholinergic effects are manifested with the antidepressants. Antipsychotics produce limited benefit, and risk of mortality and stroke.

Polypharmacy and Interactions

The prescription and use of multiple drugs to deal with multiple concomitant diseases is known as polypharmacy [69–71]. Moreover, the high prevalence of polypharmacy with aging may lead to an increased risk of inappropriate use of drugs, under-use of effective treatments, medication errors, poor adherence, different interactions, and adverse drug reactions [72–77]. The latter is related to the fact that elderly people are frailer and highly sensitive to pharmacotherapy, as a consequence of their changes in pharmacokinetic and pharmacodynamic parameters [78, 79]. Polypharmacy is an important risk factor for inappropriate medication prescribing, which is very frequent among elderly people [73, 80].

Moreover, polypharmacy is often the consequence of a prescribing cascade, which is caused by failure to recognize a new medical event as an adverse drug reaction [81, 82]. In this case, another drug is unnecessarily prescribed to treat the adverse event instead of withdrawing the responsible drug, creating a vicious circle and adding further risks [43].

In elderly people, polypharmacy has been associated with many adverse clinical outcomes, such as drug interactions and adverse drug reactions, disability and cognitive impairment, falls and fractures, malnutrition, hospitalization and institutionalization, mortality, and rising healthcare costs [73, 75, 83–94].

Polypharmacy in psychopharmacology is very important, although clinical guidelines advise otherwise. This is a result of increase of the primary drug, prevention of adverse reactions from the main medication, and treatment of comorbidities [95].

Drug–drug interactions may or may not be clinically significant. Clinically significant drug–drug interactions are events in which the pharmacodynamic or pharmacokinetic characteristics of a drug are modified by the aggregate of another drug, which can often increase serious adverse reactions or attenuate the efficacy of the first drug [96, 97].

Pharmacodynamic drug–drug interactions occur when concomitantly administered medications share similar target sites of action, producing either an additive or an antagonistic effect that can respectively enhance or weaken the physiologic effect of the primary drug [96, 98]. Clinically significant pharmacodynamic interactions can produce extrapyramidal symptoms, central nervous system depression, seizures, serotonin syndrome, and QT-interval prolongation [98, 99].

Pharmacokinetic drug–drug interactions are modifications of absorption, distribution, metabolism, and elimination of a drug by the addition of a second drug, increasing or decreasing the serum concentration of the primary drug; these interactions are often difficult to predict [100]. In these interactions, the drug absorption often changes as result of psysico-chemical changes in the primary drug, leading to decreased absorption [99, 101]. Additionally, transport of a large number of drugs across the intestinal wall are regulated by transporter proteins; principal among these is P-glycoprotein, which may play a significant role in determining blood concentrations and bioavailability of many drugs [99, 102]. Inhibition of P-glycoprotein by drugs such as verapamil result in decreased translocation of the drug back into the intestinal lumen, and a subsequent increase in systemic exposure of the drug, leading to a potential increase risk in adverse effects or enhanced efficacy [103].

Drug–drug interactions involving changes in drug distribution process a theoretical risk due to differences in protein affinity and displacement from the drug of its binding proteins [96]. While interactions involving drug displacement of their plasma-binding protein can result in elevation in plasma concentrations of the displaced drug, the clinical significance of these interactions is limited, since displacement from binding proteins results in an increase in unbound plasma concentrations, facilitating increased metabolism and clearance of the displaced drug [104].

Of the many interactions with psychotropic drugs, a minority are potentially hazardous. Most interactions are pharmacodynamic, resulting in augmented or antagonistic action at a receptor for different mechanisms in the same tissue. The most important pharmacokinetic interactions are due to effects on metabolism or renal excretion. The major enzymes involved belong to the cytochrome P450 system. Genetic variations in the CYP system produce poor, extensive or ultrarapid metabolisers. The most frequent hazardous interaction results from enzyme inhibition, but the probability of interaction depends on the initial levels of the enzyme activity and the availability of an alternative metabolic route of elimination of the drug. The most serious interactions with psychotropic drugs result in profound sedation, central nervous system toxicity, large changes in blood pressure, ventricular arrhythmias, an increased risk of dangerous side-effects, or a decrease in therapeutic effects of one of the interacting drugs [105].

The number of psychotropic drugs has expanded tremendously over the past few decades, with a proportional increase in drug–drug interactions. The majority of psychotropic agents are biotransformed by hepatic enzymes, which can lead to significant drug–drug interactions. Most drug–drug interactions of psychotropics occur at the metabolic level, involving the hepatic cytochrome P450 enzyme system [106].

Moreover, among the elderly population interaction between some SSRI antidepressants, especially fluvoxamine, and medication for the prevention of cardiovascular or cerebrovascular accidents, such as statins (atorvastatin, simvastatin or lovastatin), can produce effects adverse such as myopathy [107]. Antipsychotic drugs are used mostly for the treatment of behavioral and psychological symptoms in delirium cases. The increased mortality in elderly patients with dementia who receive antipsychotics suggests exercising caution before prescribing antipsychotics to treat delirium [10, 108].

Conclusions

Taking into account the findings exposed previously, some reflections and proposals to achieve an adequate use of psychotropic drugs in older people are presented in conclusion. The proposals are summarized in Table 22.1.

1. The risks associated with the inadequate use of groups of psychotropic drugs in this population group should be taken into account before prescribing. For this reason, it is necessary to improve the competence of physicians with regard to use and misuse of psychoactive drugs.
2. Health professionals should contribute to the reduction of prescription and excessive and unnecessary consumption of psychotropic drugs in older patients, promote de-prescription, encourage patients to face their difficulties without the help of medication, and search for other solutions, such as the adoption of hygienic–sanitary measures, reducing both health costs and risks to patient health.
3. The continuing and high use of benzodiazepines and the rapid increase in the consumption of serotonin/noradrenaline reuptake inhibitors, despite their side-effect profile, and the dramatic increase in antidepressant prescriptions, despite doubts about their efficacy in mild to moderate depression, are very worrying and require the attention of all members of the health system.
4. Several population-based studies have reported significant harm associated with drugs interactions in elderly patients. Increased awareness and interventions aimed at reducing exposure and minimizing the risks associated with potentially harmful drug combinations are necessary.

Table 22.1 Proposals

1. Improve competence with regard to use and misuse of psychoactive drugs
2. Promote deprescription
3. Encourage patients to face their difficulties without drugs
4. Adopt hygienic–sanitary measures to reduce health risks and costs
5. Form an interdisciplinary geriatric team.
6. Implement or improve pharmacist consultation and units of elderly care
7. Prevent drug–drug interactions, carefully selecting psychotropic medications, avoiding those with multiple targets, eliminating the administration of unnecessary medications, including over-the-counter medications.
8. Include drug–drug interactions as a possible differential diagnosis
9. Reduce or withdraw drugs to improve the quality and safety of pharmacological treatment in the elderly
10. Reduce exposure and minimize the risks associated with potentially harmful drug combinations
11. Contribute to decreasing both the continued use of benzodiazepines and the rapid increase of the consumption of serotonin noradrenaline reuptake inhibitors
12. Avoid drugs with narrow therapeutic window, non-linear pharmacokinetic, or long half-lives because they are prone to interactions
13. Administer new drugs at low doses and introduce slowly in patients with renal or hepatic impairment, confusion, or sedation
14. Use tables and software to detect the potential interactions of psychotropic drugs and in consequence improve patient outcomes
15. Know the mechanisms of action, adverse effects, and possible interactions of psychotropic drugs, with the aim of avoiding serious adverse reactions
16. Create lists of drugs, herbal products, and foods for each patient to detect and monitor interactions, and to reduce adverse reactions
17. Avoid the influence and pressures of the pharmaceutical industry, which increase the medicalization of the elderly population

5. Iatrogenic diseases in the elderly are very significant and frequent due to the addition of multiple factors, such as the increase in longevity and the proportion of elderly in the population, polypharmacy, multiple physicians, chronic diseases, hospitalizations, and all issues arising from medical or surgical procedures that increase the risks of iatrogenic disease in the elderly. The iatrogenic diseases can have significant psychomotor and social consequences. Some possible interventions to prevent these problems are: to form a geriatric interdisciplinary team, implement or improve pharmacist consultation and elderly care units, and implement well-planned measures for their best performance.
6. To prevent drug–drug interactions, it is very important to carefully select psychotropic medications, avoiding those with multiple targets, to eliminate the administration of unnecessary medications, including the over-the-counter medications. It is important to include drug–drug interactions as a possible differential diagnosis. It must be taken into account that some drugs are more prone to interactions due to their narrow therapeutic window, non-linear pharmacokinetic, or long half-lives. Patients with renal or hepatic impairment, confusion, or sedation are more sensitive to interactions. Therefore, the addition of new medications should be initiated at low doses and be administered slowly. Utilization of interaction tables and software may also be very useful. Clinicians can improve patient outcomes if the potential interactions of psychotropic drugs are considered and are monitored with concomitant medicines during the treatment.
7. Psychiatrists not only need a thorough knowledge of psychiatric disorders. They also need to know the mechanism of action of drugs, and the role of CYP450s in aiming to provide an optimal patient care. Psychiatrists have to identify possible interactions and in this way avoid serious adverse reactions.
8. Clinicians also must encourage patients to make and to present to the health service a list of all the prescribed or non-prescribed drugs, herbal products, and foods that they are receiving. The health service should detect potential drug–drug interactions, and monitor the existent interactions to reduce the adverse reactions and to improve life quality of patients.
9. Any intervention to reduce the total number of drugs and to withdraw psychotropic medication could improve the quality and safety of pharmacological treatment in the elderly population.
10. Although psychotropic drugs have a relevant role in the symptoms that cause certain mental diseases, these medicines present numerous problems related with adverse reactions, interactions, and inappropriate use. Moreover, the elderly have been and currently continue to be the main targets of medicalization with psychotropic drugs, drugs that are being used with other purposes than those of treating a disease. On the other hand, it should not be forgotten that much of this confusing and problematic situation in the use of psychodrugs in the elderly is the result of the strong commercial interests involved.

References

1. Elliott RA. Problems with medication use in the elderly: an Australian perspective. J Pharm Pract Res. 2006;36:58–66.
2. Shah BM, Hajjar ER. Polypharmacy, adverse drug reactions, and geriatric syndromes. Clin Geriatr Med. 2012;28:173–86.
3. Elliott RA, Booth JC. Problems with medicine use in older Australians: a review of recent literature. J Pharm Pract Res. 2014;44:258–71.
4. Landi F, Russo A, Liperoti R, Cesari M, Barillaro C, Pahor M, Bernabei R, Onder G. Anticholinergic drugs and physical function among frail elderly population. Clin Pharmacol Ther. 2007;81:235–41.
5. Cao YJ, Mager DE, Simonsick EM, Hilmer SN, Ling SM, Windham BG, Crentsil V, Yasar S, Fried LP, Abernethy DR. Physical and cognitive perfor-

mance and burden of anticholinergics, sedatives, and ACE inhibitors in older women. Clin Pharmacol Ther. 2008;83:422–9.
6. Fox C, Richardson K, Maidment ID, Savva GM, Matthews FE, Smithard D, Coulton S, Katona C, Boustani MA, Brayne C. Anticholinergic medication use and cognitive impairment in the older population: the Medical Research Council cognitive function and ageing study. J Am Geriatr Soc. 2011;59:1477–83.
7. Lampela P, Lavikainen P, Garcia-Horsman JA, Bell JS, Huupponen R, Hartikainen S. Anticholinergic drug use, serum anticholinergic activity, and adverse drug events among older people: a population-based study. Drugs Aging. 2013;30:321–30.
8. Lowry E, Woodman RJ, Soiza RL, Hilmer SN, Mangoni AA. Drug burden index, physical function, and adverse outcomes in older hospitalized patients. J Clin Pharmacol. 2012;52:1584–91.
9. Kersten H, Hvidsten LT, Gløersen G, Wyller TB, Wang-Hansen MS. Clinical impact of potentially inappropriate medications during hospitalization of acutely ill older patients with multimorbidity. Scand J Prim Health Care. 2015;33(4):243–51.
10. Montero Bancalero FJ. Considerations towards psychoactive drugs in professional and medical students in Spain and medical professionals in Mexico and Colombia. A comparative study (PhD Thesis). Department of Social Clinical Psychology and Experimental. University of Huelva (Spanish) [Internet]. 2014 [Cited 2016 Jan 7]; pp. 1–197. Available from: http://rabida.uhu.es/dspace/bitstream/handle/10272/8855/ Consideraciones_hacia_los_psicofarmacos.pdf?sequence=2.
11. Somers M, Rose E, Simmonds D, Whitelaw C, Calver J, Beer C. Quality use of medicines in residential aged care. Aust Fam Physician. 2010;39:413–6.
12. Basger BJ, Chen TF, Moles RJ. Application of a prescribing indicators tool to assist in identifying drug-related problems in a cohort of older Australians. Int J Pharm Pract. 2012;20:172–82.
13. Hollingworth SA, Siskind DJ. Anxiolytic, hypnotic and sedative medication use in Australia. Pharmacoepidemiol Drug Saf. 2010;19:280–8.
14. Hollingworth SA, Siskind DJ, Nissen LM, Robinson M, Hall WD. Patterns of antipsychotic medication use in Australia 2002–2007. Aust NZ J Psychiatry. 2010;44:372–7.
15. Blogg LC, Suzuki N, Roberts M, Clifford RM. Prescribing benzodiazepines in residential aged-care facilities. J Pharm Pract Res. 2012;42:287–90.
16. Spina E, Scordo MG. Clinically significant drug interactions with antidepressants in the elderly. Drugs Aging. 2002;19(4):299–320.
17. Pasina L, Marengoni A, Ghibelli S, Suardi F, Djade CD, Nobili A, Franchi C, Guerrini G. A multicomponent intervention to optimize psychotropic drug prescription in elderly nursing home residents: an Italian multicenter, prospective, Pilot Study. Drugs Aging. 2016;33(2):143–9.
18. Craftman ÅG, Johnell K, Fastbom J, Westerbotn M, von Strauss E. Time trends in 20 years of medication use in older adults: findings from three elderly cohorts in Stockholm, Sweden. Arch Gerontol Geriatr. 2016;63:28–35.
19. Vasudev A, Shariff SZ, Liu K, Burhan AM, Herrmann N, Leonard S, Mamdani M. Trends in psychotropic dispensing among older adults with dementia living in long-term care facilities: 2004–2013. Am J Geriatr Psychiatry. 2015;23(12):1259–69.
20. Etchepare F, Pambrun E, Bégaud B, Verdoux H, Tournier M. Compliance of psychotropic drug prescription with clinical practice guidelines in older inpatients. Fundam Clin Pharmacol. 2016;30(1):82–92.
21. Administrative Secretariat for Coordination and International Cooperation of the Secretariat of Programming for the Prevention of Drug Abuse and Drug Trafficking. A specific look at the problem of consumption of psychotropic drugs in Argentina 2012 (Spanish). [Internet] 2013. Available from: http://www.observatorio.gob.ar/especificos/ especificos-%20adicionales/Una%20mirada%20especifica%20sobre%20el%20 consumo%20de%20psicofarmacos%20en%20Argentina.2012.pdf.
22. Leiderman EA, Mugnolo JF, Bruscoli N, Massi J. Psychotropic medication consumption in the Buenos Aires general population 2006 (Spanish). Vertex. [Internet]. 2006 [Cited 2015 Dec 20];17(66):85–91. Available from: http://www.editorialpolemos.com.ar/ docs/vertex/vertex66.pdf.
23. Schatzberg AF, Cole JO, DeBattista C. Manual of clinical psychopharmacology. Washington, DC: American Psychiatric Pub; 2010. p. 1–720.
24. Spiegel R. Psychopharmacology: an introduction. Chichester: Wiley; 2003.
25. National Administration of Drugs, Food and Medical Technology (ANMAT). Definition of psychotropic drugs. Drug regulations (Spanish) [Internet]. 2010 [Cited 2016 Jan 24]. Available from: http://www.anmat.gov.ar/webanmat/Legislacion/Medicamentos/ Disposicion_ANMAT_885- 2010_Definiciones_Psicotropicos.PDF.
26. Stahl SM. Stahl's essential psychopharmacology: neuroscientific basis and practical applications. Cambridge: Cambridge University Press; 2008.
27. Pérez Álvarez M, González PH. The invention of mental disorders: listening to the drug or patient? (Spanish). Madrid: Alianza Editorial; 2007.
28. Stolerman IP. Encyclopedia of psychopharmacology, vol. 2. Berlin/Heidelberg: Springer; 2010. Available from: https://www.politicalavenue.com/PDF/Encyclopedia%20of%20PsychoPharmacology%20(2%20vols)%20-%20I.%20Stolerman%20(Springer,%202010)%20BBS.pdf
29. De Wet BS. Classification of psycho-active drugs. Paper presented at the 48th South African Medical Congress (MASA); 1971. Available from: http://journals.co.za/docserver/fulltext/m_samj/46/26/31813.pdf?expires=1489265554&id=id&accname=guest&checksum=5EEEF3A67355236C2C7BD11991228E1B

30. Velasco Martín A, Álvarez González FJ. Compendium of psychoneuropharmacology (Spanish). 1st Ed. Ed. Madrid: Díaz de Santos S.A. Ed.; 1988. p. 55. Available from: http://www.editdiazdesantos.com/libros/velasco-martin-alfonso-compendio-de-psico-neurofarmacologia-C01000860101.html#contenido.
31. World Health Organization Collaborating Centre for Drug Statistics Methodology. Anatomical Therapeutic Chemical (ATC) index with defined daily doses (DDDs). [Internet]. [Accessed 20 Jan 2016]. Available from: http://www.whocc.no/atc_ddd_index/
32. Argentine National Observatory on Drugs. Administrative Secretariat for Coordination and International Cooperation of the Secretariat of Programming for the Prevention of Drug Abuse and Drug Trafficking Misuse of psychotropic medications in everyday life. An exploratory study on social representations and usage patterns (Spanish); 2007. Available from: http://www.observatorio.gov.ar/investigaciones/MedicVidaCot.pdf.
33. Panini A, Garraza M, Teves M, Giraudo E, Calderón C. Chapter 15: Use of psychotropic drugs: between the medicalization and rationality. In: Pascual Ángel G, Humberto Luís Mesones A, editors. Neurosciences and psychiatry update. bridging the divide. Cham: Springer. p. 207–15.
34. Fava GA, Gatti A, Belaise C, Guidi J, Offidani E. Withdrawal symptoms after selective serotonin reuptake inhibitor discontinuation: a systematic review. Psychother Psychosom. 2015;84:72–81.
35. Offidani E, Guidi J, Tomba E, Fava GA. Efficacy and tolerability of benzodiazepines versus antidepressants in anxiety disorders: a systematic review and meta-analysis. Psychother Psychosom. 2013;82: 355–62.
36. Chouinard G, Chouinard V-A. New classification of selective serotonin reuptake inhibitor withdrawal. Psychother Psychosom. 2015;84:63–71.
37. Starcevic V, Brakoulias V, Viswasam K, Berle D. Inconsistent portrayal of medication dependence, withdrawal and discontinuation symptoms in treatment guidelines for anxiety disorders. Psychother Psychosom. 2015;84:379–80.
38. Fava GA. The hidden costs of financial conflicts of interest in medicine. Psychother Psychosom. 2016;85:65–70.
39. Christensen K, Doblhammer G, Rau R, Vaupel JW. Ageing populations: the challenges ahead. Lancet. 2009;374(9696):1196–2008.
40. Kinsella K, Phillips DR. Global aging: the challenge of success. Popul Bull. 2005;60:3–42.
41. Aalami OO, Song HM, Fang TD, Nacamuli RP. Physiological features of aging persons. Arch Surg. 2003;138(10):1068–76.
42. McLean AJ, Le Couteur DG. Aging biology and geriatric clinical pharmacology. Pharmacol Rev. 2004;56(2):163–84.
43. Nobili A, Garattini S, Mannuccio MP. Review: multiple diseases and polypharmacy in the elderly: challenges for the internist of the third millennium. J Comorbidity. 2011;1:28–44.
44. Marengoni A, Angleman S, Melis R, Mangialasche F, Karp A, Garmen A, et al. Aging with multimorbidity: a systematic review of the literature. Ageing Res Rev. 2011;10(4):430–9.
45. Sousa RM, Cleusa CP, Acosta D, Albanese E, Guerra M, Huang Y, et al. Contribution of chronic diseases to disability in elderly people in countries with low and middle incomes: a 10/66 Dementia Research Group population-based survey. Lancet. 2009;374(9794):1821–30.
46. Zhang M, Holman CDAJ, Preen DB, Brameld K. Repeat adverse drug reactions causing hospitalization in older Australians: a population-based longitudinal study 1980–2003. Br J Clin Pharmacol. 2007;63:163–70.
47. Pit SW, Byles JE, Cockburn J. Prevalence of self-reported risk factors for medication misadventure among older people in general practice. J Eval Clin Pract. 2008;14:203–8.
48. Miller GC, Britth HC, Valenti L. Adverse drug events in general practice patients in Australia. Med J Aust. 2006;184:321–4.
49. Vuong T, Marriott JL, Kong DCM, Siderov J. Implementation of a community liaison pharmacy service: a randomised controlled trial. Int J Pharm Pract. 2008;16:127–35.
50. Gnjidic D, Cumming RG, Le Couteur DG, Handelsman DJ, Naganathan V, Abernethy DR, et al. Drug burden index and physical function in older Australian men. Br J Clin Pharmacol. 2009;68:97–105.
51. Gnjidic D, Hilmer SN, Blyth FM, Naganathan V, Cumming RG, Handelsman DJ, et al. High-risk prescribing and incidence of frailty among older community-dwelling men. Clin Pharmacol Ther. 2012;91:521–8.
52. Wilson NM, Hilmer SN, March LM, Cameron ID, Lord SR, Seibel MJ, et al. Associations between drug burden index and physical function in older people in residential aged care facilities. Age Ageing. 2010;39:503–7.
53. Wilson NM, Hilmer SN, March LM, Cameron ID, Lord SR, Seibel MJ, et al. Associations between drug burden index and falls in older people in residential aged care. J Am Geriatr Soc. 2011;59:875–80.
54. Best O, Gnjidic D, Hilmer SN, Naganathan V, McLachlan AJ. Investigating polypharmacy and drug burden index in hospitalized older people. Intern Med J. 2013;43:912–8.
55. Beer C, Hyde Z, Almeida OP, Norman P, Hankey GJ, Yeap BB, et al. Quality use of medicines and health outcomes among a cohort of community dwelling older men: an observational. Br J Clin Pharmacol. 2011;71:592–9.
56. Leach M, Pratt N, Roughead EE. Medicine use among older Australians before and after hip fracture. J Pharm Pract Res. 2013;43:265–8.
57. Preston JD, O'Neal JH, Talaga MC. Handbook of clinical psychopharmacology for therapists. Oakland: New Harbinger Publications; 2013.
58. Atypical neuroleptics in elderly patients: acute kidney injury. Prescrire Int. 2015;165:267.

59. Aizenberg D, Weizman A, Weiss A, Akopian M, Amitai M, Beloosesky Y. The association in elderly hospitalized patients, between psychotropic drugs and hip fractures resulting from falls. Exp Aging Res. 2015;41(5):546–55.
60. Jacquin-Piques A, Sacco G, Tavassoli N, Rouaud O, Bejot Y, Giroud M, Robert P, Vellas B, Bonin-Guillaume S. Psychotropic drug prescription in patients with dementia: nursing home residents versus patients living at home. J Alzheimers Dis. 2015;49(3):671–80.
61. Jesus C, Jesus I, Agius M. What evidence is there to show which antipsychotics are more diabetogenic than others? Psychiatr Danub. 2015;27(Suppl 1):S423–8.
62. Varma S, Sareen H, Trivedi JK. The geriatric population and psychiatric medication. Mens Sana Monogr. 2010;8(1):30–51.
63. Woolcott JC, Richardson KJ, Wiens MO, Patel B, Marin J, Khan KM, Marra CA. Meta-analysis of the impact of 9 medication classes on falls in elderly persons. Arch Intern Med. 2009;169(21):1952–60.
64. Bloch F, Thibaud M, Dugue B, et al. Psychotropic drugs and falls in the elderly people: updated literature review and meta-analysis. J Aging Health. 2011;23(2):329–46.
65. Hill KD, Wee R. Psychotropic drug-induced falls in older people. A review of interventions aimed at reducing the problem. Drugs Aging. 2012;29:15–30.
66. Tinetti ME. Preventing falls in elderly persons. N Engl J Med. 2003;341:42–9.
67. Multiple causes of falls and the role of drugs. Drug therapy information Bulletin of Navarra (Spanish) [Internet]. 2013 [cited 2016 Feb 1];21(2). Available from: http://www.navarra.es/NR/rdonlyres/61C2FB0A-1915-414B-AC23-E60E6A172E87/ 261 957/Bit_v21n3.pdf.
68. Stephenson CP, Karanges E, McGregor IS. Trends in the utilization of psychotropic medications in Australia from 2000 to 2011. Aust N Z J Psychiatry. 2013;47(1):74–87.
69. Fulton MM, Allen ER. Polypharmacy in elderly: a literature review. J Am Acad Nurse Pract. 2005;17(4):123–32.
70. Hajjar ER, Cafiero AC, Hanlon JT. Polypharmacy in elderly patients. Am J Geriatr Pharmacother. 2007;5(4):345–51.
71. Veehof LJG, Meyboom-de Jong B, Haaijer-Raskamp FM. Polypharmacy in the elderly – a literature review. Eur J Gen Pract. 2000;6(7):98–106.
72. Bressler R, Bahal JJ. Principles of drug therapy for elderly patients. Mayo Clin Proc. 2003;78(12):1564–77.
73. Mangoni AA, Jackson SHD. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. Br J Clin Pharmacol. 2004;57(1):6–14.
74. Corsonello A, Pedone C, Corica F, Mussi C, Carbonin P, Antonelli Incalzi R, et al. Concealed renal insufficiency and adverse drug reactions in elderly hospitalized patients. Arch Intern Med. 2005;165(7):790–5.
75. Gurwitz JH. Polypharmacy: a new paradigm for quality drug therapy in the elderly? Arch Intern Med. 2004;164(18):1957–9.
76. Steinman MA, Landefeld CS, Rosenthal GE, Berthenthal D, Sen S, Kaboli PJ. Polypharmacy and prescribing quality in older people. J Am Geriatr Soc. 2006;54(10):1516–23.
77. Hilmer SN, Gnjidic D. The effects of polypharmacy in older adults. Clin Pharmacol Ther. 2009;85(1):86–98.
78. Kuijpers MA, van Marum RJ, Egberts AC, Jansen PA. Relationship between polypharmacy and underprescribing. Br J Clin Pharmacol. 2008;65(1):130–3.
79. Simonson W, Feinberg JL. Medication-related problems in the elderly. Defining the issues and identifying solutions. Drugs Aging. 2005;22(7):559–69.
80. Linjakumpu T, Hartikainen S, Klaukka T, Veijola J, Kivelä SL, Isoaho R. Use of medications and polypharmacy are increasing among the elderly. J Clin Epidemiol. 2002;55(8):809–17.
81. Rochon PA, Gurwitz JH. Optimising drug treatment for elderly people: the prescribing cascade. Br Med J. 1997;315(7115):1096–9.
82. Laroche ML, Charmes JP, Nouaille Y, Picard N, Merle L. Is inappropriate medication use a major cause of adverse drug reactions in the elderly? Br J Clin Pharmacol. 2007;63(2):177–86.
83. Spinewine A, Schmader KE, Barber N, Hughes C, Lapane KL, Swine C, et al. Appropriate prescribing in elderly people: how well can it be measured and optimised? Lancet. 2007;370(9582):173–84.
84. Crentsil V, Ricks MO, Xue QL, Fried LP. A pharmacoepidemiologic study of community-dwelling, disabled older women: factors associated with medication use. Am J Geriatr Pharmacother. 2010;8(3):215–24.
85. Page 2nd RL, Ruscin JM. The risk of adverse drug events and hospital-related morbidity and mortality among older adults with potentially inappropriate medication use. Am J Geriatr Pharmacother. 2006;4(4):297–305.
86. Hanlon JT, Pieper CF, Hajjar ER, Sloane RJ, Lindblad CI, Ruby CM, et al. Incidence and predictors of all and preventable adverse drug reactions in frail elderly persons after hospital stay. J Gerontol A Biol Sci Med Sci. 2006;61(5):511–5.
87. Onder G, Pedone C, Landi F, Cesari M, Della Vedova C, Bernabei R, et al. Adverse drug reactions as cause of hospital admissions: results from the Italian Group of Pharmacoepidemiology in the Elderly (GIFA). J Am Geriatr Soc. 2002;50(12):1962–8.
88. Lai SW, Liao KF, Liao CC, Muo CH, Liu CS, Sung FC, et al. Polypharmacy correlates with increased risk for hip fracture in the elderly: a population-based study. Medicine (Baltimore). 2010;89(5):295–9.
89. Ziere G, Dieleman JP, Hofman A, Pols HA, van der Cammen TJ, Stricker BH. Polypharmacy and falls in the middle age and elderly population. Br J Clin Pharmacol. 2006;61(2):218–23.

90. Boyle N, Naganathan V, Cumming RG. Medication and falls: risk and optimization. Clin Geriatr Med. 2010;26(4):583–605.
91. Haider SI, Johnell K, Thorslund M, Fastbom J. Trends in polypharmacy and potential drug–drug interactions across educational groups in elderly patients in Sweden for the period 1992-2002. Int J Clin Pharmacol Ther. 2007;45(12):643–53.
92. Nobili A, Pasina L, Tettamanti M, Lucca U, Riva E, Marzona I, et al. Potentially severe drug interactions in elderly outpatients: results of an observational study of an administrative database. J Clin Pharm Ther. 2009;34(4):337–86.
93. Koh Y, Kutty FBM, Li SC. Drug-related problems in hospitalized patients on polypharmacy: the influence of age and gender. Ther Clin Risk Manag. 2005;1(1):39–48.
94. Jyrkka J, Enlund H, Korhonen MJ, Sulkava R, Hartikainen S. Polypharmacy status as an indicator of mortality in an elderly population. Drugs Aging. 2009;26(12):1039–48.
95. Sarkar P, Chakraborty K, Misra A, Shukla R, Swain SP. Pattern of psychotropic prescription in a tertiary care center: a critical analysis. Indian J Pharmacol. 2013;45(3):270–3.
96. Ereshefsky L. Drug–drug interactions with the use of psychotropic medications. Interview by Diane M. Sloan. CNS Spectr. 2009;14:1–8.
97. Johnson JA, Bootman JL. Drug-related morbidity and mortality and the economic impact of pharmaceutical care. Am J Health Syst Pharm. 1997;54(5):554–8.
98. Yap KY, Tay WL, Chui WK, Chan A. Clinically relevant drug interactions between anticancer drugs and psychotropic agents. Eur J Cancer Care (Engl). 2011;20:6–32.
99. Schellander R, Donnerer J. Antidepressants: clinically relevant drug interactions to be considered. Pharmacology. 2010;86:203–15.
100. Strain JJ, Chiu NM, Sultana K, Karim A, Caliendo G, Mustafa S. Psychotropic drug versus psychotropic drug-update. Gen Hosp Psychiatry. 2004;26:87–105.
101. Baxter K. Stockley's drug interactions. 9. London: Pharmaceutical Press; 2010.
102. Zolk O, Fromm MF. Transporter-mediated drug uptake and efflux: important determinants of adverse drug reactions. Clin Pharmacol Ther. 2011;89:798–805.
103. Muller F, Fromm MF. Transporter-mediated drug-drug interactions. Pharmacogenomics. 2011;12:1017–37.
104. Sansom LN, Evans AM. What is the true clinical significance of plasma protein binding displacement interactions? Drug Saf. 1995;12:227–33.
105. Chadwick B, Waller DG, Edwards JG. Potentially hazardous drug interactions with psychotropics. Adv Psychiatr Treat [Internet]. 2005 [cited 2016 Jan 16];11(6):440–49. Available from: www.apt.rcpsych.org/content/11/6/440.
106. Madhusoodanan S, Velama U, Parmar J, Goia D, Brenner R. A current review of cytochrome P45 interactions of psychotropic drugs. Ann Clin Psychiatry. 2014;26(2):120–38.
107. Andrade C. Selective serotonin reuptake inhibitor drug interactions in patients receiving statins. J Clin Psychiatry. 2014;75(2):e95–9.
108. Drach LM. Psychopharmalogical treatment of delirium in the elderly (German). Med Monatsschr Pharm. 2014;37(4):124–31. quiz 133–124.

Part III

Neurosciences, Learning, Teaching and the Role of Social Environment

23

Child Cognitive Stimulation Programs: Various Modalities of Intervention in Socially Vulnerable Contexts

Celina Korzeniowski and Mirta Susana Ison

Abstract

The past two decades have seen increasing interest in the design and implementation of intervention programs aimed at stimulating cognitive control capacities in children growing up in disadvantaged socioeconomic conditions, based on numerous studies that have reported a negative impact of poverty on child development. This chapter reviews the scope, limitations, and methodological challenges of the various modalities of intervention aimed at strengthening such capacities in children at social risk: cognitive training, computerized games, curriculum adaptations, and parent and teacher training. In addition, it presents two cognitive stimulation programs that were adapted to the school curriculum and whose goal was to promote executive functions (EFs) in Argentine children, with a view to improving their school competence. One of the interventions had a brief duration (15 sessions) and was carried out with 90 schoolchildren aged 9–12. The other one was longer (30 sessions) and involved 178 children aged 6–10. The results indicated that both experiences were effective in increasing EF in schoolchildren, with an associated improvement of their reading, writing, and calculating competences. However, the improvement varied in intensity and scope depending on the duration of the intervention and the age of the children. It is concluded that

C. Korzeniowski • M.S. Ison (✉)
Human, Social and Environmental Science Institute of the National Scientific and Technological Scientific Centre (INCIHUSA-CONICET- CCT Mendoza), Mendoza, Argentina

Research Institute of the School of Psychology, Aconcagua University, Mendoza, Argentina

School of Psychology, Aconcagua University, Mendoza, Argentina
e-mail: mison@mendoza-conicet.gob.ar; mison@uda.edu.ar

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_23

implementing ecological interventions that enrich the everyday practices of children at social risk is a way to narrow the persisting academic, economic, and socio-cultural gap associated with poverty conditions.

Keywords
Executive functions • Cognitive stimulation • Intervention modality • Poverty • School performance

Rationale of Neurocognitive Interventions

Executive functions (EFs) describe a set of high-order cognitive abilities that monitor and regulate the behavior, emotions, and cognitions required to attain goals and to solve problems [1]. These functions have a hierarchical relationship to more elementary cognitive processes, such as perception and memory, and control and supervise their functioning. As a result, goals can be attained by selecting actions and thoughts that go beyond information and integrate that information temporarily [2]. Executive functions involve cognitive control processes, such as planning, decision-making, keeping information in memory and managing that information, inhibiting undesirable thoughts, feelings, and actions, and shifting flexibly from one task to another [3].

Recent research studies agree that the cognitive control process is supported by neuroanatomical observations about the hierarchical organization of the human cortex, where an integration zone in prefrontal areas receives afferences from other portions of the nervous system, and sends control information to the posterior cortex and subcortex [4]. Therefore, EFs involve the joint participation of different cortical regions, and limbic and basal structures that work dynamically and in coordination over the prefrontal cortex, shaping a whole set of interconnected neural networks [5].

EFs develop from a delicate and continuous interaction between brain maturation and environmental influences from early childhood into adulthood [6, 7]. The literature reports several sensitive periods throughout EF evolution, that is, time windows when brain plasticity increases and becomes more vulnerable to the influence of experiences [8, 9].

To have a successful school life and to build positive social relations, a child needs cognitive control abilities to not yield to temptation and to avoid inappropriate behavior, to keep him/herself focused, to inhibit distractors, to process information, to identify new connections between elements, to think creatively, to recognize different perspectives, and to find solutions to new problems. In summary, the suitable development of self-regulating capabilities plays a significant role in how a child learns and is also a predictor of future achievements. Longitudinal studies suggest that self-regulating, flexible, and creative behavior during childhood is associated with improved health, better academic achievements, a higher employment status, and a lower incidence of disruptive social behavior, addictions, conduct disorders, and criminal behavior in adulthood [10].

Based on these studies, interest in the design and implementation of intervention programs targeted toward stimulating EFs in children with and without disorders has grown in the past 20 years [11]. Along these lines, stimulating cognitive abilities in children who grow in adverse socioeconomic conditions has been recently brought to focus [12–14], as a result of extensive supporting research into the negative impact of poverty on child development [15–19].

In connection with EFs, children from deprived areas have been reported to underperform in attention, working memory, cognitive flexibility, planning, oral fluency, and problem-solving abilities [15–19]. Similarly, poverty modulators have been identified, such as overcrowding, stress, poor housing conditions, parents with a low level of education, impoverished language interactions between parents and children, and lack of cognitive stimulation at home [15, 17, 19].

This research work has provided grounds to design targeted and effective interventions. Empirical evidence underlines that cognitive control abilities are malleable, and can be trained and even improved by suitable experiences [12–14, 20]. It has also been reported that interventions promote a reorganization of the neural networks that support the functioning of these abilities [21, 22].

In summary, the effects of poverty on neurocognitive development are reversible. The plasticity of the child's brain is the key element for enrichment, inasmuch as it creates different sensitive periods in which suitable and continuing cognitive stimulation can generate benefits.

Brain Plasticity

The human brain has the dynamic capacity to change its structure and the way it functions throughout a lifetime. This capacity to change is closely linked to the human being's ability to adapt and survive and can be observed in different vital situations, from the spontaneous functional recovery of the brain after a lesion, to the neural networks' reorganization, to sculpting processes that result from learning [23].

Electron microscopic studies of synapses and neuroimages of the grey and white matters of the human brain reveal that postnatal brain development extends over time, is organized into a hierarchical progression, and presents considerable regional variability [24, 25]. Extended development and the pruning of synaptic connections are deemed to be the main mechanisms that shape brain plasticity. However, an equally important additional factor has recently been identified: the existence of polymorphisms in some human genes that take part in the genetic modification associated to environmental conditions [26, 27].

At present, there is a field of neuroscience in constant development that intends to clarify how cognitive stimulation variations, frequently associated to socioeconomic fluctuations, affect the development and efficiency of the neural networks involved in cognitive functioning [28, 29]. Considerable insight has been gained from research with animal and human models to understand the vulnerability of the nervous system in adverse settings and how it reorganizes when intervention strategies are deployed.

Since 1950, studies with animal models have shown how synaptic connections can be enhanced by a stimulating environment. Animal breeding in settings with higher sensory, cognitive, and motor stimuli reflect an increased expression of the cell signs involved in synapse formation and proliferation. These settings enable dendritic growth, and increase gliogenesis and synaptic density, especially in the hippocampus and cortex, while promoting the integration of new neurons into existing functional circuits [30, 31]. These changes are associated with improved performance in learning and memory tasks [30, 32].

Research in humans has identified that impoverished environments affect cognitive development through different paths, one of them being stress. It has been reported that prenatal stress increases the risk of abnormal fetal growth and premature birth, while altering neurodevelopment (i.e., reduced hippocampus volume). This has been related to a higher incidence of mental disorders, poor school performance, inattention, behavioral problems, and a lower IQ during childhood [21, 33–35].

In the post-natal stage, stress has been identified as having a negative impact on infant development by decreasing parents' sensitivity and care. Upbringing by parents who are irritable or have depression or anxiety is impaired by an increased frequency of punitive, inconsistent, and negligent conducts [36, 37]. This negative parenting behavior leads to an insecure attachment by children to caregivers, which brings about emotional problems, behavior disorders, and poor cognitive and school performance during childhood [13]. Conversely, good parenting is an excellent protection factor in impoverished and stressful environments, and is associated with a higher resilient capacity and better cognitive performance in children [37].

Research by Posner [22, 38] supports the above, as it suggests that attentional systems seem to be epigenetically modulated by environmental factors and the individual's own experience. His studies show that children who are favored by higher quality parenting and certain

genetic traits (haplotype) perform executive attention tasks more efficiently and have a better self-regulating ability than children who do not meet these conditions [22, 38].

In brief, a study of the interaction between brain maturity and the characteristics of development contexts has identified certain cognitive processes as potential targets for intervention, based on their higher vulnerability to modulation by socio-environmental factors and their role in infant development. At present, research on brain plasticity is rising to a new challenge: to move forward in the study of brain plasticity mechanisms by analyzing the impact of intervention programs and cognitive training.

Modalities of Intervention Programs

Cognitive neuroscience has designed and implemented a series of intervention modalities intended to train cognitive control functions across different child populations. In general, implemented strategies propose a systematic application of these processes by using complex and novel activities that increase in difficulty. Most of these studies have shown moderate improvement in children with or without disorders. In some cases, the change is just limited to trained functions; however, evidence also shows that achievements can extend to other cognitive abilities or other significant areas in the life of children. Some interventions have even recorded changes in brain activation patterns [22, 24].

According to past experiences, children with poorer EF performance benefit the most from these interventions [10]. Drawing on this conclusion, we posit that early interventions in children at social risk are a key factor to promote cognitive and socioemotional development [10, 15–17, 39–44].

Different intervention modalities and strategies are available: (a) individual cognitive training, (b) computer-based programs, (c) interventions embedded into school curricula, and (d) psychoeducational workshops for parents and teachers. With a view to increasing the ecologic validity of interventions, design has moved from a child-centered approach toward programs that can be implemented in natural development contexts and which, consequently, involve the participation of the significant adults from the child's environment in the actual implementation of techniques and strategies. At present, efforts are recognized to enrich children's daily activities, such as sports, music, and martial arts, with specific guidelines that promote EF development.

Individual Cognitive Training: Paper-and-Pencil Versus Computer-Based Modalities

Individual cognitive training strategies are based on the systematic application of a series of cognitive exercises for a variable period of time (weeks or months). Activities are adjusted according to the child's age, but increase in difficulty in order to put cognitive control processes to work. Some interventions focus on a certain process in particular [22], while others try to tackle a larger number of EFs through the mixed training of several functions at the same time [43, 44]. At first, these programs were implemented in controlled settings, such as a lab, but, today, they are implemented at school, in health care centers, and at home.

The paper-and-pencil modality for cognitive training uses a notebook for tasks, activities, and games, such as maze-solving, finding similarities and differences, putting cartoon sequences into the correct order, problem solving, and memory games [23]. The child performs these tasks with the aid of a trainer, who will adjust the levels of difficulty and provide any necessary support to promote the achievement of the intended goals. In Argentina, these interventions have had very positive overall results, promoting EF enrichment in children of preschool [14, 42] and [12, 13, 40, 41, 43, 44] school ages.

This type of intervention is not frequently found in other parts of the world [39], where, in general, the computer-based format of individual cognitive training is implemented [11, 22, 38] using tailor-made software with experimental paradigms (i.e., spatial and visual search paradigm) or

neuropsychological tests (i.e., Stroop). This software can adjust the level of difficulty according to the child's performance and offer immediate feedback. They are developed as games, with an attractive graphic design and interactive proposal to spark a child's motivation, and a collaborative attitude.

Rueda et al. (2005) have conducted valuable research in this regard. This team has designed a computerized program to train attentional networks (ANT), and has noticed that after short training periods children perform better in terms of attention tasks and show a more mature brain activation pattern [22, 38].

Similar experiences have also been implemented in Argentina [45–48]. In 2003, our team pioneered this field by developing a software test called Computer-Based Attention Test for Children [TAI, 45] to stimulate perceptual discrimination and attention in children. This test includes different subtests targeted to the exercise of focused and sustained attention by means of visual search and identification tasks.

Software efficacy has been tested in trials with 7- to 12-year olds who had been previously diagnosed with attentional dysfunction (AD). Overall results suggest that school-age children who took part in computer-based training improved their focused and sustained attention abilities as compared to their performance prior to intervention and control group results [12, 20, 40, 41]. A new software release has already been developed, including a working-memory-stimulating module [46].

Collectively, these findings reveal that individual cognitive training is a very promising tool for strengthening EF in children. It has several advantages over other designs, e.g. rigorous control, decreased influence of alien variables, individual monitoring of each child's progress, and adjustment of the levels of training difficulty. Computer-based training has other benefits as well, such as recording of performance and reaction times, automatic adjustment of difficulty levels, immediate result display, constant software update, and maybe, in the near future, free software access. The major limitation of these interventions is their transfer to untrained cognitive functions and daily activities. The design of strategies that can train multiple cognitive functions jointly and synergistically to achieve a broader scope of results is the next challenge to be faced.

School Curricula Adjustments

One strength of neuroscience intervention methods is the possibility of implementation in natural contexts, such as at school, at home, and in community settings. Based on this assumption, school curricula adjustments have been devised to embed cognitive training activities in school curricula. In developed countries, these have been implemented on a national scale under the umbrella of educational policies. "Tools of the Mind" [49] is a proven example, recording actual improvement of children's cognitive functioning and school performance.

"Tools of the Mind" is a school curriculum adjustment for preschool children designed by Brodova and Leong (2007), based on Vigotsky's approach to social pretend play. This intervention was implemented in kindergartens taking care of children from low-income families. At first, it was included as an extra academic activity, but researchers later found out that it had to be embedded into school activities if benefits were to generalize to untrained skills.

In this way, a special school curriculum was designed for children to role-play daily using any necessary materials, with trained teachers leading the activity. When children are performing, they put their EFs at work. They need to control themselves not to act out of character, remember their own and others' roles, and be flexible enough to adjust as other children improvise. The teacher is there to stimulate the use of internal language and provide young children with the scaffolding they need to reach the intended goals. As children make progress in the development of their cognitive control functions, scaffolding is gradually removed and more challenging tasks are proposed. This experience had favorable results as preschool participants improved EFs, particularly in regard to cognitive flexibility, as well as academic outcomes and school success [10].

Similar school interventions have been implemented in Argentina, although drawing on paper-and-pencil cognitive exercises. This modality was used in groups and combined with group games, even enriched with dietary supplements in some cases [12–14, 20, 40–44, 50]. Overall, results reflect improved cognitive control abilities in participant children, such as attention, inhibitory control, working memory, cognitive flexibility, metacognition, oral fluency, and planning. Furthermore, some studies have reported that these gains were successfully transferred to some untrained cognitive, emotional, and academic abilities, thus increasing internal resources in children at risk surrounded by a challenged environment [12, 13, 43, 50].

Therefore, early stimulation and advancement of cognitive control abilities by intervention programs can be a means to improve school learning and strengthen the child's integral development. The continuity of these interventions is critical in order to maintain and enhance the attained goals [12, 13].

Curricular adjustments make it possible to reach this goal because they can be implemented as early as preschool and continued throughout schooling. These interventions have multiple advantages, such as enabling the completion of longitudinal follow-up studies, reaching many children, and opening the door for the involvement of teachers and school staff to enrich interventions and encourage the implementation of new school practices. On the contrary, some limitations of this methodology include the difficulty of implementing strict controls, setting up random groups, taking diversity into consideration, and adjusting strategies to the difficulty level encountered by each child. The current challenge for those who design these interventions is to link learning sciences and neurosciences to enable a true articulation of knowledge between both disciplines.

Parental Training

An interesting line of study in the advancement of EF in children has been the introduction of training for parents as a module of interventions. Collaborative interactions between parents and their children are particularly important in the child's cognitive development. Several research studies have demonstrated that quality parenting, the use of language in the scaffolding offered to the child, the parents' educational and occupational levels, parenting stress, and maternal depression or anxiety are some factors among many which can model the relationship between parents and children and have a positive or negative impact on the child's cognitive development [15, 37, 51–53]. Therefore, helping parents to improve communication with their children, advancing the development of critical thinking skills, providing techniques to manage family stress, and guidelines to enhance children's development and learning have been some of the goals set for the training of disadvantaged parents [54].

On a global scale, the Head Start Parent Involvement Project [55] has been implemented in New York since 1990 within the framework of the Head Start federal project, and is worth highlighting. This comprehensive program was established as a longitudinal analysis of parent involvement in the Head Start program. Results show that parents who have the most active participation in program activities improve bonding with their children; they support the child's personal autonomy and minimize strict and punitive upbringing practices; they can successfully create more favorable learning environments; they help their children more with their school homework; and they look for better jobs. Children, in turn, improve social skills, particularly collaborative attitudes and school readiness [55].

A similar line of study has undertaken the training of parents who are heads of household from deprived socioeconomic settings [24]. Throughout 8 weeks, these parents learn strategies to reduce stress, improving communication with their children and helping them use critical thinking. Pre- and post-intervention outcomes are promising, because parents who participate in this experience can successfully reduce stress. By interacting with their children, they tend to increase the number of opportunities for dialog and verbal

communication as primary ways to lead interaction. Furthermore, children show changes as compared to children whose parents did not participate in the experience. Changes are measured using standardized language, intellectual quotient, and memory and attention tests [24].

Our team has given psychoeducational workshops for parents of children with attention disorder. Our first experience involved workshops for parents of children with ADHD. Participant parents reported that they could learn techniques and strategies to tackle the symptoms shown by their children, which was associated with decreased inattention, oppositional behavior, and organizational difficulties. Probably, the major achievement of this program was that adults felt encouraged to change their attitude toward their children's disorder. Parents started to interpret their children's behavior better and to highlight their children's strengths, which supported the development of positive interactions [56].

Subsequent experiences involved the design of psychoeducational workshops for parents of children suffering from attentional dysfunction. Participant mothers described favorable changes in child management and mother–child relationships, as communication and strategies to find solutions together improved. These changes were associated with reduced inattention behavior in children, as reported by their mothers and measured with neuropsychological tests against performance prior to the intervention [57].

In summary, interventions focusing on parental action show enormous potential, and enhance child-centered programs implemented during the school day. These interventions have many advantages, such as training the adults who take care of the child as a way to enlarge the family's educational resources, enabling a positive interaction environment, and supporting improved stress management. One of the major limitations of this method is maintaining sustained participation by low-income parents. These adults frequently face economic, family, and social difficulties that restrict their chances of participation and their interest in the activity. Another limitation is the low number of educational and health policies that propose the implementation of these practices at school or health care centers. Challenges are multiple. However, they boil down to designing creative and flexible strategies that can become an integral part of children and their parents' daily activities, do not take up much of their time, and have an actual potential for providing substantial benefits.

Cognitive Intervention Programs: Experiences in Argentina

Experience 1

Our team has been carrying out research on attentional processes, executive functions, and socio-cognitive abilities in children at-risk and not-at-risk for poverty since 1997 [12, 33, 40, 41, 46]. Within this research framework, several programs for cognitive stimulation during childhood have been implemented in Argentine state-run schools [12, 40, 41, 43, 44, 46, 50].

In 2009, we designed an intervention program to advance cognitive control and socio-emotional abilities in disadvantaged children. This program was implemented at deprived urban schools in the province of Mendoza; it recruited 309 schoolchildren participants and consisted of four modules.

- Module 1: cognitive stimulation. Paper-and-pencil exercises were designed to stimulate attentional control, inhibitory control, working memory, planning, categorization and cognitive flexibility (see Fig. 23.1a, b). This module included 15 intervention sessions that were conducted twice a week. The full class participated in the proposed tasks with the presence of their responsible teacher.
- Module 2: workshops on interpersonal resolution conflicts. Workshops were held in groups to stimulate cognitive functions for the resolution of interpersonal problems. Two workshops per school course were given, including dramatization of problem situations, role playing, guided discussions, and final sharing of insights.

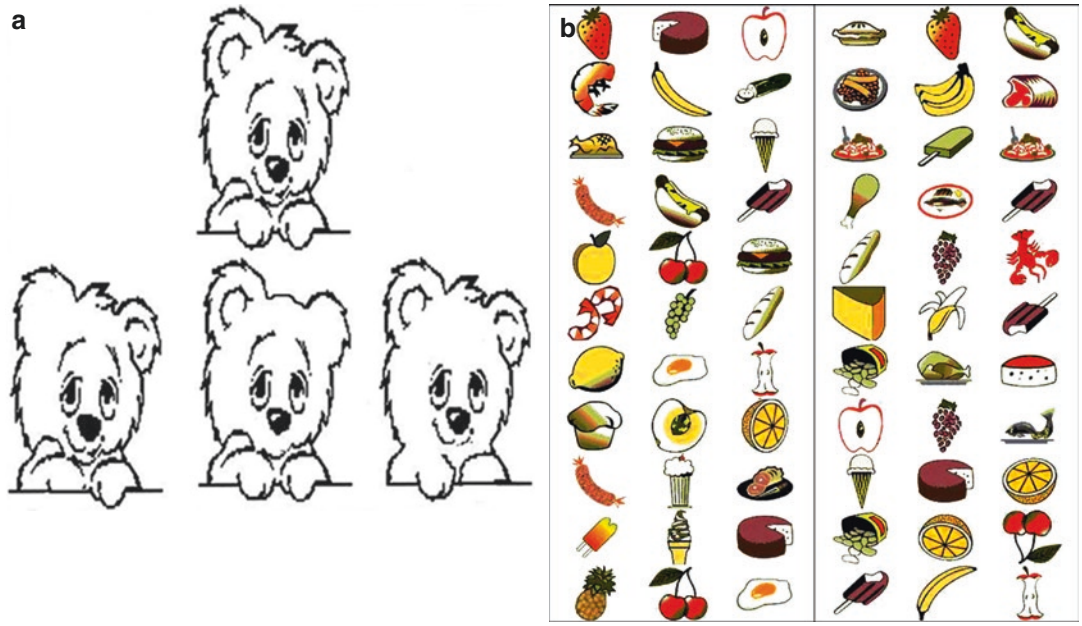


Fig. 23.1 (**a**) Focused attention task. The child is asked to find the three differences between each stimulus and the model. (**b**) Working memory task. The child is asked to cross out the identical drawings in both rectangles and circle the identical drawings in the same rectangle

- Module 3: psychoeducational workshops for parents. Information was provided to parents about cognitive development and school learning in children. Upbringing guidelines were proposed and discussed, and specific strategies were delivered to parents so that they could support the stimulation work performed at school from home.
- Module 4: workshops for teachers. Teachers were trained on how to deliver specific exercises to favor EF development and cognitive abilities oriented to solving interpersonal conflicts between students.

Our intervention program was implemented in different formats and modalities, according to the age of children. This paper presents the results from a group of 90 elementary school children from fourth to seventh grade (9- to 12-year-olds), as this intervention focused on strengthening cognitive control abilities and assessing their impact on school competencies.

Two groups were set up, the intervention group and the control group. Cognitive and school performances were quite similar at baseline. The four program modules were given to school children, parents, and teachers in the intervention group for 3 months. An assessment conducted upon completion of the intervention showed that trained children outperformed their own baseline performance and that of controls in terms of focused attention, sustained attention, cognitive abilities to solve interpersonal problems, and math competence.

The primary finding was that the strengthening of cognitive-attentional resources in children was associated with a significant increase in the ability to solve math calculations [43]. The specificity of this relationship supports earlier studies highlighting the role of focused and sustained attention, especially in terms of math skills [58, 59].

Maybe the strengthening of other cognitive control abilities could have improved other school competencies. However, some program

weaknesses restricted the scope for intervention, such as the short duration of cognitive training, school absenteeism, and limited participation of parents in training.

Experience 2

The outcome of the above experience led us to develop a new program to value the possible impact of cognitive training on school performance more accurately. A more extensive intervention was then designed to promote cognitive control abilities in primary school 6- to 9-year-olds from first to third grade [50]. The selection of this age group was supported by a set of research studies that show this to be a sensitive period in EF development [6–9] and which report closer links between EF and school performance [58, 60].

This experience was carried out in two deprived urban schools in the province of Mendoza, with 178 disadvantaged first- to third-grade primary school children from 6 to 10 years of age. A control group and an intervention group were set up. Cognitive and school performances were quite similar at baseline.

This cognitive training program was divided into 30 sessions, each displaying activities and games targeted to achieving a synergistic stimulation of different executive functions during the same session. Activities included crossing numbers or letters out, finding differences, attentive listening, games with rules, putting cartoon sequences into the correct order, completing sequences, solving problems, classification tasks, divided attention exercises, and tasks for performance self-evaluation (see Fig. 23.2a, b). Activities were proposed for each school course, and included the participation of each responsible teacher. Contents were

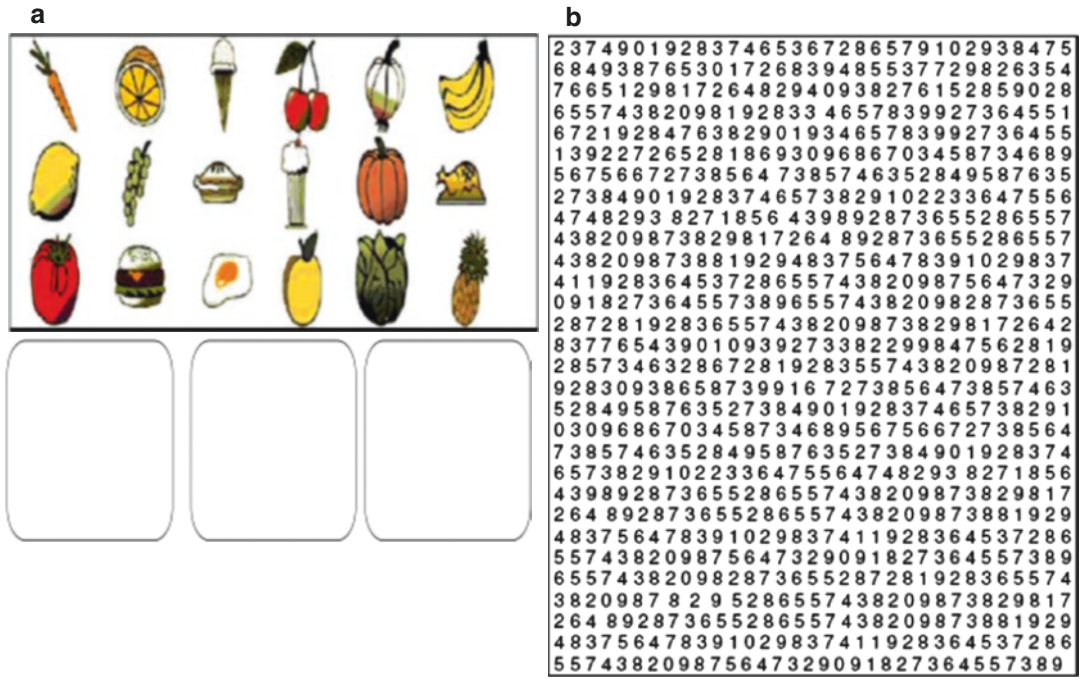


Fig. 23.2 (**a**) Categorization task. The child is asked to group the images by an essential characteristic and give a name to each group. (**b**) Divided attention task. The child is asked to cross out numerals "3" and circle numerals "7" at the same time

taken from the school monthly planning in order to integrate our program to school curricula. In addition, psychoeducational workshops for parents and teachers were held.

Results suggest that the school children who received cognitive training improved performance in terms of cognitive flexibility, planning, inhibitory control, and metacognition as compared to their baseline values and to children in the control group. These gains were supported by the teachers' perception about the executive functioning of children in the classroom. Teachers reported that the children who participated in this training showed better abilities to plan and organize their school tasks, decreased impulsivity, and increased metacognitive capacity to think about their own school performance as compared to children who did not participate in this experience. It was further observed that trained children strengthened their competencies in regard to tasks involving word reading and writing compared to controls [50].

Finally, parents and teachers reported that their participation in psychoeducational workshops was a positive learning experience. Parents pointed out that they learned new specific guidelines and strategies to strengthen the cognitive development of their children through daily activities, such as reading a story and using educational games. They further reported that the implementation of these strategies at home was associated with better cognitive and school abilities in children. In addition, teachers said they learned new techniques, strategies, and specific games to stimulate their students' EFs during school routines. The most valuable lesson from this experience is that teachers themselves designed activities to use with their students. Children were enriched by these practices, improved school performance, and appeared to be more attentive and focused, according to teachers' reports [50].

In brief, these data suggest that cognitive training was effective. Ecological validity was supported by gains observed in cognitive abilities, as children actually transferred them to their daily activities at school, thus strengthening school performance. In addition, data reflect the importance of training the primary adults who live with the children as a way to supplement cognitive stimulation tasks and enhance the practices and interactions that children put at work every day.

Conclusions and Methodology Challenges

Valuable insight has been gained from intervention programs designed to strengthen cognitive control abilities in children at social risk.

One of their primary contributions is that intensive and systematic interventions that start in the early stages of child development produce the best outcomes in terms of cognitive performance if continued over time. The effectiveness of interventions can be enhanced by adding a wide range of tasks involving different sensory stimulation paths.

Embedding interventions in school curricula is perhaps the most promising modality of these programs. Among the primary outcomes were active involvement by a larger number of children, reduced stress in the classroom, and enriched play, self-confidence, and social and emotional development. Similarly, this modality boosts EF development and school performance, as it can be initiated at an early age and sustained over the children's full school life. Training for parents and teachers is another valuable benefit of this intervention modality.

Fully understanding the achievements of cognitive interventions involves reflecting upon their limitations. Two weaknesses deserve special attention. First, evidence shows that the positive effects of interventions tend to decrease progressively if proposed activities and learned conducts are not taken care of by means of subsequent refresher modalities. Second, gains from interventions have been reported to improve solely trained abilities, with a limited effect on other social, emotional, and academic competencies. Therefore, new methodological challenges will need to be faced when designing future cognitive stimulation programs for children.

In our view, the biggest efforts will have to advance the integral development of children at social risk by creating ecological interventions targeted to enhancing the children's daily practices and strengthening self-regulating abilities. Intervention programs can thus become a valuable tool to bridge the persistent academic, economic, and socio-cultural gap associated with poverty.

Translated by

Isolda Rodríguez Villegas. ENS en Lenguas Vivas "Sofía B. de Spangenberg". IES en Lenguas Vivas "Juan R. Fernández". Universidad CAECE, Mar del Plata, Argentina. email: idiomasrv@gmail.com

References

1. Diamond A. Executive functions. Ann Rev Psychol. 2013;64(1):135–68.
2. Marino JC. Actualización en tests neuropsicológicos de funciones ejecutivas. Revista Argentina de Ciencias del Comportamiento. 2010;2:34–45.
3. Barker JE, Semenov AD, Michaelson L, Provan LS, Snyder HR, Munakata Y. Less-structured time in children's daily lives predicts self-directed executive functioning. Front Psychol. 2014;5:1–16.
4. Fuster JM. The prefrontal cortex — an update: time of the essence. Neuron. 2001;30:319–33.
5. Verdejo-García A, Bechara A. Neuropsicología de las funciones ejecutivas. Psicothema. 2010;22(2):227–35.
6. Davidson M, Amsoa D, Anderson LC, Diamond A. Development of cognitive control and executive functions from 4 to 13 years: evidence from manipulations of memory, inhibition, and task switching. Neuropsychology. 2006;44:2037–78.
7. Flores-Lázaro JC, Castillo-Preciado RE, Jiménez-Miramonte NA. Desarrollo de funciones ejecutivas de la niñez a la juventud. Anales de Psicología. 2014;30(2):463–73.
8. Hughes C. Changes and challenges in 20 years of research into the development of executive functions. Infant Child Dev. 2011;20:251–71.
9. Romine C, Reynolds C. A model of the development of frontal lobe functioning: findings from a meta-analysis. Appl Neuropsychol. 2005;12(4):190–201.
10. Diamond A, Lee K. Interventions shown to aid executive function development in children 4 to 12 years old. Science. 2011;333:959–64.
11. Diamond A, Barnett WS, Thomas J, Munro S. Preschool program improves cognitive control. Science. 2007;318:1387–8.
12. Ison MS. Propuesta de intervención para estimular habilidades socio-cognitivas en escolares argentinos en condiciones de vulnerabilidad social. En Saforcada E, Mañas M, Aldarondo E, Comp. Neurociencias, Salud y Bienestar Comunitario. San Luis: Nueva Editorial Universitaria; 2010. p. 111–27.
13. Richaud de Minzi MC. Fortalecimiento de recursos cognitivos, afectivos, sociales y lingüísticos en niñez en riesgo ambiental por pobreza: un programa de intervención. En: Richaud de Minzi MC, Ison MS, Comps. Avances en investigación en ciencias del comportamiento en Argentina. Mendoza: Ed. Universidad del Aconcagua; 2007. p. 145–76.
14. Colombo JA, Lipina S. Hacia un programa público de estimulación cognitiva infantil. Buenos Aires: Paidós; 2005.
15. Hackman DA, Gallop R, Evans GW, Farah MJ. Socioeconomic status and executive function: developmental trajectories and mediation. Dev Sci. 2015;18(5):686–702.
16. Noble K, McCandliss B, Farah M. Socioeconomic gradients predict individual differences in neurocognitive abilities. Dev Sci. 2007;10(4):464–80.
17. Lipina S, Segretin S, Hermida J, Prats L, Fracchia C, Lopez Camelo J, Colombo J. Linking childhood poverty and cognition: environmental mediators of non-verbal executive control in an Argentine sample. Dev Sci. 2010;16(5):697–707.
18. Musso M. Funciones ejecutivas: un estudio de los efectos de la pobreza sobre el desempeño ejecutivo. Interdisciplinaria. 2010;27(1):95–110.
19. Arán Filippetti V, Richaud de Minzi MC. A structural analysis of executive functions and socioeconomic status in school-age children: cognitive factors as effect mediators. J Genet Psychol. 2012;173(4): 393–416.
20. Ison MS. Programa de intervención para mejorar las capacidades atencionales en escolares argentinos. Int J Psychol Res. 2011;4(2):72–9.
21. Farah MJ, Shera DM, Savage JS, Betancourt L, Giannetta JM, Brodsky NL, Malmud EK, Hurt H. Childhood poverty: specific associations with neurocognitive development. Brain Res. 2006;1110: 166–74.
22. Rueda R, Posner M, Rothbart K. The development of executive attention: contributions to the emergence of self-regulation. Dev Neuropsychol. 2005;28(2): 573–94.
23. Portellano-Pérez JA. Cómo desarrollar la inteligencia: Entrenamiento neuropsicológico de la atención y las funciones ejecutivas. Madrid: Somos; 2005.
24. Stevens C, Neville H. Variabilidad en los perfiles de plasticidad neuronal en la cognición humana. En: Lipina SJ, Sigman M, Eds. La pizarra de Babel. Puentes entre neurociencia, psicología y educación. Buenos Aires: Libros Del Zorzal; 2011. p.107–32.

25. Giedd J, Blumenthal J, Jeffries N, Castellanos F, Liu H, Zijdenbos A, et al. Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci. 1999;2:861–3.
26. Kegel C, Bus A. Links between DRD4, executive attention, and alphabetic skills in a nonclinical sample. J Child Psychol Psychiatry. 2013;54(3):305–12.
27. Sheese B, Rothbart M, Posner M, White L, Fraundorf S. Executive attention and self-regulation in infancy. Infant Behav Dev. 2008;31:501–10.
28. Hackman DA, Farah MJ, Meaney MJ. Socioeconomic status and the brain: mechanistic insights from human and animal research. Neuroscience. 2010;11:651–9.
29. Noble KG, Houston SM, Kan E, Sowell ER. Neural correlates of socioeconomic status in the developing human brain. Dev Sci. 2012;15(4):516–27.
30. Sale A, Berardi N, Maffei L. Enrich the environment to empower the brain. Trends Neurosci. 2009;32: 233–9.
31. van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci. 2000;1:191–8.
32. Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature. 1997;386:493–5.
33. Ison MS, Greco C, Korzeniowski C, Morelato G. Attentional efficiency: a comparative study on Argentine students attending schools from different socio-cultural contexts. Electron J Res Educ Psychol. 2015;13(2):343–68.
34. Kishiyama MM, Boyce WT, Jiménez AM, Perry LM, Knight RT. Socioeconomic disparities affect prefrontal function in children. J Cogn Neurosci. 2009;21(6):1106–15.
35. Lupien SJ, King S, Meaney MJ, McEwen BS. Can poverty get under your skin? Basal cortisol levels and cognitive function in children from low and high socioeconomic status. Dev Psychopathol. 2001;3: 653–76.
36. Hackman DA, Farah MJ. Socioeconomic status and the developing brain. Trends Cogn Sci. 2009;13(2):65–72.
37. Mills-Koonce WR, Willoughby MT, Zvara BZ, Barnett M, Gustafsson H, Cox MJ, The Family Life Project Key Investigators. Mothers' and fathers' sensitivity and children's cognitive development in low-income, rural families. J Appl Dev Psychol. 2015;38:1–10.
38. Posner M, Rothbart M, Rueda M. Desarrollo de la autorregulación y desempeño escolar. En: Lipina SJ, Sigman M, Eds, La pizarra de Babel. Puentes entre neurociencia, psicología y educación Buenos Aires: Libros Del Zorzal; 2011. pp.133–52.
39. Röthlisberger M, Neuenschwander R, Cimeli P, Michel E, Roebers C. Improving executive functions in 5- and 6-year-olds: Evaluation of a small group intervention in prekindergarten and kindergarten children. Infant Child Dev. 2012;21:411–29.
40. Ison MS. Abordaje psicoeductivo para estimular la atención y las habilidades interpresonales en escolares argentinos. Revista de la Facultad de Psicología – Universidad de Lima. 2009;12:29–51.
41. Ison MS, Morelato G, Casals C, Maddio S, Carrada M, Espósito A, Greco C, Arrigoni F. Desarrollo de las estrategias atencionales y habilidades socio-cognitivas en niños de edad escolar. En: Vivas J, Comp. Las ciencias del comportamiento en los albores del siglo XXI. Mar del Plata: Editorial UNMDP; 2005. p. 83–97.
42. Segretin MS, Lipina SJ, Hermida MJ, Sheffield T, Nelson JM, Espy KA, Colombo JA. Predictors of cognitive enhancement after training in two samples of Argentinean preschoolers from diverse socioeconomic backgrounds. Front Psychol. 2014;5:1–20.
43. Korzeniowski C, Carrada M, Moreno C, Ison, M. Programa de entrenamiento cognitivo de 4° a 7° grado de educación primaria. Trabajo presentado en el congreso de la Asociación Argentina de Ciencias del Comportamiento, Córdoba, Argentina; 2013.
44. Espósito A, Ison M. Programas de intervención cognitiva en escolares de 2° y 3° grado. Trabajo presentado en el congreso de la Asociación Argentina de Ciencias del Comportamiento, Córdoba, Argentina; 2013.
45. Ison MS, Soria ER, Ana D. Test de Atención Infantil [unpublished manuscript]. Argentina: CONICET; 2003.
46. Ison MS. Attentional capacity in children: intervention programs for its development. In: Gargiulo PA, Mesones-Arroyo HL, editors. Psychiatry and neuroscience update: bridging the divide. New York: Springer; 2015. p. 243–52.
47. Goldin AP, Segretin MS, Hermida MJ, Paz L, Lipina SJ, Sigman M. Training planning and working memory in third graders. Mind Brain Educ. 2013;7(2): 136–46.
48. Goldin AP, Hermida MJ, Elías-Costa M, López-Rosenfeld M, Shalom DE, Segretin MS, Fernández-Slezak D, Lipina SJ, Sigman M. Far transfer to Language and Math scores of a short software-based gaming intervention. Proc Natl Acad Sci. 2014;111(17):6443–8.
49. Bodrova E, Leong D. Tools of the mind: the vygotskian approach to early childhood education. New York: Merrill/Prentice; 2007.
50. Korzeniowski C. Programa de estimulación de las funciones ejecutivas y su incidencia en el rendimiento escolar en alumnos mendocinos de escuelas primarias de zonas urbano-marginadas. [PhD thesis]. San Luis: Universidad Nacional de San Luis; 2015.
51. Blair C, Granger DA, Willoughby M, Mills-Koonce WR, Cox MJ, Greenberg MT, et al. Salivary cortisol mediates effects of poverty and parenting on executive functions in early childhood. Child Dev. 2011; 82(6):1970–84.
52. Hoff E. The specificity of environmental influence: socioeconomic status affects early vocabulary development via maternal speech. Child Dev. 2003;74(5): 1368–78.
53. Ardila A, Rosselli M, Matute E, Guajardo S. The influence of the parents' educational level on the development of executive functions. Dev Neuropsychol. 2005;28(1):539–60.

54. Korzeniowski C. ¿Cómo educar saludablemente a un niño con TDAH? Pautas psicoeducativas para padres y docentes. Alemania: Editorial Académica Española; 2012.
55. Parker F, Piotrkowski C, Kessler-Sklar S, Baker A, Peay L, Clark B. The impact of parent involvement in head start on parents and children. New York: National Council of Jewish Women; 1997.
56. Korzeniowski C, Ison M. Estrategias psicoeducativas para padres y docentes de niños con TDAH. Revista Argentina de Clínica Psicológica. 2008;XVII(1): 65–72.
57. Korzeniowski C, Ison, M. Abordaje de la disfunción atencional infantil: los padres como co-terapeutas. Trabajo presentado en el congreso Asociación Argentina de Ciencias del Comportamiento, Buenos Aires; 2011.
58. Best J, Miller P, Naglieri J. Relations between executive function and academic achievement from ages 5 to 17 in a large representative national sample. Learn Individ Differ. 2011;21(4):327–36.
59. LeFevre J, Berrigan L, Vendetti C, Kamawar D, Bisanz J, Skwarchuk S, Smith-Chant B. The role of executive attention in the acquisition of mathematical skills for children in Grades 2 through 4. J Exp Child Psychol. 2013;114:243–61.
60. Fuhs M, Nesbitt K, Farran D, Dong N. Longitudinal associations between executive functioning and academic skills across content areas. Dev Psychol. 2014;50(6):1698–709.

24 Work and Psychological Suffering: A Case Study on Customer Service Employees at an Electrical Services Company

Melisa Mandolesi, Carlos Bonantini,
Víctor Fabián Quiroga Calegari,
Maria Romina Cattaneo, and Miguel Gallegos

Abstract

This chapter communicates some results of an ongoing research project. The study presented here was conducted in an energy company of the city of Rosario, Santa Fe, Argentina, with the employees in the customer service area. It is considered of great importance to analyze the effects that working in customer service has on this sector's employees, who must meet the demands of users and, not infrequently, must deal with customers' annoyance with regard to the service or the costs.

This work continues research that had allowed us to make an instrument which aims to determine psycho-social-labor vulnerability and its impact on mental health. The aim of this research is to assess psycho-social-labor vulnerability and its effects on mental health in customer service employees of the electricity services sector in Rosario, Santa Fe. To retrieve the data and evaluate it, we used our own standardized instrument. It was applied to a statistically significant sample of a target population group of 98 workers who do customer services in six branch offices. Finally, a focus group with some selected workers was created; the sample was chosen by purpose and availability. The results showed us levels of psychological distress and suffering. We provide details of the findings in the information analysis.

Keywords

Psycho-social-labor vulnerability • Psychological suffering • Mental health

M. Mandolesi (✉) • C. Bonantini
V.F. Quiroga Calegari • M.R. Cattaneo
Faculty of Psychology – National University of Rosario, Riobamba 250 bis, Rosario, Santa Fe 2000, Argentina
e-mail: melisamandolesi@gmail.com; cbonantini@gmail.com; quirogavictor@hotmail.com; mrcattaneo@hotmail.com

M. Gallegos, PhD
Faculty of Health Sciences (Faculty of Psychology), National University of Rosario (UNR), National Council of Scientific and Technical Research (CONICET), Riobamba 250 bis, Rosario, Santa Fe 2000, Argentina
e-mail: maypsi@yahoo.com.ar

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_24

Introduction

This chapter is a continuation of the one called "Social Representation and Imagery of Labor: Evaluation Process of the Psycho-social-labor Vulnerability (PSLV) and Its Relation with Mental Health", published in *Neurosciences and Psychiatry. Bridging the Difference* edited by Gargiulo and Mesones Arroyo (2015). For this reason, in this chapter, the operational concepts used for the research are expressed very briefly, inviting the reader to consult that other chapter if interested in deepening such concepts.

The study of PSLV began around 2009. This concept arose after several investigations on the concept of social vulnerability made between 2002 and 2004, a period that came after a deep economic and social crisis in Argentina. This crisis led our team to be interested in establishing the impact of working conditions and/or the lack of employment on workers' mental health.

In subsequent years, our research team dedicated itself to conducting several studies with the aim of establishing what might be the different ways in which this impact appeared, also picking up research by other authors [1–4] that showed the high degree of psychological suffering of workers.

These developments were taken up later by our research team, in the project called "Psycho-social-labor vulnerability: Conceptualization and measurement. Its effects on mental health", which took place between the years 2008 and 2012. In this period, we worked on the demarcation of the concept of PSLV [5–7] and the design of a screening tool that aims to determine, quickly and accurately, the PSLV in workers of different professions [8].

In 2013, a new research study derived from this project, called "Assessing the psycho-social-labor vulnerability and its impact on mental health in a population of workers in the electricity sector in Rosario", was approved and subsidized by the Secretary of University Politics belonging to the Ministry of Education of Argentina (SPU Resolution number 3272/13). This time, the proposition was to make a pilot test of the tool previously created by our team, with customer service workers, to further refine it and make a last implementation of the final tool for PSLV.

Under an agreement with the union, we got access to an electrical company that allowed us to apply the instrument to 300 subjects in customer service tasks, for a first pilot test period. It allowed us, starting from these data, to conclude the preparation of a definitive instrument for measuring and screening PSLV. It was applied in the first stage of the final study, conducted during 2014, to a sample of 26 workers in the customer service area in a power services company.

In this chapter, we start with the definition of the main theoretical constructs used during the development of this research, then we describe the final PSLV instrument, and then finally present the data collected with it during the first stage of the final study of this research.

The aim of the study, as noted previously, is to inquire about the existence of indicators of PSLV and its possible implications for the workers' mental health, and specifically, to analyze the psychological suffering of these workers as a possible consequence of the tensions generated by the characteristics of the job.

In this respect, we consider it important to describe the findings and conclusions arrived at this early stage, aiming to collaborate in the debate in this field of study. We believe that these contributions will allow us and others to develop new ideas and concepts to apply in the field of customer service workers and services sectors, which are increasingly important in our post-industrial society.

Definition of the Main Theoretical Constructs Used in the Research

We consider it necessary to begin by defining the basic concept of this research.

PSLV is an integrative concept, which aims to overcome the purely physical perspective of vulnerability, to incorporate the implications that both the social aspects and the work environment have on an individual's mental health.

It is a process that involves an invisible web of social and labor relations that affect the daily

lives of workers. We believe that to make visible this invisible frame is necessary in order to move forward with a strategy to understand this network of variables.

We understand PSLV as a construct that determines an interdisciplinary field of research, studying the risks faced by people linked to the world of work. These risks are related to potential problems and possible consequences of the different work processes on mental health, from the perspective of work but also from a non-work point of view.

We have established the importance of considering this construct as a specific element of psychosocial vulnerability [6, 9], in order to develop a new way of looking into the design and implementation of comprehensive plans for addressing social and health problems affecting the working population.

As was stated, we consider that the importance of studying PSLV is based on determining its effects on mental health and psychological suffering.

To conceptualize mental health, we refer to the National Mental Health Law No. 26,657 of Argentina (Regulatory Decree 603/2013), which establishes in the 3rd article that "In the framework of this law, mental health is recognized as a component determined by historical, socioeconomic, cultural, biological, and psychological processes; the preservation and improvement of it involves a dynamic of social construction linked to the realization of everyone's human and social rights". This law, passed in 2010, completes, to our understanding, the definition made in the Guidelines of the National Mental Health Plan published in 1984 for the Ministry of Public Health of Argentina [10, 11].

Taking into account these contributions, we define mental health as a complex process which involves a wide number of elements linked to an individual's human rights and their integration into culture, under the empowerment of social actors in this process. This is a definition highly related to disease, disorders, and psychological suffering process, with which has a dialectical relationship.

Traditionally, psychology has made use of the terms health and disease/disorder on the same terms as the medical system, rather than focusing on the subjective experience of these processes which should matter most to our discipline. In this article, we differentiate the concept of illness, disease, or mental disorder from the concept of psychological suffering.

We define psychological suffering, following Augsburger as a "conscious or unconscious condition caused by grief or pain that every living being experiences facing a concrete situation" [12]. The author proposes, taking up some developments made by Freud, that psychological suffering threatens subjects from three different sites: from the subject's own body, from the outside world, and from links with other human beings. Freud stated that this ultimate source of suffering is perhaps the most painful. "Thus, the spaces of social belonging, integration into a community of interests and feelings with others, are marked by the paradox of being simultaneously guarantees and security providers at the same production site of pain and suffering" [12].

We consider it relevant to state the main difference between psychological suffering and the notion of mental illness. Psychological suffering refers specifically to issues related with being and existing, linked to the interaction with other human beings, and allows us to reflect on what happens when feelings of displeasure arises in that linkage with others, and the daily life of individuals is affected. At the same time, it should be stated that these situations which cause psychological distress need to be taken into account within the social relationships in which they are generated, and not as traditional pathological processes. "The emergence of psychological suffering does not necessarily lead to disease, it can both precede it, as well as diverge from it" [12].

Finally, we chose this concept because it allows us to incorporate the subjective dimension of the disorder or condition, knowing that often the perception and articulation of discomfort by a subject may not be accompanied by signs or symptoms discernible by third parties.

In many cases, working becomes a major source of psychological suffering for the subjects. As previously proposed [10], the concept of work traditionally refers to every human's productive

activity aimed at changing nature and in the process transforming itself. After what we considered was a necessary redefinition of this concept, our research team added to the above: "in order to satisfy basic human needs and continuously improve life quality". However, in the discussion on what "work" really means in contemporary society, we cannot stop thinking about the importance of its relationships with worker's psychological suffering and mental health; that is why this time we delve into one of its dimensions: the quality of working life (QWL) concept.

The study of QWL has been addressed mainly from two major theoretical and methodological perspectives: environmental QWL and psychological QWL. According to Segurado Torres and Agulló Thomas [13], the perspective of the environment quality of working life aims to achieve an improvement of the quality of working life through the achievement of organizational interests. The center of their analysis would be the whole organization, understood as a system, analyzing the various subsystems within it. On the other side, the perspective of the psychological quality of working life is interested in the workers, developing an analysis of some specific elements that make up the different work situations in which the individual is directly involved. This theoretical perspective emphasizes the importance of the subjective issues in working life and, therefore, gives the worker a prominent role [13, p.828].

The inquiry carried out in our research can be considered within this second perspective. Studying working life quality in an organization, from the worker's point of view, requires an analysis focused on the individual, and on the ways in which they experience and perceive their work environment. Fernandez Rios, in the same article [13], defines QWL as the "degree of personal and professional satisfaction in the performance of the job and in the workplace, which is given by a certain type of leadership and management, working conditions, compensation, attraction, and interest in the activities and level of personal and team accomplishment".

With regard to the variables that shape this construct from the psychological and workers' perspective, these are traditionally: individual experiences in the workplace, perceptions, and the level of motivation and satisfaction of individuals. Specifically, following the research carried out by Segurado Torres and Agulló Thomas [13], over the decades psychological QWL studies have focused on: work motivation; linking and needing to maintain a balance between work life and personal life; work satisfaction; organizational efficiency and productivity; socioeconomic environment conditions; physical, psychological, and social well-being; relationships; worker participation in the operation of the organization and planning of their tasks; autonomy and decision-making of individuals on their respective jobs; comprehensive development of the worker; change strategies for optimizing the organization; methods of human resources management; working conditions and environment; the worker as a resource and not as a business cost or a mere producer.

The same authors suggest the importance of researching the variables related to security and stability in employment, occupational risk prevention, personal and professional recognition of staff workers, communication and feedback channels, the participation of workers in the company's benefits, career development, continued education programs, teamwork, culture, and corporate image, among others.

Methodological Strategy

As noted, this research is part of a line that out team has been following since 2008.

The company in which we conducted this study is a services organization, a public utility industry whose product is essential for the daily life of citizens. It has two offices (one in Santa Fe the provincial capital, and one in Rosario, the most important city in the province) and a set of offices in different locations around the province. We worked with a target population of 98 customer service workers in six centers in different parts of the city of Rosario, Argentina. We selected, through a simple random sampling, a sample consisting of 26 workers to whom the different data collection techniques, subsequently characterized in this section, were applied.

For access to the field, two agreements with the union representing workers in this category in the city of Rosario were generated. The first is an agreement between the union and the National University of Rosario, by which the parties undertake to cooperate in areas of mental health, training, and quality of working life of the union members. The second agreement, which has specific character, was signed between the National University of Rosario, the union and the Faculty of Psychology of the University, to provide an institutional framework for the research with a number of clauses that established the character of research, the objectives of the same, the ethical standards, and the characteristics of the return of results to the company and Union.

In addition, the research was conducted with the support provided by the Secretary of University Policies (SPU) of the Ministry of Education of Argentina, through the financial support granted within the framework of a public call for research projects. The University of Rosario provided the institutional counterpart.

To facilitate the entrance of the research group in the organization, the union signed a letter of cooperation. The research team and the subjects involved in the study signed a confidentiality and use of data agreement.

With regard to the methodology, we use a strategy of quantitative and qualitative triangulation. From a quantitative perspective, we applied the PSLV definitive version, which after the pilot test conducted was amended to its current version. It consists of 144 questions organized into a Likert scale of five options, ranging from (1) never to (5) always. The questions are organized around PSLV construct dimensions, namely: networking, personal and job satisfaction, training, contract type and quality of working life, health and life project, and free time.

These dimensions are directly related to the variables of the quality of working life and allowed us to investigate them, as well as other features of work and its relation to mental health.

After analyzing the information obtained with the instrument, we proceeded to the qualitative stage; for this, we prepared materials that were discussed at a focus group composed by one member from each service center. Members of the focus group were selected not randomly but according to availability.

We read the material to this group and set them an objective of discussing the statements contained in it from the perspective of their own experience in the workplace.

The qualitative material was finally completed with a discussion group composed by some interviewers (scholarship students trained for the task), in which the participants expressed their subjective impression of the whole experience, deepening in their perceptions and opinions and talking about some important items not included in the standardized quantitative instrument.

Once all instruments has been applied, the research group analyzed, interpreted, and verified all the data obtained and built during the process, to reach the conclusions and reflections that we present in this work.

The data presented here were collected and constructed in the final stage of the study, using the latest version of the instrument generated throughout the research process. To the data found through the use of the quantitative instrument we added the qualitative data built in the final stages, in order to deepen the analysis and interpretation presented.

Results

The sample (n = 26) was equally distributed between men and women (50%). Of the subjects, 76.9% had a regular partner, 19.2% had no partner and only 3.8% had no regular partner. Most respondents had stable employment contracts, and only 3.8% claimed to be hired (temporary work). The mean of ages were 43 years for men and 38 years for women.

In this company, the staff is composed of workers who belong to different generations of the same family who have spent their entire working life in the organization. In the sample, we see that the overall mean of time in the organization is 142 months, and the statistical mode is 32 months. Workers have an average of 90 months working in the same workplace.

Every employee (100%) feels that the company provides job stability; this can be confirmed by relating the data with the job characteristics in the organization, where workers said that they finished high school and remained throughout their entire working life in the same company; these are people who began their working life at the end of high school. Mode for both men and women is 18 years.

A high percentage recognizes the additional payment related with insecurity, risks, and overtime, and 76.9% believe that labor laws are respected. However, when asked about the feeling of being respected at work, we found that only 57.7% responded that they feel respected continuously, and only 34.6% feel respected at times.

Given these characteristics of the organization and, in order to research the psychological quality of working life and its relationship with mental suffering, we would like to present the workers' point of view on the following dimensions of the PSLV instrument: job satisfaction and quality of life at work, and networks.

Job Satisfaction and Quality of Working Life

What appears in a first analysis is the degree of job satisfaction and employee loyalty to the organization (Fig. 24.1). With regard to the first item, 84.6% of workers get satisfaction from going to work; the percentage of those who say are bored or frustrated with the task is very low, 7.7% in men and 7.6% in women. Although a relatively small percentage consider the job as creative (46.1%); 69.3% of workers are motivated to produce in the job, and 65.4% find the work has some meaning.

With regard to loyalty to the organization, we could say it is very high. An indicator that gives us this impression is that 88.5% feel proud to work at this company. To this we could add the proactive attitude of workers; 80.8% consider that they overcome difficulties encountered in working hours, and 53.9% say that at the face of difficulties, they can maintain the pace of work.

With regard to attention to working conditions and environment, we agree with the concept characterized by Neffa [14] as "constituted by the socio-technical and organizational production process implemented in the establishment (working

Fig. 24.1 Job satisfaction (Source: based on own data)

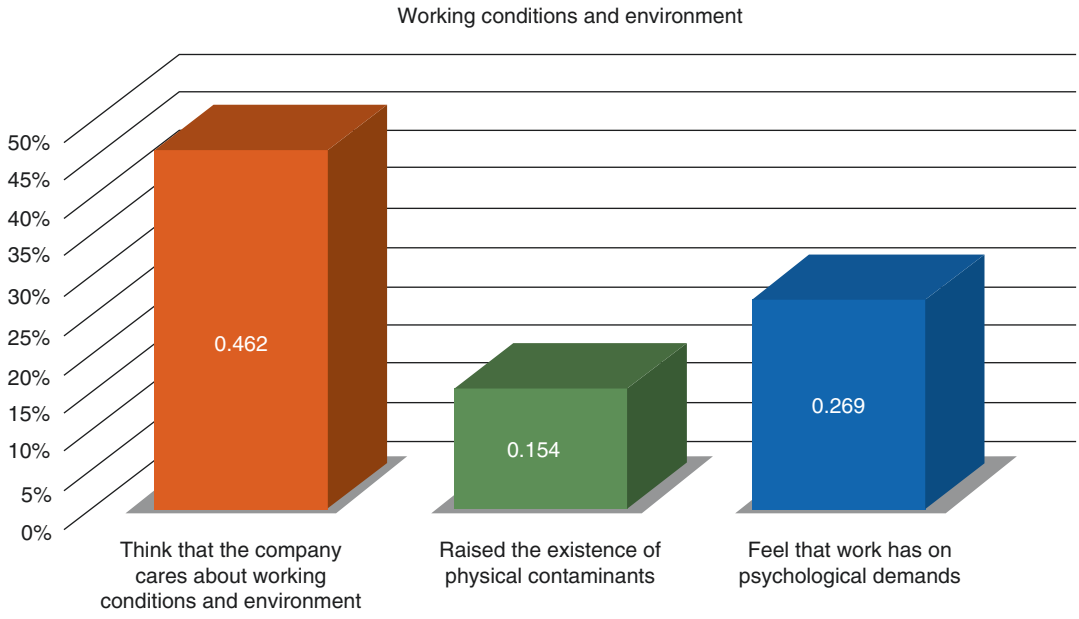


Fig. 24.2 Working conditions and environment (Source: based on own data)

conditions) and by the risk factors of the working environment". The percentage of those who think that the company cares about this is 46.2%.; 15.4% of them raised the existence of physical contaminants, and 26.9% feel that the work makes psychological demands, both physically and psychologically causing ailments and discomfort (Fig. 24.2).

The same author also raises the concept of "workload", referring to both the mental and the psychological burden. Neffa defines mental burden or mental workload as "the requirements and demands of the job in terms of cognitive activities (...). It depends on the one hand, on the structure and functioning of cognition processes and on the other hand, on the nature, amount, and frequency of the information to be perceived, captured, and processed in a given unit of time" [14]. On the other hand, he characterizes the psychological load or psycho-social aspects of workload as very closely related to the actual content of the job.

With regard to the findings related to these two forms of workload, 19.2% of respondents think that work produces mental disorders, 26.9% believe that work causes psychological discomfort, and 26.9% declare that they suffer psychological fatigue attributable to the complexity of the task. With smaller but no less significant values, 11.5% of workers think that the knowledge required for the job affects their mental health, and 7.6% believe that the knowledge required for the tasks exceeds them (Fig. 24.3).

This, in some cases, results in somatization. This can be considered as belonging to the field of psychological suffering, namely headache during working hours (23% of respondents), feelings of oppression or "my head is about to explode" (19.2%), sleep disorders (30.8%), permanent fatigue (19.2%), and nerves or moodiness (11.5%) (Fig. 24.4).

In some cases, this psychological suffering leads to psychological disorders; 3.8% of respondents expressed having felt overwhelmed or panic for no reason and another 7.6% reported having felt that the world was coming down, affecting both their relationships with couples and children.

A small number of workers (19.2%) directly attributed their psychological ailments to being in the area of customer service. However, 50% of respondents said that there are certain discomforts from working in customer service, but that they are not incessant.

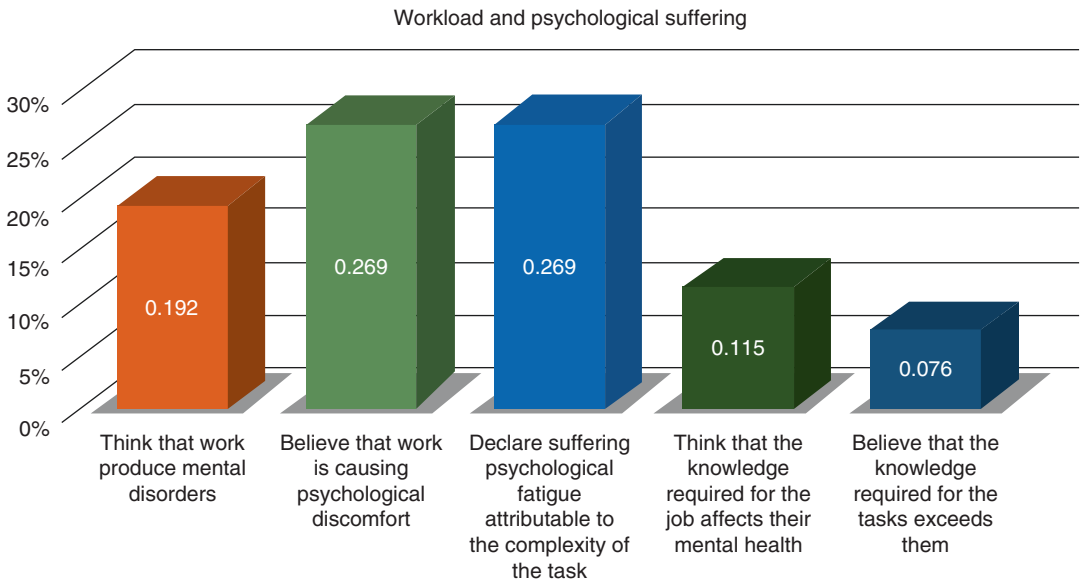


Fig. 24.3 Workload and pyschological suffering (Source: based on own data)

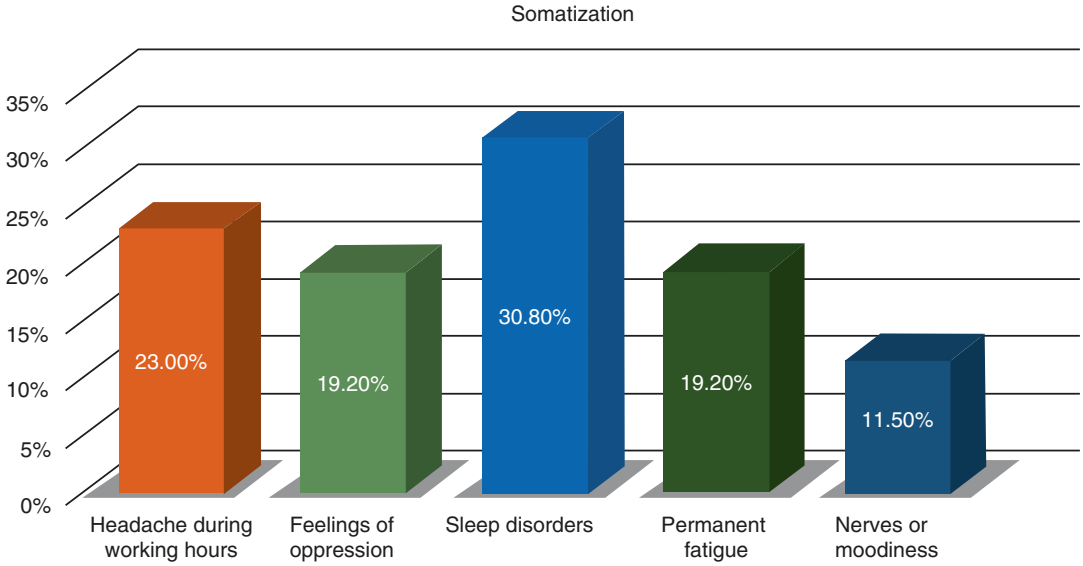


Fig. 24.4 Somatization (Source: based on own data)

Within the data referred to psychological suffering, it is interesting to note that women have higher percentages in questions regarding discomfort produced by work (14.3% women and 8.3% men) (Fig. 24.5). Most of the women (57.1%) argued that they felt emotionally exhausted by the task, a value that in men was only 25%. It also highlights that 14.3% of women

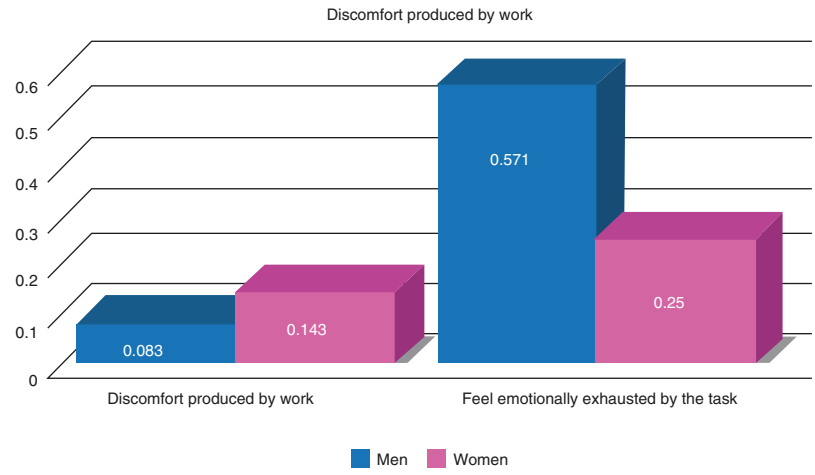


Fig. 24.5 Discomfort produced by work (Source: based on own data)

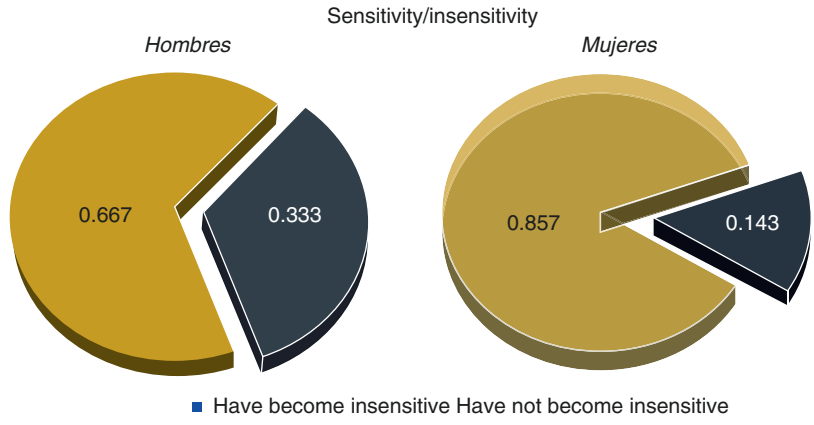


Fig. 24.6 Sensitivity and insensitivity

reported feeling treated as an object in their workplace, unlike the men, all of whom responded negatively to this question.

Other interesting differences found in relation to the gender of respondents refer to the defense mechanisms brought into play to cope with psychological suffering: since performing this tasks, 33.3% of men feel that they have become less sensitive, while only 14.3% of women feel this way (Fig. 24.6) although, curi-

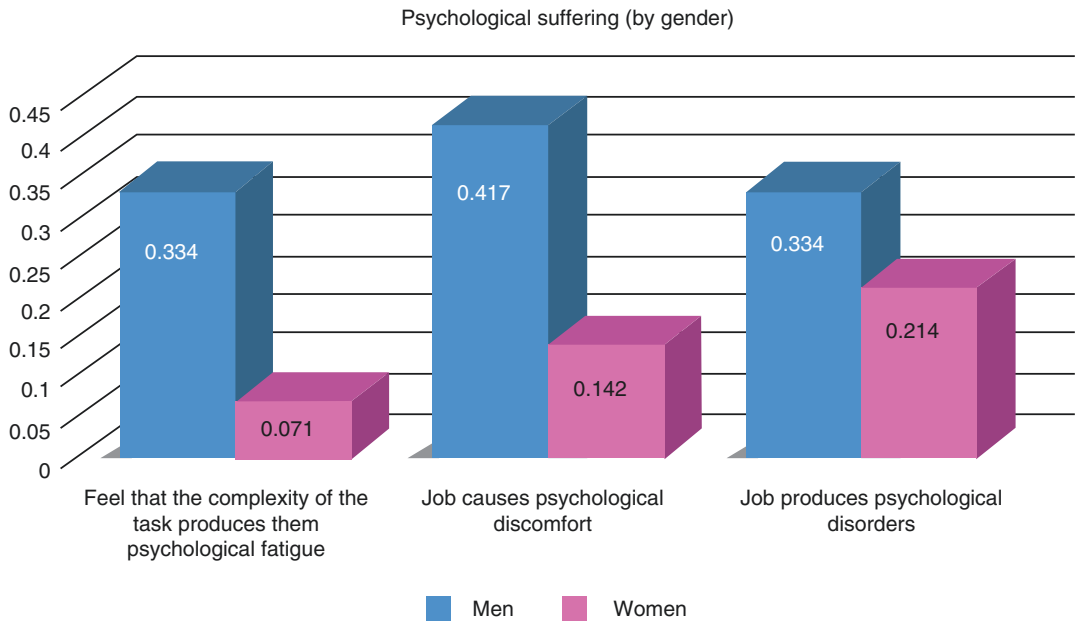


Fig. 24.7 Psychological suffering by gender (Source: based on own data)

ously, it is women in greater proportion (50%) who feel the transfer of problems from users to themselves, while this was found only in 27.3% of men.

It is also interesting to note that there is a greater psychological burden and greater involvement as a result of the task in men than in women: 33.4% of men believe that labor produces psychological disorders, and only 7.1% of women agreed (Fig. 24.7). On the other hand, 41.7% of men and 14.2% of women believe that the job causes psychological discomfort; and finally, 33.4% of men and 21.4% of women feel that the complexity of the task gives them psychological fatigue.

Networks

We address this dimension of the instrument specifically because of its close relationship with the intersubjective aspect (the outside world and links with other human beings) of psychological suffering.

As we stated earlier in this chapter — and this was also developed in previous studies [8] — among the main variables that define the construct of PSLV we can mention the "possibility of establishing social networks" (e.g., family networks, unions, neighborhood, etc.).

We classify as "social networks" forms of resistance that society of late capitalism has developed to address the growing vulnerability that social actors have to large corporations. When we talk about creating networks, we are not just thinking of the networks created in the workplace. We also refer to associations, groups, meeting spaces that citizens generate against the arbitrariness of bureaucratic power, which attempts to place them in a state of helplessness. Making networks means the possibility of partnering to work for a common destiny. Networks (land, family, professional) constitute what in other articles [7] we have considered a social capital of great importance to the formulation of employability and survival strategies (mainly in the most vulnerable sectors of the population).

In this sample, we found low percentages in the answers to questions of networks. With regard to participation and contact with organizations and neighborhood activities, 7.7% of workers participate in them, and 19.2% consider that they

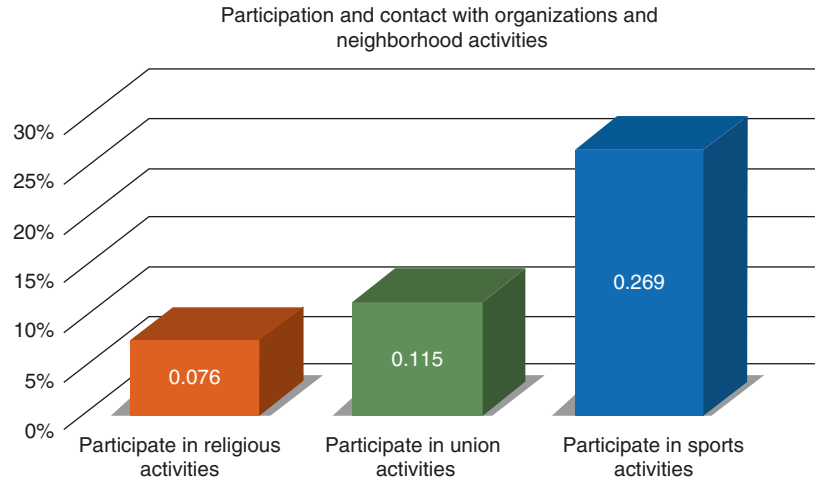


Fig. 24.8 Participation and contact with organizations and neighborhood activities (Source: based on own data)

function as a support in case of need. Some of the workers (7.6%) participate in religious activities, other (11.5%) do so in union activities, and a greater number (26.9%) participate in sports activities (Fig. 24.8).

Conclusions

So far, we have described the results of the first application of the final version of the PSLV instrument in a sample of 26 customer service employees of a public utility company.

Among the features of the company that we consider it necessary to point out is the fact that it is the property of the state, which is why many of the values found in this field have certain peculiarities. Workers involved in an organization with internal working markets, and high care, health, and social security coverage combined with high job security, have no difficulty building a life outside their jobs with adequate satisfaction rates. While the values in the dimension "networks" of the instrument are not high, it was possible to understand the involvement of workers in social activities of various kinds from the focus groups.

As we stated during the development of the main concepts addressed in the research, the fact that workers' health is traditionally reduced to a lack of disease often manes that discomforts and intermediate sufferings between health and disease extremes are ignored by mental health workers. This traditional way of thinking about health/disease without seeing that disease is the conclusion of "a series of links that progressively deteriorate health, and where working conditions and environment play a decisive role since its inception" [14], prevents to some extent the development of policies for disease prevention and health promotion which are appropriate to the problems of the workplace.

That is why we attach great importance to addressing psychological suffering as one of the elements which may or may not lead to disease. To this end, we consider that it is essential not to relate PSLV only to socioeconomic status or to any particular social actor's condition, as there is a multiplicity of factors that define this issue, including several of those associated with the concept of quality of working life (QWL).

In terms of the relationship between psychological suffering and quality of the working life of people, we consider it appropriate to state that, based on the data we could find in this first study, job security, compliance with laws and contracts, health benefits, and pension contributions do not ensure that workers consider that working conditions and environment are appropriate. One of the possible reasons for disagreement with working conditions and environment can be related to the existence of certain physical and psychological issues expressed by workers. While these discomforts do not have high values, they must be

taken into consideration when addressing psychological suffering. It is noteworthy in relation to this that about 26.9% of employees feel that the task creates excessive physical or psychological demands, and that their job causes them discomfort and inconvenience. We consider it important not to underestimate and to keep the focus on the 19.2% of workers who feel they suffer from psychological disorders. We believe this is a high percentage of workers and a level of psychological suffering that is a crucial point to generate prevention policies.

Finally, we found that the subjects reported feeling that working in customer service gives them high stress levels. This may be because in many cases, these workers are in the front office of the organization, having direct relationships with the service users, in addition to complaints about several problems inherent to the service provided by the company. They often transferred in the form of protest their own problems, as was discovered by workers focus groups. At this point, our findings are consistent with previous studies [1, 2, 4] with regard to workers' psychological suffering effects of working in customer service of any organization. We believe that this overlap on the findings indicates the need for care and prevention programs by work organizations, aiming to reduce or eliminate the negative effects of work on workers' health, and, at the same time, to create opportunities and programs promoting health, and specifically mental health, in different organizational settings.

Acknowledgements We wish to thank the collaboration in the statistical analysis of Dr. Norberto Martin.

References

1. Giovanella L. Los sistemas de salud de Argentina, Brasil y Uruguay en perspectiva comparada. [Internet] 2013. Consulted 2016 Jan 16. Available in: http://docplayer.es/530614-Los-sistemas-de-salud-de-argentina-brasil-y-uruguay-en-perspectiva-comparada.html.
2. Zeballos JL. Argentina: Efectos socio-sanitarios de la crisis, 2001–2003. [Internet] 2003. Consulted 2016 Jan 16. Available in: http://apps.who.int/iris/bitstream/10665/169025/1/2003-ARG-efectos-sociosanitarios-crisis.pdf.
3. Olivares Faúndez V, Jélvez Wilke C, Mena Miranda L, Lavarello Salinas J. Estudios sobre Burnout y Carga Mental en Conductores del Transporte Público de Chile (Transantiago). Cienc Trab. [Internet]. 2013. Consulted 2016 Jan 16;15(48):173–8. Available in: http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0718-24492013000300011&lng=es. http://dx.doi.org/10.4067/S0718-24492013000300011.
4. Zaldúa G, Bottinelli M, Paulovics M, y Nabergoi M. El trabajo bancario en contexto críticos. XII Jornadas de Investigación y Primer Encuentro de Investigadores en Psicología del Mercosur. Facultad de Psicología — Universidad de Buenos Aires. [Internet] 2005. Consulted 2016 Jan 16. Available in: http://www.aacademica.org/000-051/324.
5. Gómez Jarabo G, Simonetti G, Bonantini C, y Quiroga V. Vulnerabilidad psicosociolaboral, trabajo y salud mental. Psicopatología. 2007;27(1y2):45–70. Promolibro. Valencia.
6. Bonantini C, Simonetti G, Quiroga V, Mandolesi M, y Cattaneo R. Aspectos conceptuales, metodológicos y prácticos del estudio de la vulnerabilidad psicosociolaboral (VPSL). Aristeo. 2008;1(1):15–27. Eudeba. Buenos Aires.
7. Simonetti G, Bonantini C, Quiroga V, Lerma S, Cervigni M, Cattaneo R, Gallegos M, y Milicich M. Trabajo y salud mental. Sobre el estudio de la vulnerabilidad y sus relaciones con las políticas de salud y cambio tecnológico. Psyberia. 2009;1(2):81–92. Rosario.
8. Quiroga V, Bonantini C, Cervigni M, Cattaneo R, Gallegos M, y Mandolesi M. La vulnerabilidad psicosociolaboral como herramienta de screening. Cuadernos Sociales Iberoamericanos. 2010;10(1): 119–38. UNR Editora.
9. Bonantini C, Simonetti G, Quiroga V, Mandolesi M, y Cattaneo R. El desempleo como factor de discapacidad. Anales de discapacidad y salud mental. 2009;9:71–82. Madrid.
10. Bonantini C, Cervigni M, Mandolesi M, Quiroga V, y Gallegos M. Social representation and imagery of labor: evaluation process of the psychosocial and labor vulnerability and its relation with mental health. In: Gargiulo PÁ, Mesones Arroyo HL, editors. Psychiatry and neuroscience update. Switzerland: Springer; 2015. p. 253–64.
11. Goldenberg M. Lineamientos para un Plan Nacional de Salud Mental. Informe final sobre la asesoria en salud mental al ministerio de salud publica — Organizacio paamericana de la salud y OPS/OMS. Buenos Aires; 1984.
12. Ausburger C. De la epidemiología psiquiátrica a la epidemiología en salud mental: el sufrimiento psíquico como categoría clave. Cuadernos Médicos Sociales. 2012;81:61–75.
13. Segurado Torres, A y Agulló Thomas, E. Calidad de vida laboral: hacia un enfoque integrador desde la Psicología Social. Psicothema 2002;14(4):828–836.
14. Neffa J. ¿Qué son las condiciones y medio ambiente de trabajo? Propuesta de una perspectiva. Buenos Aires: Hvmanitas; 2002.

Part IV

Explaining Pathological Human Behaviors: From Brain Disorders to Psychopathology

Neuropathological Background of MK-801 for Inducing Murine Model of Schizophrenia

25

Ane Murueta-Goyena Larrañaga,
Arkaitz Bengoetxea Odrioizola,
Pascual Ángel Gargiulo,
and José Vicente Lafuente Sánchez

Abstract

Schizophrenia is a complex psychiatric disorder with a developmental component that compromises neural circuits. Understanding the neuropathological basis of schizophrenia remains a major challenge for establishing new therapeutic approaches. In this review, causal factors for abnormal brain development in schizophrenia are discussed, with particular focus on N-methyl-D-aspartate (NMDA) receptor hypofunction and GABAergic circuit-mediated neurotransmission. Changes in interneuron structure and function have been reported in schizophrenia, and current evidence points to a specific involvement of interneuronal NMDA receptor signaling. Furthermore, altered gamma-band oscillations in schizophrenic patients drew attention to a possible deficit in fast-spiking parvalbumin-expressing interneurons, which play an essential role in regulating complex interaction between pyramidal cells, and represent a key to the understanding of network operations. Here, we describe the major biochemical, neuropathological, and cognitive deficits present in schizophrenic human individuals, and the faithfulness of animal models for mimicking those impairments. In NMDA receptor antagonism-based animal models, repeated injections of MK-801 (dizocilpine) during early postnatal brain development, disrupt the excitation/inhibition balance.

A. Murueta-Goyena Larrañaga, MSc (✉)
Laboratory of Clinical and Experimental Neurosicence (LaNCE), Department of Neuroscience, University of the Basque Country UPV/EHU, Leioa, Spain

University of the Basque Country,
Laboratory of Clinical and Experimental Neuroscience, Department of Neuroscience,
Faculty of Medicine and Nursing,
Barrio Sarriena s/n, Leioa, Bizkaia 48940, Spain
e-mail: ane.muruetagoyena@ehu.eus

A.B. Odrioizola • J.V. Lafuente Sánchez
Laboratory of Clinical and Experimental Neurosicence (LaNCE), Department of Neuroscience, University of the Basque Country UPV/EHU, Leioa, Spain

P.Á. Gargiulo
Laboratory of Neurosciences and Experimental Psychology, CONICET, Area of Pharmacology, Department of Pathology, Faculty of Medical Sciences, National University of Cuyo, Mendoza, Argentina

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_25

A unifying hypothesis to explain the altered brain function in this model is a specific perturbation of GABAergic cells that results in long-term structural brain changes and modified network activity in adulthood, especially when MK-801 is administered during neurodevelopment. Subsequent impairment in cognition, particularly working memory and associative memory, are extremely relevant for schizophrenia research.

Keywords
Schizophrenia • MK-801 • NMDA • Animal model • Neurodevelopment

Schizophrenia is a disabling psychiatric disorder whose etiopathogenesis is still unclear. Brain research has been mostly focused on neurological diseases but in recent decades, research on the neurobiological basis of mental illnesses has emerged. This lack of information has restrained research from advancing in the understanding of the neurobiological basis of schizophrenia, and has led clinicians to make the diagnosis based on some symptom clusters. Schizophrenia is characterized by positive, negative, and cognitive symptoms. Positive symptoms represent abnormal mental functions, such as hallucinations and delusions. Negative symptoms include social isolation, decreased motivation, and flattened affect. Cognitive symptoms are related to poor executive function, particularly that involving attention and memory. The typical onset of these symptoms starts between late adolescence and early adulthood [1], although neurodevelopmental processes play an important role in schizophrenia. Currently, antipsychotic drugs are effective in reducing positive symptoms but have minimal beneficial effects on cognition. Poor cognitive functions affect the everyday life of schizophrenic patients and contribute most to chronic disability and unemployment [2–4]. However, no current treatment or therapy can successfully manage cognitive deficits.

Within the past two decades, numerous efforts in understanding the underlying etiology of cognitive dysfunction of schizophrenia have been made. For that purpose, understanding the neurobiology and circuitry of the forebrain, which support cognitive processes, is of major interest. Several theories regarding the etiology of schizophrenia have been proposed, including, but not limited to, genetic predisposition [5–9], prenatal infection [10, 11], environmental influences [12, 13], or a combination of these [14]. Brain imaging and post-mortem studies have shown anatomical changes in schizophrenic patients, primarily in prefrontal cortex and temporal lobe structures, such as decreased cortical volume [15, 16], altered circuitry and connectivity [17], and changes at the neuronal level [18] that contribute to core cognitive dysfunctions in schizophrenia.

Cognitive dysfunction is pervasive and is independent of other symptoms [3]. The most characteristic finding is the decreased ability in working memory tasks, especially when a high degree of information needs to be processed [19, 20]. The dorsolateral prefrontal cortex (DLPFC) is implicated in working-memory deficits, and brain imaging studies have consistently demonstrated alterations in activation of DLPFC in schizophrenic individuals, particularly during cognitive tasks [21–23]. In addition, hippocampal circuitry is altered both regionally and extra-regionally [24]. Within these circuits, GABAergic connections are of particular interest. Local interconnections of GABAergic interneurons onto pyramidal cells show alterations at the synaptic level, resulting in cognitive deficits [25, 26]. Furthermore, afferent and efferent connections between hippocampus and DLPFC also seem to be affected [27, 28]. The aberrant plasticity of the hippocampal–prefrontal cortex pathway may explain

the deficits of cognitive processes that require spatial and temporal information [28, 29].

Postmortem studies further support the idea that GABAergic alterations in the prefrontal cortex and hippocampus are implicated in the etiology of schizophrenia. In particular, immunostaining studies reveal a selective decrease of the calcium-binding protein parvalbumin in brains of schizophrenic patients compared with controls, with no concurrent loss of calretinin- or calbindin-immunoreactive interneurons [30–32]. The reason why the decrease of GABAergic interneurons is mostly limited to parvalbumin-containing cells is unclear, but it has been suggested that developmental changes in parvalbumin (PV) expression could make them vulnerable to dysfunction. Recent new studies of postmortem brains indicate that GABAergic interneurons containing the neuropeptide somatostatin are also decreased in orbital regions of the forebrain in schizophrenic patients [33].

Maturation of GABAergic Interneurons

GABAergic interneurons are the main source of cortical inhibition in the mammalian brain [34] and account for 10–25% of total number of neurons, depending on the brain region [35]. The high diversity of GABAergic interneurons in their morphology and functional properties has made the classification challenging. Suffice it to say that GABAergic interneurons play an important role in regulating and orchestrating the activity of pyramidal cells. They also shape cortical plasticity, synaptic wiring, and oscillations during prenatal and postnatal development [35]. An important characteristic of GABAergic circuit development is its long duration. The maturation begins early in embryonic stages and proceeds in several steps before fully-developed cell features are acquired. This involves the maturation of GABA release and reuptake, the ability of neurons to form synapses at defined developmental stages, and the expression of particular proteins that regulate cell signaling to eventually acquire mature electrophysiological properties.

Interneurons containing the calcium-binding protein PV seem to be the most affected GABAergic interneurons in schizophrenia. In particular, the selective downregulation of PV and GAD67 (glutamic acid descarboxilase 67) in the prefrontal cortex is the most consistent finding [36–38]. PV+ interneurons can be classified into two morphologically differentiable groups: basket cells and chandelier cells. Basket cells usually synapse in pyramidal cell somas and proximal dendrites, and chandelier cells form axo–axonic connections with the axon initial segment of pyramidal cells. One single basket cell can target large population of pyramidal cells, thereby exerting powerful postsynaptic modulation of excitatory output. In turn, pyramidal cells provide feedback input to parvalbumin positive cells, and this closed loop seems to evoke gamma oscillations, the physiological correlate for proper sensory integration and cognitive functions [39, 40]. Although another mechanism for gamma oscillations generation has also been proposed, the involvement of basket cells in gamma oscillation generation is known to be critical [41]. Schizophrenic patients show reduced power in gamma oscillation in frontal lobe during working memory tasks, in auditory cortex after a train of clicks, and in visual cortex when the scenery needs a perceptual organization [42]. Rhythmic brain activity in gamma-band seems to be necessary to transfer information between brain regions, and a lack of proper communication between brain regions is believed to underlie the pathophysiology of schizophrenia [42]. Given that PV and GAD67 expression is regulated by cortical activity [43, 44], the absence of both in PV+ neurons of schizophrenic patients indicates a lack or decreased activity of PV+ neurons. This would shift brain activity balance towards excitation. The dysregulation of local circuitry could be explained by abnormal neurodevelopmental changes.

Early in neurodevelopment, GABA acts as a depolarizing neurotransmitter, due to chloride accumulation in the cytoplasm. Around the end of the first postnatal week, the expression of

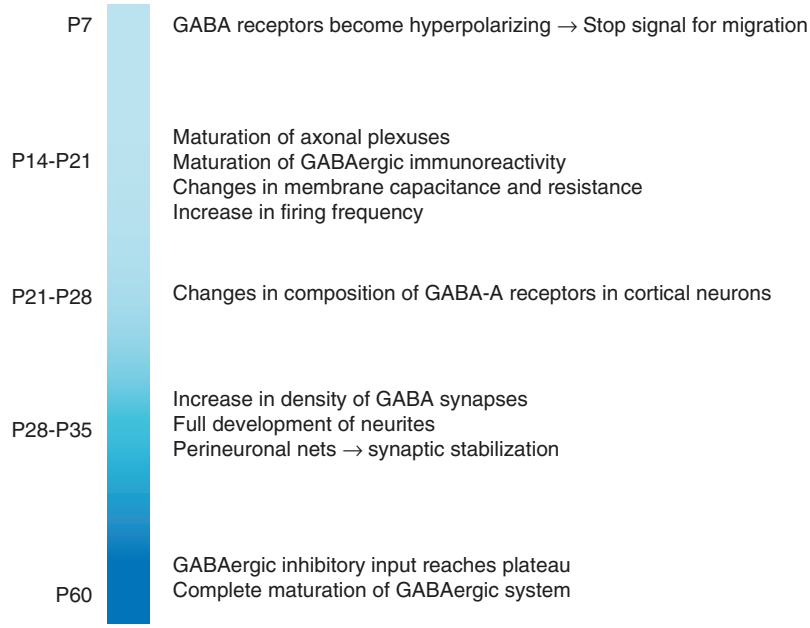


Fig. 25.1 Major steps of GABAergic interneuron maturation and neurotransmission

potassium-chloride cotransporter KCC2 dramatically increases in the forebrain, and GABA becomes hyperpolarizing [45]. The expression of KCC2 is regulated by local neuronal activity, so it subserves as a stop signal for further migration [46]. Between the second and third postnatal weeks, several changes in GABAergic interneurons take place: (1) maturation of GABAergic immunoreactivity [47], (2) appearance of adult-like electrophysiological properties (increase in firing frequency, high-frequency subthreshold membrane potential oscillations, and changes in membrane resistance) [35, 48], and (3) maturation of axonal plexuses of cortical interneurons [48]. Parvalbumin-expressing cells fully develop neurites at approximately 4–5 weeks [48]. In the first month, there is an increase in GABAergic synapses. The composition of $GABA_A$ receptors also changes during development in cortical neurons, and adult form subunits are found at 3–4 weeks [49]. Subunit switch is paralleled with the maturation of GABAergic inhibitory postsynaptic potentials (IPSPs) in the neocortex and hippocampus of parvalbumin-expressing fast-spiking cells [48]. $GABA_B$ receptors, a G protein-coupled receptor, also express different subunits during postnatal development [50] (Fig. 25.1). Developmentally regulated subunit expression has important functional implications in physiological properties.

Inhibition carried out by GABAergic interneurons is low during neurodevelopment, and acquires adult features in late adolescence or early adulthood [35]. The increase in inhibitory tone is correlated with the development of perineural nets (PNN) [51]. Perineural nets are proteoglycans that wrap certain type of neurons, and are thought to give homeostatic balance to highly active neurons. They are developed in an activity-dependent manner and act as physiological buffers for ions. PNN are particularly present in fast-spiking (FS) neurons for their high activity patterns. The opening and closure of critical periods are regulated by the level of maturation of inhibitory neurons, which in part is determined by the presence of PNN, as they offer synaptic stability [51].

There is evidence of perturbed maturation of GABAergic interneurons in schizophrenia. Impaired interneuron migration during development has been demonstrated through several findings. Various authors have found increased density of interneurons

in the superficial white matter of schizophrenic patients [52, 53]. Following knockdown of DISC-1, a gene implicated in schizophrenia susceptibility, tangential migration of MGE-derived interneurons is altered [54]. In addition, neuroregulin-1 (NRG-1) and its receptor ErB4 are also associated with increased risk of schizophrenia, and ErB4 is exclusively expressed in inhibitory interneurons, particularly in PV+ cells [55]. Altered connectivity in local excitation/inhibition circuitry has also been shown: less expression of the α1 subunit at basket cell–pyramidal cell synapses, and overexpression of the α2 subunit at chandelier cell–pyramidal cell synapses [56]. There are decreased axo–axonic synapses and less glutamatergic synaptic input onto parvalbumin-expressing basket cells in mice models [57]. These findings support the hypothesis that parvalbumin-expressing cells get improperly connected during development, resulting in aberrant network activity and plasticity in adulthood. Moreover, the substantial changes that GABAergic system undergoes at late adolescence and the onset of schizophrenic symptoms have the same age-dependency profile.

Animal Models of Schizophrenia

Animal models have been useful for unraveling the pathophysiological mechanism and treatment development in many areas of medicine. The critical obstacle in using animal models for studying psychiatric disorders is rooted in the poor understanding of their neural basis. Moreover, schizophrenia is considered a uniquely human disorder, as it mostly affects perception, thinking, language, and attention. Modeling those features in lower mammals has been controversial, but the high prevalence of schizophrenia (approximately 1% of general population), and the debilitating effects of the disease justify a great effort to study it. In this way, animal models are an indispensable tool.

Several approaches have been taken to model schizophrenia in rodents. Animal models can represent diseases from three different perspectives: (1) reproducing etiopathogenetic factors, (2) simulating signs and symptoms, or (3) predictability of response to treatment. We refer to these approaches as construct validity, face validity, and predictive validity respectively. The faithfulness of each type of validity varies considerably, and the utility of the proposed model depends greatly on the goals of each study. Oftentimes, models with high predictive validity are used for the development of new pharmacological treatments, but animal models with high construct validity provide a better framework for studying pathological processes and outcomes. Given the complexity of the nervous system and the lack of valid pathognomonic biological markers, phenotypes, or genotypes of schizophrenia, a heuristic model that would encompass different aspects of schizophrenia would be desirable [58]. These aspects should include anatomical, neurochemical, behavioral, and cognitive features of schizophrenia, but it is rare that a single model addresses multiple phenomena [58]. The best replicated neurobiological findings are thinning of prefrontal and temporal region cortices [59], and decreased expression of calcium-binding protein parvalbumin and GAD67 enzyme in cortical interneurons [60–64].

Animal models of schizophrenia have been categorized to date in three main groups: neurodevelopmental models, genetic models, and pharmacological models [58, 59, 65–69]. Neurodevelopmental models include obstetrical complications such as gestational malnutrition or prenatal exposure to influenza virus. Early stressors such as maternal separation and social isolation [70–72] have also been used, but usually combined with genetic or pharmacological approaches, also named "two-hit models" [73]. Neonatal brain lesions in ventral hippocampus have been widely performed, and present face validity in terms of damaged brain structures, although the disturbance is far more severe than in schizophrenia [74–77]. In any case, the causative role of any of these approaches is dubious, and thus the construct validity. Schizophrenia is highly heritable, and a genetic component of the disease is beyond discussion. Genes interact with environmental factors, and depending on that interaction

the disorder may or may not emerge. Animal genetic models have been perfectly reproduced for some diseases, which give strong construct and face validity. However, given the large number of genes involved in schizophrenia, and their complex interplay [78] with stochastic and environmental factors, it is unlikely that a faithful model can be built based entirely on this approach. Genes that have been involved with increased risk of schizophrenia are dysbindin, neuroregulin-1 (NRG-1), and disrupted-in-schizophrenia 1 (DISC-1), among others [35, 68]. With regard to pharmacological approaches, early studies showed that D2 dopamine receptor antagonists reduced positive symptoms of schizophrenia. Therefore, a dysfunction of dopaminergic neurotransmission has been the most enduring theory as the underlying cause of schizophrenia. Despite the longevity of the dopaminergic hypothesis and its face validity in schizophrenia research, it is now believed that dopaminergic dysfunction is a consequence rather than the cause [79]. Animal models of non-competitive NMDA antagonists are currently the most characterized pharmacological approach. Phencyclidine (PCP) and ketamine have been shown to induce psychosis in healthy humans and to exacerbate positive symptoms of schizophrenic patients [80]. This suggested the involvement of NMDA receptors in the pathophysiology of schizophrenia. The effect of non-competitive NMDA antagonists is not completely understood, but seems to have complex interactions in glutamatergic, dopaminergic, and GABAergic neurotransmission [81]. Altered glutamate transmission and NMDA receptors have been related to negative and cognitive symptoms observed in schizophrenia. NMDA antagonists, unlike dopamine, have strong construct validity for studying cognitive and attention deficits of schizophrenia [65, 67, 71]. Some authors have suggested that NMDA antagonists fail to take into account neurodevelopmental processes, mainly because acute doses of NMDA antagonist have been used in the literature, and short-term consequences measured rather than long-term effects. Nevertheless, in recent years repeated subchronic/chronic administration of NMDA antagonists has been used during the early postnatal period to model cognitive deficits of schizophrenia, and long-term behavioral aspects evaluated (during adolescence and adulthood) [69, 82]. MK-801, also known as dizocilpine, is the most potent and selective drug among non-competitive NMDA antagonists, and therefore widely used to model schizophrenia in rodents [83].

NMDA Hypofunction and Brain Maturation

Glutamate activates intracellular cascades via ionotropic and metabotropic receptors. Glutamate is integral in neurodevelopment. It regulates synaptogenesis, network plasticity, dendritic arborization, neuronal progenitor propagation, and migration [84]. NMDA receptors are the only glutamatergic excitatory receptors postnatally, as functional AMPA receptors are absent at the beginning of the postnatal period [85]. Glutamate can act via ionotropic and metabotropic receptors. Glutamate ionotropic receptors, N-methyl-D-aspartate receptor (NMDAR), are the target in schizophrenia research. Hypofunction of NMDAR plays a role not only in psychiatric diseases like schizophrenia, but also in Alzheimer's disease or autism [86]. The differences in clinical and neuropathological presentations might account for the timing and cause of NMDAR hypofunction.

NMDARs are made of four subunits, forming a heterotetramer composed by NR1 subunit and the facultative NR2 (A, B, C or D) or NR3 (A or B). NR3 subunits are mainly found in early development. NR2 subunits regulate the channel gating. NR2A subunit is the most abundant throughout the nervous system, but NR2B is predominant in forebrain and hippocampus. Depending on the combination of NMDA subunits, the electrophysiological properties of NMDA receptors vary. NR1-NR2B combinations have longer excitatory postsynaptic potentials in vitro than NR1-NR2A complexes [87]. NMDA receptor subunits are also involved in synaptic plasticity: a shift in subunit expression in a particular receptor potentially changes its functional properties. In fact, NR2B incorporation could increase the time period for synaptic coincidence, thereby enhancing synaptic efficacy and probably memory function. NMDAR

subunits also differ in their binding sites: NR1 subunits have glycine binding sites, and NR2 subunits glutamate binding sites. Glycine acts as a co-agonist, meaning that its binding to NMDAR is a prerequisite for the activation of NMDA receptor, together with the removal of the magnesium block. D-serine can also function as a co-agonist when it binds to glycine-B sites of NMDAR. At resting membrane potentials, magnesium ions enter the channel pore and prevent further ion permeation. A membrane depolarization is necessary to dislodge and repel magnesium block, to allow ion flow through the channel. In addition to the heterotetramer, NMDA receptors have postsynaptic densities (PSD), a set of proteins that give structural and functional stability to glutamatergic synapses.

NMDA receptors are tightly related to brain maturation. In PFC, functional NMDARs are expressed in tangentially migrating interneuron precursors [88]. Depending on the electrophysiological properties of interneurons, NMDAR mediated-currents vary. Regular-spiking (RS) interneurons maintain NMDA-mediated currents constant through development, whereas fast-spiking (FS) interneurons have a large decay [89]. This decrease is more prominent from postnatal weeks 2–4 and from weeks 12–15 [89]. NMDAR mediated currents of FS-cells decrease approximately from 75% in juvenile rats to 25% in adult animals. This is probably secondary to changes in NR2 subunits. In fact, brain circuitry maturation usually coincides with NMDAR subunit switch, marking the transition from young to adult neural processing. NR2 subunit switch is cell type-specific in prefrontal cortex, with NR2B levels remaining constant until adulthood in pyramidal cells, but with a gradual replacement from NR2B to NR2A in fast-spiking interneurons, particularly in adolescence [89, 90]. Subunits switch makes NMDARs extremely vulnerable to genetic risk factors and environmental perturbations, and both interact to affect normal brain development [91]. Similarly, NMDAR subunit expression and function in hippocampus is necessary for proper hippocampal development, with NMDAR dysregulation resulting in failures in synaptogenesis and circuit maturation [92–95].

Recent evidence supports the finding of abnormal glutamatergic transmission and NMDAR hypofunction in schizophrenic individuals [84]. Firstly, multiple genes involved in increased risk for schizophrenia are known to alter NMDAR-mediated signaling [96, 97]. Susceptibility genes for schizophrenia therefore regulate neuronal proliferation, migration, and synaptogenesis. Secondly, dysregulation of NMDAR subunits in postmortem brains of schizophrenic patients, in which NR1 subunits are decreased, further indicates perturbed NMDA function. Thirdly, transgenic mice with low NMDAR expression and animal models of NMDA antagonism present symptoms reminiscent of schizophrenia. NMDA antagonism not only produces behavioral changes, but also patterns of metabolic and neurochemical alterations of the disease [98]. Taken together, it is increasingly recognized that schizophrenia is a neurodevelopmental disorder, in which early brain development is affected [99].

Effects of MK-801 on NMDA Receptors

As stated in a previous section, failure in glutamatergic neurotransmission is known to play a role in the pathophysiology of schizophrenia. MK-801 is a noncompetitive NMDA receptor antagonist that physically blocks the receptor by inserting in the channel pore, binding to several PCP-s binding sites, and preventing the flow of cations through the channel pore. Blocking NMDA receptors results in an excessive release of glutamate that can have an impact on the blocked neuron itself and on downstream brain regions. In fact, early-life MK-801 administration has pro-apoptotic effects shortly after exposure. The ability to activate apoptotic pathways depends on the duration and severity of NMDA blockade. Doses higher than 0.25 mg/kg are necessary to induce irreversible degeneration and cell death [100]. The mechanism for activating apoptotic cell death is not well established, although NMDA receptor coupling to ERK1/2-CREB in early brain development has been proposed to be vital for neurotrophic action of

NMDA receptor. MK-801-induced apoptotic injury is believed to result from the dissociation of the NMDA receptor from the ERK1/2-CREB signaling pathway [82]. Ikonomidou et al. [100] reported that only neuronal cells committed apoptosis with no glial cell activation. Studies from the last decade, however, suggest glial impairment in the cerebral cortex of schizophrenics, including reduced glial cell size and density, and glial dysfunction in prefrontal cortex and hippocampus [101, 102]. Astrocytic glutamate metabolism, more specifically glutamate–glutamine–GABA cycle, is also perturbed after MK-801 administration. Glutamate and glutamine levels after repeated injections of high doses of MK-801 (0.5 mg/kg during 6 days) were comparable to those found in first-episode patients of schizophrenia [103]. The susceptibility for MK-801-induced damage correlates with the highest expression of NMDA receptors (around week 2) and growth spurt (peaks at P10). The excessive release of glutamate after NMDA antagonism, mediates excitotoxicity that goes beyond apoptotic pathways, affecting growth cone activity, and neurite extension and branching [104]. Neuronal injury, such as dendritic atrophy, is also seen in postmortem brains [17].

Glutamate-mediated excitotoxicity has considerable functional consequences. GABAergic interneurons are claimed to be 10 times more sensitive to NMDA receptor antagonism than pyramidal cells [105]. In particular, fast-spiking cells that express the calcium-binding protein parvalbumin (PV) seem to be the most vulnerable to damage after NMDA blockade, and their alteration is sufficient to induce behavioral traits that resemble symptoms of schizophrenia. Several studies have demonstrated that repeated MK-801 administration decreases PV immunoreactivity. Chronic NMDAR blockade in juvenile and adult rats diminishes PV+ densities in hippocampus, especially in the dentate gyrus and CA1 region, shortly after exposure [60, 62]. Neurodevelopmental models further support this finding, but usually doses higher than 0.5 mg/kg are needed to induce long-term structural and anatomical changes [106]. Such large doses not only alter hippocampal PV densities, but also PV cells in mPFC, a region involved in higher cognitive functions [61, 63, 64]. Moreover, Nakazawa et al. [41] demonstrated in transgenic mice that the lack of cortical and hippocampal NMDA receptors in GABAergic interneurons was sufficient to evoke schizophrenia-like features. Most of the altered GABAergic interneurons had, in fact, parvalbumin-positive immunoreactivity. The mechanisms by which PV+ FS-cells can be selectively susceptible to damage after MK-801 administration are unclear, although a number of hypotheses have been proposed. FS-cells express Kv1.3 channels that allow a fast repolarization of the membrane, and the ability to fire the next action potential very rapidly. The high frequency of action potential firing means that the open probability of NMDAR in FS-cells is much higher than in any other GABAergic cell or excitatory cells that fire at a slower rate. As MK-801 is an uncompetitive drug and needs the ion channel to be opened for blocking the receptor, the chances of blocking NMDAR of FS-cells is much higher. Another mechanism is the one described by Wang & Gao [107]. They demonstrated that NMDAR of presynaptic glutamatergic terminals targeting pyramidal cells and FS interneurons were distinctly affected after subchronic MK-801 exposure. Presynaptic NMDAR are critical to modulate and facilitate neurotransmitter release. Interestingly, MK-801 completely blocked presynaptic NMDA receptors in glutamatergic terminals that targeted FS interneurons, whereas new NMDA receptors were inserted in presynaptic terminals that made synaptic contact with pyramidal neurons. This mechanistic approach further confirms that synaptic mechanisms of NMDA blockade are cell-type specific. These two hypotheses are not mutually exclusive, and probably MK-801 alters NMDAR by several mechanisms, but all resulting in PV+ FS-cell underactivation, with overall disinhibition of pyramidal cell activity in cerebral cortex.

It is not known whether the NMDAR on astrocytes are blocked, how this could influence glutamate metabolism and transport or glycine release and uptake, and thereby local circuitry [108]. What has been demonstrated so far is that

astrocytes affect the glutamatergic system. Notably, the upregulation of glutamate transporter-1 (GLT-1) mRNA [109], protein, and activity [110] in astrocytes has been found in prefrontal cortex of schizophrenic patients. Animal studies revealed that NMDAR antagonist phencyclidine provokes similar findings, although the effects of MK-801 have not been studied yet [111]. Furthermore, a selective deletion of astroglial A2AR, which tightly regulates GLT-1 activity [112], decreases working memory in rodents, as measured in radial arm maze [113]. Glycine transporters (GlyT) are present in both astroglial cells and neurons. It is well established that glycine plays a pivotal role in NMDAR neurotransmission, and GlyT are closely associated with NMDAR. Although it was believed that glycine levels in synaptic cleft were enough to saturate the glycine-B sites of the NMDA receptor, it is now known that these levels are below saturation. Under physiological conditions, glycine is actively removed by the action of presynaptic and postsynaptic glycine transporters (GlyT). Augmenting glycine availability in the synaptic cleft could therefore facilitate NMDAR function, and drugs that inhibit GlyT action have been proved to be effective in improving cognition in animal models of schizophrenia induced by MK-801 (Table 25.1) [114–118].

Table 25.1 Pharmacological compounds and behavioral tasks used to demonstrate that glycine transporter inhibition improves cognitive function in MK-801-induced rodent model of schizophrenia

	GlyT inhibitor	Behavioral paradigm
Harada et al. (2012) [114]	ASP2535	Working memory in Y-maze
Shimazaki et al. (2010) [115]	NFPS	Social memory
Black et al. (2009) [116]	NFPS	Latent inhibition
Manahan-Vaughan et al. (2008) [117]	SSR103800 SSR504734	Reference memory in RAM
Karasawa et al. (2008) [118]	NFPS	NOR

NFPS N[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine, *RAM* radial arm maze, *NOR* novel object recognition test

Effects of MK-801 on Brain Circuits and Activity

Acute systemic administration of MK-801 increases mPFC activity, and the decreased signal-to-noise ratio could account for the deficits in mPFC-dependent executive functions. Unraveling the mechanisms by which this occurs could contribute to a better understanding of the dysfunctional brain. As previously suggested, a local disinhibition of mPFC secondary to PV+ FS GABAergic cell underactivation could explain increased excitability of this region. Nevertheless, it has been shown that local infusion of MK-801 in CA1 of hippocampus augments mPFC neural activity in a similar manner to systemic administration [119]. According to these findings, local disinhibition of pyramidal cells in CA1 that send glutamatergic projections to mPFC through hippocampal-PFC pathway could contribute to the overexcitation of mPFC. The hippocampal–PFC pathway supports high-order cognitive functions [120–122]. Blot et al. [29] demonstrated an aberrant form of plasticity in this pathway after acute MK-801 administration that correlated with impaired working memory and learning flexibility in rodents. NMDA receptors are involved in brain plasticity, and cellular models of learning and memory formation such as LTP or LTD are NMDAR-dependent. Alterations in plasticity could underlie the pathophysiology of schizophrenic symptoms. In fact, human studies indicate that there is impaired glutamatergic plasticity in schizophrenic brains [123], and the hippocampal–prefrontal pathway is crucial in the pathophysiology [28]. Blot et al. [29] demonstrated that a single dose of MK-801 (0.1 mg/kg) evoked long-lasting response to synaptic input from the ventral hippocampus that was independent of synchronized afferent inputs — necessary for standard LTP. The authors suggested that this was an aberrant form of plasticity. For LTP formation in mPFC of hippocampus–prefrontal pathway synapses, concurrent activation of dopaminergic and NMDA receptors is necessary. As suggested by the authors, an excessive release of glutamate and dopamine by the action of MK-801 in mPFC could be the mechanism by which the aberrant form of plasticity

takes place. Furthermore, subchronic administration of MK-801 during 14 days hindered LTP induction. According to the results of Manahan-Vaughan et al. [117], LTP induction and expression was also profoundly impaired in the dentate gyrus 1 week after administering MK-801 acutely (5 mg/kg), and consequently learning deficits were present in MWM. LTP and learning performance were rescued by application of glycine transporter-1 inhibitors discussed before. In a subsequent study of the same group, LTP impairment was also present 4 weeks after drug administration, due to hippocampal hyperactivity and changes in expression of GABA and NMDA receptors in the prefrontal cortex and hippocampus that led to poor inhibitory control of prefrontal cortex [81]. This uncoupling of hippocampal–prefrontal communication could account for learning impairments and memory dysfunction found in MK-801-treated animals.

The nucleus accumbens has been identified as another important brain region that links several findings of schizophrenic disturbances [124–126]. The vast majority of neurons in nucleus accumbens are medium spiny neurons, a special type of GABAergic inhibitory neurons that have long-range projections. They receive glutamatergic inputs from hippocampus, prefrontal cortex, and amygdala, and dopaminergic inputs from the ventral tegmental area (VTA) (Fig. 25.2). Medium spiny neurons modulate inhibitory control on thalamocortical glutamatergic neurons. Local blockade of NMDA receptors in nucleus accumbens has been demonstrated to be sufficient to augment PFC activity and impair working memory [127]. Mesolimbic pathway connects dopaminergic neurons from ventral tegmental area with GABAergic neurons of nucleus accumbens. There is a differential dysregulation of mesocortical and mesolimbic dopaminergic pathways in schizophrenia. Mesocortical pathway is hypoactivated, whereas increased firing of dopaminergic neurons in mesolimbic pathway is found. The overexcitation of mesolimbic pathway prevents proper inhibition by accumbal neurons. This results in sensory information overload

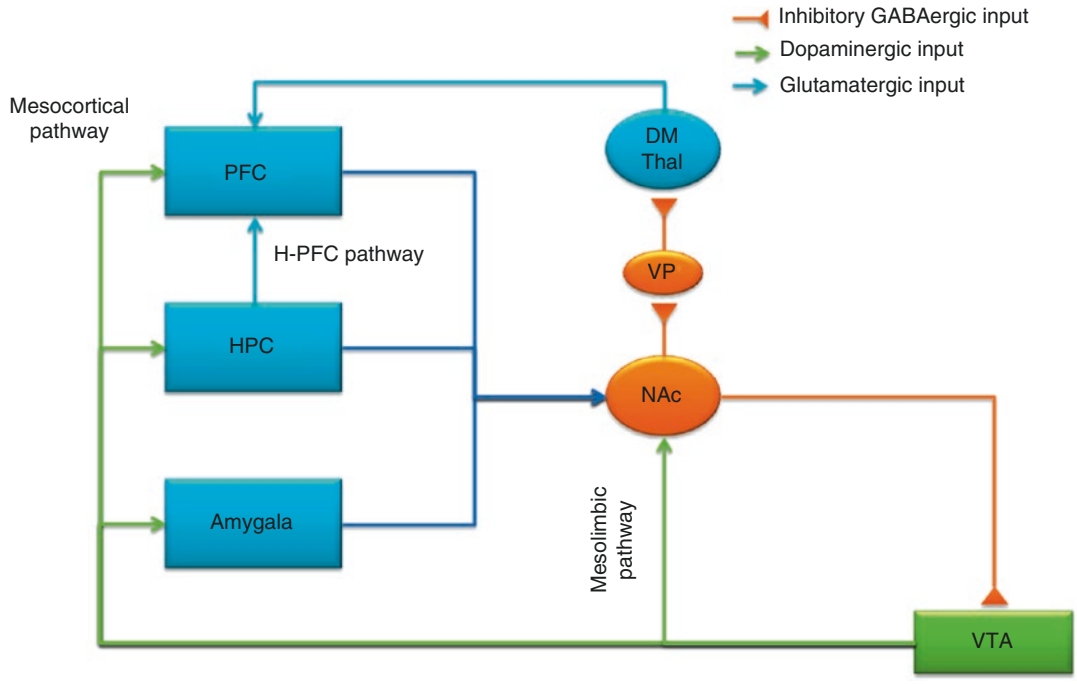


Fig. 25.2 Schematic representation of the major afferent and efferent connections of the NAc. *PFC* prefrontal cortex, *HPC* hippocampus, *DM Thal* dorsomedial thalamus, *VP* ventral pallidum, *NAc* nucleus accumbens, *VTA* ventral tegmental area

to cortex and a lack of feedback inhibition to VTA dopaminergic neurons. In turn, this will lead to an excessive release of glutamate in nucleus accumbens through the overactivation of glutamatergic neurons projecting to nucleus accumbens (Fig. 25.2).

Effects of MK-801 on Cognition

Memory function enables storage and retrieval of information over variable periods ranging from seconds to years, and is critical to daily life functioning. In schizophrenic patients, not only memory but all areas of cognition seem to be impaired, suggesting widespread cortical dysfunction. Cognitive symptoms are considered a distinct dimension of the illness, and are relatively independent of positive and negative symptoms. Cognitive deficits include impaired working, spatial, and declarative memory, attentional dysfunction, and poor cognitive flexibility. Working memory [19] and episodic memory [128] are especially sensitive to neuropsychiatric disorders, and appear to be core features of schizophrenia. Valid schizophrenia animal models should mimic at least one of these cognitive deficits.

Behavioral paradigms that evaluate working memory are non-matching to sample of objects or odors, operant tasks, and paradigms that use spatial information, such as maze tasks (delayed alternation, radial-arm maze, Morris water maze) [129]. Using these devices, deficits in working memory have been consistently shown after MK-801 administration [130–134]. Morris water maze (MWM) and radial arm maze (RAM) are the most widely performed tasks for spatial learning and memory assessment. MK-801 impairs spatial learning and memory. Concretely, acquisition, reversal learning, and working memory performance are affected in hidden-platform trials, although reference memory is spared [106, 135–139]. Spatial learning tasks are readily available both in humans and animals, and allow a direct translation of findings. In an attempt to bridge the gap between human and animal research, a variant of Morris water maze has been used in schizophrenic patients, showing decreased navigational abilities in human patients, and further confirming spatial impairment [140]. Deficits in cognitive or behavioral flexibility have been documented in schizophrenic patients [141], a type of executive function carried out by prefrontal cortex. MK-801-induced animal models have also displayed problems in cognitive flexibility measured by reversal learning in MWM, as stated previously, and by active place avoidance tasks in radial arm maze [29, 136]. It is well established that hippocampal neurons are essential for spatial navigation. Nevertheless, the neurocircuitry involved in spatial learning and memory contains different systems that collaborate in serial or parallel fashion. In this context, the role of medial prefrontal cortex is essential. Neurons from cornus ammonis 1 (CA1) send projections to frontal areas, mainly the prelimbic and cingulated cortices [142]. Disconnecting hippocampus from the medial prefrontal cortex impairs spatial memory and spatial working memory in rodents [120, 121]. It seems that spatial information is acquired by the hippocampus and then transferred to mPFC.

Novel object recognition (NOR) is one of the most widely performed preclinical cognitive tests for schizophrenia [143], and it has long been considered the analog of human episodic memory. Multiple schizophrenia-relevant studies have used NOR for exploring cognitive impairment in rodents [144, 145]. NOR is based on a rodent's natural tendency to explore new stimuli and environments. Following acute administration of MK-801, NOR is severely disrupted [146, 147, 118] but in neurodevelopmental models these results failed to be replicated. In fact, early-life repeated injections of MK-801 have no long-term consequences in NOR, using delays of 1.5 h [148], 2 h [149] and 5 h [150] between acquisition and test trials. Using NOR for evaluating declarative memory has raised some concerns, as it seems that NOR is a familiarity-based test, which is not affected in schizophrenic patients, rather than a recollection-based test [151, 152]. Tests that use associative or relational information are more closely related to human episodic memory. Such

tests need temporal and spatial precision of object memory. Li et al. [64] demonstrated that associative recognition memory was impaired in adolescence and adulthood after early-life NMDA blockade. The dissociation of results between NOR and tests that use relational information in MK-801 rodent model could be explained by neural circuitry. Brain wiring for rodent recognition memory involves several structures, but perirhinal (Prh) cortex plays a major role [153]. Although hippocampus (HPC), medial prefrontal cortex (mPFC), mediodorsal thalamus (MD) and postrhinal cortex (PostRh) participate in recognition memory, NOR is particularly sensitive to perirhinal cortex dysfunction, and not to hippocampal alterations. Rather, the role of hippocampus is to integrate object information with spatial or contextual information. Similarly, mPFC integrates spatial information from CA1 subfields of HPC with object information of Prh cortex, using NMDA-dependent synaptic plasticity [154]. Therefore, associative tasks require network interdependency across multiple structures, in which HPC–mPFC-Prh circuits are essential for memory acquisition and retrieval. Furthermore, associative memory depends on NMDA receptor neurotransmission, and hippocampal NMDAR are required for acquisition, but not retrieval, of associative memories [154]. mPFC and HPC are two critical brain regions in the pathophysiology of schizophrenia, so assessing cognitive functions dependent on the interaction of both regions will preferably reveal cognitive deficits in rodents.

Conclusions

Neonatal administration of MK-801 instills a hypofunction of NMDAR that results in a widespread apoptotic injury during synaptogenesis. The GABAergic system is particularly sensitive in the developing brain to transient NMDA blockade. Among subpopulations of GABAergic interneurons, PV+ cells seem to provide the most potent inhibitory input on pyramidal neurons by formation of synaptic contacts in soma, proximal dendrites, and axon initial segment. A decrease in PV+ interneuron population following MK-801 administration therefore has a significant impact on neuronal synchrony and information processing. Disrupted synaptic integration in early brain development results in modified network activity and plasticity in adulthood. This is corroborated by behavioral data that show a constellation of neurobehavioral sequelae that resemble symptoms of schizophrenia.

Mimicking behavioral and cognitive deficits of schizophrenia in animal models is a real challenge, and the validity of cognitive deficits in animal models largely depends on the appropriate behavioral paradigm. Cognitive impairment is best assessed by means of tasks that require mPFC–HPC interactions, a circuit that underlies episodic, working, and spatial memory. Although deficits elicited by repeated NMDA antagonism are unlikely to represent an animal model of schizophrenia *per se*, a strong body of evidence supports an MK-801-induced neurodevelopmental model of schizophrenia as a valid model for some of the essential deficits occurring in this condition.

Acknowledgements This study was supported by grants from the University of the Basque Country UPV/EHU (UFI 11/32), (EHU 14/33), and by the Government of the Basque Country (GIC IT 901/16). Murueta-Goyena A is financed by a predoctoral fellowship of the University of the Basque Country (UPV/EHU).

References

1. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. PLoS Med. 2005;2(5):e141.
2. Green MF, Kern RS, Braff DL, Mintz J. Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the "right stuff"? Schizophr Bull. 2000;26(1):119–36.
3. Gold JM. Cognitive deficits as treatment targets in schizophrenia. Schizophr Res. 2004;72(1):21–8.
4. Bouwmans C, de Sonneville C, Mulder CL, Hakkaart-van RL. Employment and the associated impact on quality of life in people diagnosed with schizophrenia. Neuropsychiatr Dis Treat. 2015;11:2125–42.
5. Bergen SE, Petryshen TL. Genome-wide association studies of schizophrenia: does bigger lead to better results? Curr Opin Psychiatry. 2012;25(2):76–82.
6. Kukshal P, Thelma BK, Nimgaonkar VL, Deshpande SN. Genetics of schizophrenia from a clinicial perspective. Int Rev Psychiatry. 2012;24(5):393–404.

7. Owen MJ. Implications of genetic findings for understanding schizophrenia. Schizophr Bull. 2012;38(5):904–7.
8. Greenwood TA, Swerdlow NR, Gur RE, Cadenhead KS, Calkins ME, Dobie DJ, et al. Genome-wide linkage analyses of 12 endophenotypes for schizophrenia from the consortium on the genetics of schizophrenia. Am J Psychiatry. 2013;170(5):521–32.
9. Singh S, Kumar A, Agarwal S, Phadke SR, Jaiswal Y. Genetic insight of schizophrenia: past and future perspectives. Gene. 2014;535(2):97–100.
10. Brown AS. Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism. Dev Neurobiol. 2012;72(10):1272–6.
11. Labouesse MA, Langhans W, Meyer U. Long-term pathological consequences of prenatal infection: beyond brain disorders. Am J Physiol Regul Integr Comp Physiol. 2015;309(1):R1–R12.
12. Bourque F, van der Ven E, Fusar-Poli P, Malla A. Immigration, social environment and onset of psychotic disorders. Curr Pharm Des. 2012;18(4):518–26.
13. Pishva E, Kenis G, van den Hove D, Lesch KP, Boks MP, van Os J, et al. The epigenome and postnatal environmental influences in psychotic disorders. Soc Psychiatry Psychiatr Epidemiol. 2014;49(3):337–48.
14. Uher R. Gene–environment interactions in severe mental illness. Front Psych. 2014;5:48.
15. Ordóñez AE, Luscher ZI, Gogtay N. Neuroimaging findings from childhood onset schizophrenia patients and their non-psychotic siblings. Schizophr Res. 2015;72(8):803–12.
16. Kubota M, van Haren NE, Haijma SV, Schnack HG, Cahn W, Hulshoff Pol HE et al. Association of IQ changes and progressive brain changes in patients with schizophrenia. JAMA Psychiatry. 2015;72(8):803–12.
17. Garey L. When cortical development goes wrong: schizophrenia as a neurodevelopmental disease of microcircuits. J Anat. 2010;217(4):324–33.
18. Liu J, Corbera S, Wexler BE. Neural activation abnormalities during self-referential processing in schizophrenia: an fMRI study. Psychiatry Res. 2014;222(3):165–71.
19. Silver H, Feldman P, Bilker W, Gur RC. Working memory deficit as a core neuropsychological dysfunction in schizophrenia. Am J Psychiatry. 2003;160(10):1809–16.
20. Barch DM. What can research on schizophrenia tell us about the cognitive neuroscience of working memory? Neuroscience. 2006;139(1):73–84.
21. Wolf DH, Satterthwaite TD, Calkins ME, Ruparel K, Elliott MA, Hopson RD. Functional neuroimaging abnormalities in youth with psychosis spectrum symptoms. JAMA Psychiat. 2015;72(5):456–65.
22. Brunoni AR, Vanderhasselt MA. Working memory improvement with non-invasive brain stimulation of the dorsolateral prefrontal cortex: a systematic review and meta-analysis. Brain Cogn. 2014;86:1–9.
23. Arnsten AF, Jin LE. Molecular influences on working memory circuits in dorsolateral prefrontal cortex. Prog Mol Biol Transl Sci. 2014;122:211–31.
24. Benes FM. Emerging principles of altered neural circuitry in schizophrenia. Brain Res Brain Res Rev. 2000;31(2-3):251–69.
25. Rotaru DC, Lewis DA, Gonzalez-Burgos G. The role of glutamatergic inputs onto parvalbumin-positive interneurons: relevance for schizophrenia. Rev Neurosci. 2012;23(1):97–109.
26. Volk DW, Lewis DA. Impaired prefrontal inhibition in schizophrenia: relevance for cognitive dysfunction. Physiol Behav. 2002;77(4-5):501–5.
27. Meyer-Lindenberg AS, Olsen RK, Kohn PD, Brown T, Egan MF, Weinberger DR, et al. Regionally specific disturbance of dorsolateral prefrontal–hippocampal functional connectivity in schizophrenia. Arch Gen Psychiatry. 2005;62(4):379–86.
28. Godsil BP, Kiss JP, Spedding M, Jay TM. The hippocampal–prefrontal pathway: the weak link in psychiatric disorders? Eur Neuropsychopharmacol. 2013;23(10):1165–81.
29. Blot K, Bai J, Otani S. The effect of non-competitive NMDA receptor antagonist MK-801 on neuronal activity in rodent prefrontal cortex: an animal model for cognitive symptoms of schizophrenia. J Physiol Paris. 2013;107(6):448–51.
30. Blum BP, Mann JJ. The GABAergic system in schizophrenia. Int J Neuropsychopharmacol. 2002;5(2):159–79.
31. Reynolds GP, Abdul-Monim Z, Neill JC, Zhang ZJ. Calcium binding protein markers of GABA deficits in schizophrenia — postmortem studies and animal models. Neurotox Res. 2004;6(1):57–61.
32. Gonzalez-Burgos G, Cho RY, Lewis DA. Alterations in cortical network oscillations and parvalbumin neurons in schizophrenia. Biol Psychiatry. 2015;77(12):1031–40.
33. Joshi D, Catts VS, Olaya JC, Shannon WC. Relationship between somatostatin and death receptor expression in the orbital frontal cortex in schizophrenia: a postmortem brain mRNA study. NPJ Schizophrenia. 2015;1:14004.
34. Jentsch TJ, Stein V, Weinreich F, Zdebik AA. Molecular structure and physiological function of chloride channels. Physiol Rev. 2002;82(2):503–68.
35. Le Magueresse C, Monyer H. GABAergic interneurons shape the functional maturation of the cortex. Neuron. 2013;77(3):388–405.
36. Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA. Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. Arch Gen Psychiatry. 2000;57(3):237–45.
37. Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, et al. Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J Neurosci. 2003;23(15):6315–26.

38. Zhang ZJ, Reynolds GP. A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. Schizophr Res. 2002;55(1–2):1–10.
39. Singer W. The formation of cooperative cell assemblies in the visual cortex. J Exp Biol. 1990;153:177–97.
40. Uhlhaas PJ. Dysconnectivity, large-scale networks and neuronal dynamics in schizophrenia. Curr Opin Neurobiol. 2013;23(2):283–90.
41. Nakazawa K, Zsiros V, Jiang Z, Nakao K, Kolata S, Zhang S, et al. GABAergic interneuron origin of schizophrenia pathophysiology. Neuropharmacology. 2012;62(3):1574–83.
42. Williams S, Boksa P. Gamma oscillations and schizophrenia. J Psychiatry Neurosci. 2010;35(2):75–7.
43. Qin ZH, Zhang SP, Weiss B. Dopaminergic and glutamatergic blocking drugs differentially regulate glutamic acid decarboxylase mRNA in mouse brain. Brain Res Mol Brain Res. 1994;21(3-4):293–302.
44. Gierdalski M, Jablonska B, Smith A, Skangiel-Kramska J, Kossut M. Deafferentation induced changes in GAD67 and GluR2 mRNA expression in mouse somatosensory cortex. Brain Res Mol Brain Res. 1999;71(1):111–9.
45. Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, et al. The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature. 1999;397(6716):251–5.
46. Bortone D, Polleux F. KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner. Neuron. 2009;62(1):53–71.
47. Del Rio JA, Soriano E, Ferrer I. Development of GABA-immunoreactivity in the neocortex of the mouse. J Comp Neurol. 1992;326(4):501–26.
48. Doischer D, Hosp JA, Yanagawa Y, Obata K, Jonas P, Vida I, et al. Postnatal differentiation of basket cells from slow to fast signaling devices. J Neurosci. 2008;28(48):12956–68.
49. Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci. 1992;12(11):4151–72.
50. Fritschy JM, Meskenaite V, Weinmann O, Honer M, Benke D, Mohler H. GABAB-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. Eur J Neurosci. 1999;11(3):761–8.
51. Bitanihirwe BK, Woo TU. Perineuronal nets and schizophrenia: the importance of neuronal coatings. Neurosci Biobehav Rev. 2014;45:85–99.
52. Eastwood SL, Harrison PJ. Interstitial white matter neurons express less reelin and are abnormally distributed in schizophrenia: towards an integration of molecular and morphologic aspects of the neurodevelopmental hypothesis. Mol Psychiatry. 2003;8(9):769. 821–31
53. Joshi D, Fung SJ, Rothwell A, Weickert CS. Higher gamma-aminobutyric acid neuron density in the white matter of orbital frontal cortex in schizophrenia. Biol Psychiatry. 2012;72(9):725–33.
54. Steinecke A, Gampe C, Valkova C, Kaether C, Bolz J. Disrupted-in-schizophrenia 1 (DISC1) is necessary for the correct migration of cortical interneurons. J Neurosci. 2012;32(2):738–45.
55. Fazzari P, Paternain AV, Valiente M, Pla R, Luján R, Lloyd K, et al. Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. Nature. 2010;464(7293):1376–80.
56. Volk DW, Pierri JN, Fritschy JM, Auh S, Sampson AR, Lewis DA. Reciprocal alterations in pre- and postsynaptic inhibitory markers at chandelier cell inputs to pyramidal neurons in schizophrenia. Cereb Cortex. 2002;12(10):1063–70.
57. Zheng K, An JJ, Yang F, Xu W, Xu ZQ, Wu J, et al. TrkB signaling in parvalbumin-positive interneurons is critical for gamma-band network synchronization in hippocampus. Proc Natl Acad Sci U S A. 2011;108(41):17201–6.
58. Lipska BK, Weinberger DR. To model a psychiatric disorder in animals: schizophrenia as a reality test. Neuropsychopharmacology. 2000;23(3):223–39.
59. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. Nat Neurosci. 2010;13(10):1161–9.
60. Rujescu D, Bender A, Keck M, Hartmann AM, Ohl F, Raeder H, et al. A pharmacological model for psychosis based on N-methyl-D-aspartate receptor hypofunction: molecular, cellular, functional and behavioral abnormalities. Biol Psychiatry. 2006;59(8):721–9.
61. Abekawa T, Ito K, Nakagawa S, Koyama T. Prenatal exposure to an NMDA receptor antagonist, MK-801 reduces density of parvalbumin-immunoreactive GABAergic neurons in the medial prefrontal cortex and enhances phencyclidine-induced hyperlocomotion but not behavioral sensitization to methamphetamine. Psychopharmacology (Berl). 2007;192(3):303–16.
62. Braun I, Genius J, Grunze H, Bender A, Möller HJ, Rujescu D. Alterations of hippocampal and prefrontal GABAergic interneurons in an animal model of psychosis induced by NMDA receptor antagonism. Schizophr Res. 2007;97(1–3):254–63.
63. Coleman Jr LG, Jarskog LF, Moy SS, Crews FT. Deficits in adult prefrontal cortex neurons and behavior following early post-natal NMDA antagonist treatment. Pharmacol Biochem Behav. 2009;93(3):322–30.
64. Li JT, Zhao YY, Wang HL, Wang XD, Su YA, Si TM. Long-term effects of neonatal exposure to MK-801 on recognition memory and excitatory-inhibitory balance in rat hippocampus. Neuroscience. 2015;308:134–43.
65. Marcotte ER, Pearson DM, Srivastava LK. Animal models of schizophrenia: a critical review. J Psychiatry Neurosci. 2001;26(5):395–410.
66. Jones CA, Watson DJG, Fone KCF. Animal models of schizophrenia. Br J Pharmacol. 2011;164(4):1162–94.
67. van der Staay FJ, Arndt SS, Nordquist RE. Evaluation of animal models of neurobehavioral disorders. Behav Brain Funct. 2009;5:11.
68. Salgado JV, Sandner G. A critical overview of animal models of psychiatric disorders: challenges and

perspectives. Rev Bras Psiquiatr. 2013;35(Suppl 2):S77–81.
69. Bubeníková-Valesová V, Horácek J, Vrajová M, Höschl C. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. Neurosci Biobehav Rev. 2008;32(5):1014–23.
70. Jones GH, Hernandez TD, Kendall DA, Marsden CA, Robbins TW. Dopaminergic and serotonergic function following isolation rearing in rats: study of behavioural responses and postmortem and in vivo neurochemistry. Pharmacol Biochem Behav. 1992;43(1):17–35.
71. Geyer MA, Wilkinson LS, Humby T, Robbins TW. Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. Biol Psychiatry. 1993;34(6):361–72.
72. Wilkinson LS, Killcross SS, Humby T, Hall FS, Geyer MA, Robbins TW. Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition. Neuropsychopharmacology. 1994;10(1):61–72.
73. Gilabert-Juan J, Belles M, Saez AR, Carceller H, Zamarbide-Fores S, Moltó MD, et al. A "double hit" murine model for schizophrenia shows alterations in the structure and neurochemistry of the medial prefrontal cortex and the hippocampus. Neurobiol Dis. 2013;59:126–40.
74. Daenen EW, Wolterink G, Van Der Heyden JA, Kruse CG, Van Ree JM. Neonatal lesions in the amygdala or ventral hippocampus disrupt prepulse inhibition of the acoustic startle response; implications for an animal model of neurodevelopmental disorders like schizophrenia. Eur Neuropsychopharmacol. 2003;13(3):187–97.
75. Rueter LE, Ballard ME, Gallagher KB, Basso AM, Curzon P, Kohlhaas KL. Chronic low dose risperidone and clozapine alleviate positive but not negative symptoms in the rat neonatal ventral hippocampal lesion model of schizophrenia. Psychopharmacology (Berl). 2004;176(3–4):312–9.
76. Marquis JP, Goulet S, Doré FY. Neonatal ventral hippocampus lesions disrupt extra-dimensional shift and alter dendritic spine density in the medial prefrontal cortex of juvenile rats. Neurobiol Learn Mem. 2008;90(2):339–46.
77. Tseng KY, Lewis BL, Hashimoto T, Sesack SR, Kloc M, Lewis DA, et al. A neonatal ventral hippocampal lesion causes functional deficits in adult prefrontal cortical interneurons. J Neurosci. 2008;28(48):12691–9.
78. Green JG, McLaughlin KA, Berglund PA, Gruber MJ, Sampson NA, Zaslavsky AM, et al. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: associations with first onset of DSM-IV disorders. Arch Gen Psychiatry. 2010;67(2):113–23.
79. Grace AA. Gating of information flow within the limbic system and the. Brain Res Brain Res Rev. 2000;31(2–3):330–41.
80. Lahti AC, Weiler MA, Tamara Michaelidis BA, Parwani A, Tamminga CA. Effects of ketamine in normal and schizophrenic volunteers. Neuropsychopharmacology. 2001;25(4):455–67.
81. Grüter T, Wieschollеck V, Dubovyk V, Aliane V, Manahan-Vaughan D. Altered neuronal excitability underlies impaired hippocampal function in an animal model of psychosis. Front Behav Neurosci. 2015;9:117.
82. Lim AL, Taylor DA, Malone DT. Consequences of early life MK-801 administration: long-term behavioural effects and relevance to schizophrenia research. Behav Brain Res. 2012;227(1):276–86.
83. Wong EH, Kemp JA, Priestley T, Knight AR, Woodruff GN, Iversen LL. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. Proc Natl Acad Sci U S A. 1986;83(18):7104–8.
84. Snyder MA, Gao WJ. NMDA hypofunction as a convergence point for progression and symptoms of schizophrenia. Front Cell Neurosci. 2013;7:31.
85. Ben-Ari Y, Khazipov R, Leinekugel X, Caillard O, Gaiarsa JL. GABAA, NMDA and AMPA receptors: a developmentally regulated 'ménage à trois'. Trends Neurosci. 1997;20(11):523–9.
86. Newcomer JW, Farber NB, Olney JW. NMDA receptor function, memory, and brain aging. Dialogues Clin Neurosci. 2000;2(3):219–32.
87. Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron. 1994;12(3):529–40.
88. Soria JM, Valdeolmillos M. Receptor-activated calcium signals in tangentially migrating cortical cells. Cereb Cortex. 2002;12(8):831–9.
89. Wang HX, Gao WJ. Cell type-specific development of NMDA receptors in the interneurons of rat prefrontal cortex. Neuropsychopharmacology. 2009;34(8):2028–40.
90. Wang H, Stradtman 3rd GG, Wang XJ, Gao WJ. A specialized NMDA receptor function in layer 5 recurrent microcircuitry of the adult rat prefrontal cortex. Proc Natl Acad Sci U S A. 2008;105(43):16791–6.
91. Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev. 2000;24(4):417–63.
92. Roberts AC, Díez-García J, Rodriguiz RM, López IP, Luján R, Martínez-Turrillas R, et al. Downregulation of NR3A-containing NMDARs is required for synapse maturation and memory consolidation. Neuron. 2009;63(3):342–56.
93. Brigman JL, Wright T, Talani G, Prasad-Mulcare S, Jinde S, Seabold GK, et al. Loss of GluN2B-containing NMDA receptors in CA1 hippocampus and cortex impairs long-term depression, reduces dendritic spine density, and disrupts learning. J Neurosci. 2010;30(13):4590–600.
94. Gambrill AC, Barria A. NMDA receptor subunit composition controls synaptogenesis and synapse stabilization. Proc Natl Acad Sci U S A. 2011;108(14):5855–60.

95. Gray JA, Shi Y, Usui H, During MJ, Sakimura K, Nicoll RA. Distinct modes of AMPA receptor suppression at developing synapses by GluN2A and GluN2B: single-cell NMDA receptor subunit deletion in vivo. Neuron. 2011;71(6):1085–101.
96. Moghaddam B. Bringing order to the glutamate chaos in schizophrenia. Neuron. 2003;40(5):881–4.
97. Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry. 2005;10(1):40–68.
98. Morris BJ, Cochran SM, Pratt JA. PCP: from pharmacology to modelling schizophrenia. Curr Opin Pharmacol. 2005;5(1):101–6.
99. Lewis DA, Gonzalez-Burgos G. Neuroplasticity of neocortical circuits in schizophrenia. Neuropsychopharmacology. 2008;33:141–65.
100. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vöckler J, Dikranian K, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science. 1999;283(5398):70–4.
101. Cotter DR, Pariante CM, Everall IP. Glial cell abnormalities in major psychiatric disorders: the evidence and implications. Brain Res Bull. 2001;55(5):585–95.
102. Kondziella D, Brenner E, Eyjolfsson EM, Sonnewald U. How do glial–neuronal interactions fit into current neurotransmitter hypotheses of schizophrenia? Neurochem Int. 2007;50(2):291–301.
103. Kondziella D, Brenner E, Eyjolfsson EM, Markinhuhta KR, Carlsson ML, Sonnewald U. Glial–neuronal interactions are impaired in the schizophrenia model of repeated MK801 exposure. Neuropsychopharmacology. 2006;31(9):1880–7.
104. Ringler S, Aye J, Byrne E, Anderson M, Turner C. Effects of disrupting calcium homeostasis on neuronal maturation: early inhibition and later recovery. Cell Mol Neurobiol. 2008;28(3):389–409.
105. Grunze HC, Rainnie DG, Hasselmo ME, Barkai E, Hearn EF, McCarley RW, et al. NMDA-dependent modulation of CA1 local circuit inhibition. J Neurosci. 1996;16(6):2034–43.
106. van der Staay FJ, Rutten K, Erb C, Blokland A. Effects of the cognition impairer MK-801 on learning and memory in mice and rats. Behav Brain Res. 2011;220(1):215–29.
107. Wang HX, Gao WJ. Prolonged exposure to NMDAR antagonist induces cell-type specific changes of glutamatergic receptors in rat prefrontal cortex. Neuropharmacology. 2012;62(4):1808–22.
108. Takahashi N, Sakurai T. Roles of glial cells in schizophrenia: possible targets for therapeutic approaches. Neurobiol Dis. 2013;53:49–60.
109. Matute C, Melone M, Vallejo-Illarramendi A, Conti F. Increased expression of the astrocytic glutamate transporter GLT-1 in the prefrontal cortex of schizophrenics. Glia. 2005;49(3):451–5.
110. Simpson MD, Slater P, Deakin JF. Comparison of glutamate and gamma-aminobutyric acid uptake binding sites in frontal and temporal lobes in schizophrenia. Biol Psychiatry. 1998;44(6):423–7.
111. Fattorini G, Melone M, Bragina L, Candiracci C, Cozzi A, Pellegrini Giampietro DE, et al. GLT-1 expression and Glu uptake in rat cerebral cortex are increased by phencyclidine. Glia. 2008;56(12):1320–7.
112. Matos M, Augusto E, Santos-Rodrigues AD, Schwarzschild MA, Chen JF, Cunha RA, et al. Adenosine A2A receptors modulate glutamate uptake in cultured astrocytes and gliosomes. Glia. 2012;60(5):702–16.
113. Matos M, Shen HY, Augusto E, Wang Y, Wei CJ, Wang YT, et al. Deletion of adenosine A2A receptors from astrocytes disrupts glutamate homeostasis leading to psychomotor and cognitive impairment: relevance to schizophrenia. Biol Psychiatry. 2015;78(11):763–74.
114. Harada K, Nakato K, Yarimizu J, Yamazaki M, Morita M, Takahashi S, et al. A novel glycine transporter-1 (GlyT1) inhibitor, ASP2535. Eur J Pharmacol. 2012;685(1–3):59–69.
115. Shimazaki T, Kaku A, Chaki S. D-Serine and a glycine transporter-1 inhibitor enhance social memory in rats. Psychopharmacology (Berl). 2010;209(3):263–70.
116. Black MD, Varty GB, Arad M, Barak S, De Levie A, Boulay D, et al. Procognitive and antipsychotic efficacy of glycine transport 1 inhibitors (GlyT1) in acute and neurodevelopmental models of schizophrenia: latent inhibition studies in the rat. Psychopharmacology (Berl). 2009;202(1–3):385–96.
117. Manahan-Vaughan D, Wildförster V, Thomsen C. Rescue of hippocampal LTP and learning deficits in a rat model of psychosis by inhibition of glycine transporter-1 (GlyT1). Eur J Neurosci. 2008;28(7):1342–50.
118. Karasawa J, Hashimoto K, Chaki S. D-Serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. Behav Brain Res. 2008;186(1):78–83.
119. Jodo E, Suzuki Y, Katayama T, Hoshino KY, Takeuchi S, Niwa S, et al. Activation of medial prefrontal cortex by phencyclidine is mediated via a hippocampo–prefrontal pathway. Cereb Cortex. 2005;15(5):663–9.
120. Wang GW, Cai JX. Disconnection of the hippocampal–prefrontal cortical circuits impairs spatial working memory performance in rats. Behav Brain Res. 2006;175(2):329–36.
121. Wang GW, Cai JX. Reversible disconnection of the hippocampal–prelimbic cortical circuit impairs spatial learning but not passive avoidance learning in rats. Neurobiol Learn Mem. 2008;90(2):365–73.
122. Churchwell JC, Kesner RP. Hippocampal–prefrontal dynamics in spatial working memory: interactions and independent parallel processing. Behav Brain Res. 2011;225(2):389–95.
123. Hasan A, Nitsche MA, Rein B, Schneider-Axmann T, Guse B, Gruber O, et al. Dysfunctional long-term potentiation-like plasticity in schizophrenia revealed by transcranial direct current stimulation. Behav Brain Res. 2011;224(1):15–22.
124. Gargiulo PA, Siemann M, Delius JD. Visual discrimination in pigeons impaired by glutamatergic

blockade of nucleus accumbens. Physiol Behav. 1998;63(4):705–9.
125. Gargiulo PA, Acerbo MJ, Krug I, Delius JD. Cognitive effects of dopaminergic and glutamatergic blockade in nucleus accumbens in pigeons. Pharmacol Biochem Behav. 2005;81(4):732–9.
126. Gargiulo PA, Landa De Gargiulo AI. Glutamate and modeling of schizophrenia symptoms: review of our findings: 1990–2014. Pharmacol Rep. 2014;66(3):343–52.
127. Baiardi G, Ruiz AM, Beling A, Borgonovo J, Martínez G, Landa AI, et al. Glutamatergic ionotropic blockade within accumbens disrupts working memory and might alter the endocytic machinery in rat accumbens and prefrontal cortex. J Neural Transm (Vienna). 2007;114(12):1519–28.
128. Pause BM, Zlomuzica A, Kinugawa K, Mariani J, Pietrowsky R, Dere E. Perspectives on episodic-like and episodic memory. Front Behav Neurosci. 2013;7:33.
129. Dudchenko PA. An overview of the tasks used to test working memory in rodents. Neurosci Biobehav Rev. 2004;28(7):699–709.
130. Li JT, Su YA, Guo CM, Feng Y, Yang Y, Huang RH, Si TM. Persisting cognitive deficits induced by low-dose, subchronic treatment with MK-801 in adolescent rats. Eur J Pharmacol. 2011;652(1–3):65–72.
131. Zemanova A, Stankova A, Lobellova V, Svoboda J, Vales K, Vlcek K, et al. Visuospatial working memory is impaired in an animal model of schizophrenia induced by acute MK-801: an effect of pretraining. Pharmacol Biochem Behav. 2013;106:117–23.
132. Su YA, Huang RH, Wang XD, Li JT, Si TM. Impaired working memory by repeated neonatal MK-801 treatment is ameliorated by galantamine in adult rats. Eur J Pharmacol. 2014;725:32–9.
133. Kim TW, Kang HS, Park JK, Lee SJ, Baek SB, Kim CJ. Voluntary wheel running ameliorates symptoms of MK-801-induced schizophrenia in mice. Mol Med Rep. 2014;10(6):2924–30.
134. Li ML, Yang SS, Xing B, Ferguson BR, Gulchina Y, Li YC, et al. LY395756, an mGluR2 agonist and mGluR3 antagonist, enhances NMDA receptor expression and function in the normal adult rat prefrontal cortex, but fails to improve working memory and reverse MK801-induced working memory impairment. Exp Neurol. 2015;273:190–201.
135. Stuchlik A, Rezacova L, Vales K, Bubenikova V, Kubik S. Application of a novel Active Allothetic Place Avoidance task (AAPA) in testing a pharmacological model of psychosis in rats: comparison with the Morris Water Maze. Neurosci Lett. 2004;366(2):162–6.
136. Lobellova V, Entlerova M, Svojanovska B, Hatalova H, Prokopova I, Petrasek T, et al. Two learning tasks provide evidence for disrupted behavioural flexibility in an animal model of schizophrenia-like behaviour induced by acute MK-801: a dose–response study. Behav Brain Res. 2013;246:55–62.
137. Vales K, Bubenikova-Valesova V, Klement D, Stuchlik A. Analysis of sensitivity to MK-801 treatment in a novel active allothetic place avoidance task and in the working memory version of the Morris water maze reveals differences between Long–Evans and Wistar rats. Neurosci Res. 2006;55(4):383–8.
138. Singer P, Hauser J, Llano Lopez L, Peleg-Raibstein D, Feldon J, Gargiulo PA, Yee BK. Prepulse inhibition predicts working memory performance whilst startle habituation predicts spatial reference memory retention in C57BL/6 mice. Behav Brain Res. 2013;242:166–77.
139. Nozari M, Mansouri FA, Shabani M, Nozari H, Atapour N. Postnatal MK-801 treatment of female rats impairs acquisition of working memory, but not reference memory in an eight-arm radial maze; no beneficial effects of enriched environment. Psychopharmacology (Berl). 2015;232(14):2541–50.
140. Hanlon FM, Weisend MP, Hamilton DA, Jones AP, Thoma RJ, Huang M, et al. Impairment on the hippocampal-dependent virtual Morris water task in schizophrenia. Schizophr Res. 2006;87(1–3):67–80.
141. Han K, Young Kim I, Kim JJ. Assessment of cognitive flexibility in real life using virtual reality: a comparison of healthy individuals and schizophrenia patients. Comput Biol Med. 2012;42(8):841–7.
142. Ferino F, Thierry AM, Glowinski J. Anatomical and electrophysiological evidence for a direct projection from Ammon's horn to the medial prefrontal cortex in the rat. Exp Brain Res. 1987;65(2):421–6.
143. Young JW, Powell SB, Risbrough V, Marston HM, Geyer MA. Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. Pharmacol Ther. 2009;122(2):150–202.
144. Dere E, Huston JP, De Souza Silva MA. The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav Rev. 2007;31(5):673–704.
145. Lyon L, Saksida LM, Bussey TJ. Spontaneous object recognition and its relevance to schizophrenia: a review of findings from pharmacological, genetic, lesion and developmental rodent models. Psychopharmacology (Berl). 2012;220(4):647–72.
146. King MV, Sleight AJ, Woolley ML, Topham IA, Marsden CA, Fone KC. 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation — an effect sensitive to NMDA receptor antagonism. Neuropharmacology. 2004;47(2):195–204.
147. Nilsson M, Hansson S, Carlsson A, Carlsson ML. Differential effects of the N-methyl-d-aspartate receptor antagonist MK-801 on different stages of object recognition memory in mice. Neuroscience. 2007;149(1):123–30.
148. Lim AL, Taylor DA, Malone DT. A two-hit model: behavioural investigation of the effect of combined neonatal MK-801 administration and isolation rearing in the rat. J Psychopharmacol. 2012;26(9):1252–64.

149. Stefani MR, Moghaddam B. Transient N-methyl-D-aspartate receptor blockade in early development causes lasting cognitive deficits relevant to schizophrenia. Biol Psychiatry. 2005;57(4):433–6.
150. Baier PC, Blume A, Koch J, Marx A, Fritzer G, Aldenhoff JB, Schiffelholz T. Early postnatal depletion of NMDA receptor development affects behaviour and NMDA receptor expression until later adulthood in rats — a possible model for schizophrenia. Behav Brain Res. 2009;205(1):96–101.
151. Achim AM, Lepage M. Is associative recognition more impaired than item recognition memory in schizophrenia? Brain and Cogn. 2003;53:121–4.
152. Danion JM, Huron C, Vidailhet P, Berna F. Functional mechanisms of episodic memory impairment in schizophrenia. Can J Psychiatry. 2007;52:693–701.
153. Winters BD, Bussey TJ. Glutamate receptors in perirhinal cortex mediate encoding, retrieval, and consolidation of object recognition memory. J Neurosci. 2005;25(17):4243–51.
154. Barker GRI, Warburton EC. Object-in-place associative recognition memory depends on glutamate receptor neurotransmission within two defined hippocampal–cortical circuits: a critical role for AMPA and NMDA receptors in the hippocampus, perirhinal, and prefrontal cortices. Cereb Cortex. 2015;25(2):472–81.

The Effect of 17β-Estradiol and Its Analogues on Cognition in Preclinical and Clinical Research: Relevance to Schizophrenia

26

Alyssa M. Sbisa, Maarten van den Buuse, and Andrea Gogos

Abstract

Epidemiological and clinical evidence suggests estrogen plays a role in the development and severity of schizophrenia, and a growing body of literature indicates estrogen therapy is a feasible treatment option. Current pharmacological treatments for schizophrenia primarily address the positive symptoms and fail to adequately address the cognitive deficits; thus, novel treatments require exploration. The sex steroid hormone 17β-estradiol has been extensively studied as a treatment for schizophrenia, and selective estrogen receptor modulators (SERMs) have been more recently investigated as other potential candidates. This chapter aims to critically analyse the current evidence for the clinical applicability of 17β-estradiol and the SERM raloxifene for the treatment of schizophrenia, with particular emphasis on treating cognitive symptoms.

Keywords

Schizophrenia • Cognition • Estrogen • Estradiol • SERMs • Raloxifene • Positive symptoms • Psychoneuroendocrinology • Information processing

A.M. Sbisa, BAppSc (Hons) (✉)
Florey Institute of Neuroscience and Mental Health, University of Melbourne, 30 Royal Parade, Parkville, Melbourne, VIC 3052, Australia

School of Psychology and Public Health, La Trobe University, Bundoora, VIC 3086, Australia

M. van den Buuse
School of Psychology and Public Health, La Trobe University, Bundoora, VIC 3086, Australia

A. Gogos
Florey Institute of Neuroscience and Mental Health, University of Melbourne, 30 Royal Parade, Parkville, Melbourne, VIC 3052, Australia
e-mail: andrea.gogos@florey.edu.au

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_26

Introduction

Estrogen is a potent gonadal steroid that can have dynamic effects in the brain. A rich literature encompassing both preclinical and clinical studies describes the effect that estrogen can exert on cognition, mood, and behaviour. The past two decades have seen an increased interest in the role of estrogen in the pathophysiology and treatment of schizophrenia [1–3]. This chapter aims to critically analyse the current evidence for the utility of 17β-estradiol and its analogues as a form of therapy for schizophrenia, with particular focus on the feasibility of the treatment for the cognitive deficits associated with the disorder.

Schizophrenia

Schizophrenia is a complex neuropsychiatric disorder that will affect approximately seven to eight individuals per 1,000 during their lifetime [4]. The disorder is characterised by three broad categories of symptoms: positive, negative, and cognitive symptoms [5]. The positive and negative symptoms are often considered the most debilitating; however, cognitive deficits are the best predictor of functional outcome [6, 7]. Current antipsychotic drugs are not suitable for the effective treatment of the cognitive dysfunctions associated with schizophrenia; thus, novel treatments require exploration [8]. It has previously been theorised that second-generation antipsychotics can provide some cognitive benefit in patients [9]. However, more recent research suggests observed outcomes could be attributed to poor study design, practise effects, and inappropriate doses of medication [10].

Cognitive Underperformance in Schizophrenia

Cognition is a broad term referring to the mental processes related to acquiring knowledge and understanding [11]; measurable areas of cognition can include learning, processing speed, memory, and reasoning. In this chapter we focus on information processing and learning and memory. It is well established that individuals with schizophrenia suffer from working memory problems [12]. A meta-analysis by Forbes et al. in 2009 [12] concluded there are large deficits in all domains of working memory (central executive, visuospatial, phonological) in schizophrenic patients compared with healthy controls.

Cognitive and intellectual underperformance has been consistently identified as a risk factor for schizophrenia. Decline in cognitive ability precedes the onset of clinical symptoms by nearly a decade [13]. A meta-analysis of neurocognitive function has found schizophrenia patients perform on average 1.5–2.5 standard deviations below the norm on neurocognitive tests of attention, motor performance, memory, and general intelligence [10]. Importantly, even when the positive and negative symptoms of schizophrenia are in remission, cognitive deficits remain [14]. This demonstrates the robustness of the cognitive dysfunctions associated with the disorder, in addition to the limited capability of current pharmacological treatments to improve function [15].

Gender Differences in Schizophrenia

The onset of schizophrenia generally occurs in late adolescence or young adulthood [16]. Examining gender differences in schizophrenia has found an earlier onset of approximately 4–6 years in males compared with females. Further, females also have a second peak of incidence at 45–50 years of age, suggested to be a period of low hormone levels due to menopause [16]. Course of illness, severity of symptoms, and response to antipsychotic medication differ between sexes, with females having a better outcome than males [17–19]. Presentation of the illness is also considered to differ, with men affected by more negative symptoms and women suffering more affective symptoms [18]. Likewise, neuroanatomy is considered dissimilar between men and women with schizophrenia; males are thought to have more brain structural abnormalities than females, including enlarged ventricles and decreased temporal lobe volume [18].

Sex hormones are postulated to be a prominent factor in the dissimilar presentation of illness between men and women. Symptom severity in women with schizophrenia and risk of relapse increases during the postpartum period, after menopause, and during the luteal phase of the menstrual cycle, all times of reduced hormone levels [20–23]. In a 1959 study by Dalton and colleagues [24], it was reported that of their sample of 276 women admitted to psychiatric hospitals, 46% were admitted during or immediately before menstruation, a period of low plasma estrogen. More recent research further corroborates a negative correlation between estrogen levels and psychosis [25, 26]. A relationship has also been discovered between earlier puberty and later onset of schizophrenia [27]. Additionally, it has been theorised that improved pharmacological response with lower doses of typical antipsychotics in women of child-bearing age, compared with postmenopausal women, is due to the enhancing effect of estrogen via its antidopaminergic properties [18].

The Role of Estrogens in Schizophrenia

Estrogen can exert potent effects in numerous regions of the brain, consequently affecting mood, cognition, and behaviour [28, 29]. Physicians first noticed the benefits of hormones for psychiatric illness and menopausal symptoms over 100 years ago [30]. In the past 50 years, research into estrogen therapy has markedly advanced. Its medical utility has been explored in breast [31] and prostate cancer [32], and osteoporosis [33]. Moreover, researchers continue to study the use of estrogens for the delay or alleviation of neurodegenerative diseases, and treatment of psychiatric disorders including treatment-resistant depression [34], perimenopausal and postpartum depression [35, 36], bipolar affective disorder [37], and schizophrenia [2].

Evidence suggests that steroidal hormones, such as estrogen, exert their effects over the entire lifetime, protecting the brain from certain insults [38]. Accumulating evidence has led to the hypothesis that reoccurring hormone influxes in women serve as a protective factor in the initial development of schizophrenia [39]. The hypothesised role of estrogen in schizophrenia is not a recent theory. In 1961, Diczfalusy and Lauritzen [40] reviewed studies measuring estrogen concentration in the blood and urine of women with schizophrenia [40, 41]. In seven of the eight studies examined, low estrogen levels were detected [40]. This has contributed to the hypoestrogenism hypothesis, which posits that low 17β-estradiol in schizophrenia is either a trigger or initial vulnerability leading to development of the mental illness, or conversely, an outcome of the disease itself [41]. While these studies are over 70 years old, admittedly used small sample sizes, and applied laboratory methods now considered outdated, the research is of significance due to its occurrence during the pre-antipsychotic era [41]. The current use of antipsychotic treatment in women with schizophrenia, particularly typical antipsychotics, affords difficulty in reliably assessing hormone levels due to hyperprolactinemia [42]. A review of research into antipsychotic-induced hyperprolactinemia in schizophrenia patients found 67% of the sample of women had abnormal levels of prolactin [42]. Elevated prolactin levels occur when antipsychotics block dopamine D_2 receptors on the anterior pituitary gland [42], and consequently can suppress gonadal function.

Despite the potential confounding effect of antipsychotics, research continues to demonstrate that low estrogen plasma concentration correlates with an increased risk of symptoms of schizophrenia [41]. For example, in pre-menopausal hospital patients with schizophrenia, Bergemann et al. [43] found a significant increase in admissions during the 3 days prior to and following the first day of menses. Further, in a more recent study Bergemann et al. [44] assessed plasma 17β-estradiol concentration in schizophrenia patients, while also controlling for antipsychotic treatment; hypoestrogenism occurred in approximately 60% of their sample. In addition, there was a significant difference in prolactin levels between the patients in the typical and atypical antipsychotic groups, however, no difference between

the two atypical antipsychotic groups (clozapine and olanzapine). Ultimately, Bergemann et al. concluded that low serum levels of 17β-estradiol exist independent of antipsychotic-induced elevation of prolactin levels [44].

Gender differences in schizophrenia, and comparison of endocrinological function in schizophrenic women compared to the healthy population, provides a case for the estrogen hypothesis. Evidence including lower baseline levels of circulating estrogen, amenorrhea, [45], the association between earlier onset of puberty and later onset of the disorder [27], and superior response to antipsychotic medication in women compared to men, suggests estrogen can exert a protective effect in schizophrenia, and consequently may serve as an appropriate form of treatment. Molecular findings further strengthen the clinical observations and evidence for the estrogen hypothesis [46]. Most notably, Weickert et al. [47] discovered that estrogen receptors are altered in the brains of individuals with schizophrenia, consequently affecting their ability to respond to endogenous estrogen. Clinical and molecular findings over the past 50 years provide a substantial argument for the use of estrogen therapy in women with schizophrenia; however, the most appropriate estrogenic compound for long-term use is yet to be determined.

17β-Estradiol

There exist numerous forms of endogenous estrogen; however, 17β-estradiol is considered the most potent form. Although it is often considered the primary 'female sex hormone', it is present in both sexes [48]. While 17β-estradiol is predominantly produced in the ovaries to regulate the menstrual cycle in females, it is also created by non-endocrine tissues, including fat, breast, and neural tissues [49]. It is important to note that reference to estrogen treatment, particularly in early research, can broadly refer to numerous estrogenic compounds including estrone, diethylstilbestrol, equilin, 17β-estradiol, and ethinylestradiol. From here on, this chapter will specifically focus on the estrogen 17β-estradiol, unless otherwise stated.

The Effect of 17β-Estradiol on Cognition in Clinical Studies

A considerable volume of literature has been published on the facilitative effect of 17β-estradiol on cognitive performance. Researchers identified a connection between ovarian hormones and cognition after discovering that fluctuating levels across stages of the human menstrual cycle were accompanied by changes in cognitive performance [50]. This has contributed to the hypothesis that estrogen maintains a certain level of cognitive function in women [51]. This theory has been substantiated in postmenopausal women receiving hormone replacement therapy [52], and in preclinical studies using ovariectomised (OVX) rodents and 17β-estradiol replacement [53]. Clinical research outcomes, however, are inconsistent and results vary widely dependent on sample characteristics. Collectively, current evidence indicates that 17β-estradiol has the ability to facilitate cognition; however, this outcome is dependent on factors including treatment dose [54], cognitive task and brain region [55], sex, endogenous levels of hormones [56], treatment window [51], and mental and physical health [57].

General consensus regarding endogenous 17β-estradiol in naturally cycling women is that verbal and fine motor skill task performance improves when estrogen levels are elevated, while decreased levels assist in spatial task performance [51]. For example, Hampson [61] found verbal fluency and articulation was improved in young women during the mid-luteal (high estrogen) phase of the menstrual cycle. Sundström et al. [58] however, have concluded in their meta-analysis that there is no consistent pattern found among the existing studies of verbal ability and menstrual cycle [62–66]. It is important to note, however, that while the collective literature is inconsistent for verbal memory and menstrual cycle, exogenous estrogen has been found to benefit verbal memory [60].

As Sundström et al. [58] identify, many of the studies reviewed in their meta-analysis have poor design and low power. Additionally, days of the menstrual cycle during which participants were

tested varied between studies, which can result in diverse hormone levels and dissimilar outcomes between research findings, despite testing within the same menstrual period. Although researchers can examine the relationship between certain cognitive abilities and menstrual phases, it is important to consider the effects of individual hormones within the menstrual phases. Studies demonstrating a proclivity for enhanced verbal ability during the mid-luteal phase often attribute the effect to estrogen; however, this period involves a rise in both 17β-estradiol and progesterone. Only in recent years have researchers taken this factor into consideration. For example, Maki et al. [64] assayed hormones to determine whether a correlation exists between levels of 17β-estradiol, progesterone, and cognitive measures. In a sample of young women, they [64] found verbal scores were positively associated with 17β-estradiol levels, while spatial ability scores were negatively related. However, correlations between progesterone and cognition were not statistically significant. While it is possible to investigate relationships between endogenous hormones and cognitive tasks in humans, it is difficult to account for potential hormonal interactions. Fortunately, preclinical research investigating exogenous 17β-estradiol and cognition can further elucidate the outcomes seen in clinical studies.

Estrogen Therapy in the Clinical Population

Generally, there is no clinical necessity for healthy women of childbearing age to be treated with 17β-estradiol. Although the effects of estrogen-based contraceptives in healthy young women have been investigated [67, 68], the contraceptive formulations do not include 17β-estradiol, but rather synthetic derivatives (e.g., ethinylestradiol). Therefore, research examining the effect of 17β-estradiol treatment has primarily been concerned with neurodegenerative diseases [69], psychiatric disorders [2], and the postmenopausal population [70]. Perhaps the most notable study in this area of research is the Women's Health Initiative (WHI) memory study, a large double-blind, randomised placebo-controlled trial, which investigated cognitive function in a postmenopausal sample [71]. Following cognitive testing of 1,416 women receiving hormone replacement therapy, researchers found there was no beneficial effect of estrogen on cognition [71]. Importantly, participants partaking in the WHI study were treated with conjugated equine estrogens (CEE). Conjugated estrogens primarily contain estrone and equilin, with lesser quantities of 17β-estradiol [56]. This is important to note, as 17β-estradiol and the main component of CEE, estrone, have shown opposing effects on cognition in preclinical research [56]. Estrone has been found to impair spatial working memory [72], while 17β-estradiol has the opposite effect [73]. The discrepancy between CEE and 17β-estradiol is often not outlined in research administering CEE as estrogen therapy, which has consequently contributed to the incorrect conclusion that 17β-estradiol is not beneficial, or is even harmful, for cognitive function in postmenopausal women. Research concerning estrogen treatment and CEE in the postmenopausal population will not be detailed further in this chapter; however, see Fischer et al. [28] for an overview of hormone therapy relevant to cognition in postmenopausal women. Similarly, Gogos et al. [68] recently reviewed literature concerning ethinylestradiol-based oral contraceptives and their effect on cognition in pre-menopausal women.

Estrogen Therapy in Schizophrenia

A growing body of literature provides evidence that estrogen treatment in conjunction with antipsychotics is beneficial for treating the positive symptoms of schizophrenia [2, 74, 75]. An initial pilot study by Kulkarni et al. [76] discovered that the 17β-estradiol derivative, ethinylestradiol, taken orally daily for 8 weeks, significantly improved positive symptoms compared to the antipsychotic-only group. Later, trialling a transdermal method of administration, the same investigators determined that schizophrenia patients receiving adjunctive 17β-estradiol had significant

improvements in positive symptoms compared to the placebo group [75].

Although research has found low levels of endogenous estrogen correlate with more severe negative symptoms [77], few studies thus far have demonstrated significant changes in negative symptoms following 17β-estradiol treatment. Conversely, the beneficial effect of estrogen treatment for the positive symptoms of schizophrenia has been replicated [2, 74–76, 78, 79]. However, there have also been instances of inability to replicate. For example, Bergemann et al. [80] failed to demonstrate the beneficial effect of 17β-estradiol on positive or negative symptoms in their placebo-controlled double-blind study. Similarly, Lindamer et al. [81], using a cross-sectional sample, found no effect of estrogen on positive symptomatology in postmenopausal women with schizophrenia, however, negative symptoms were improved. Importantly, Bergemann et al. [80] used a combined 17β-estradiol and progestin oral treatment, with different compounds and doses dependent on the phase of menstrual cycle. Lindamer et al. [81] did not administer pharmacological intervention, but rather used a cross-sectional sample of women with schizophrenia who had received hormone replacement therapy for at least 1 year, and women with schizophrenia receiving no hormone replacement therapy.

While research into 17β-estradiol therapy thus far suggests that it aids the positive symptoms of schizophrenia [2, 76, 78], the effect on cognitive symptoms remains less clear. Examining endogenous estrogen, researchers have determined a relationship between 17β-estradiol and performance in certain cognitive tasks in women with schizophrenia. A study by Hoff et al. [82] found that improvements in verbal memory, perceptual motor speed, and spatial memory were positively correlated with 17β-estradiol levels. Ko and colleagues [77] determined a similar trend; they divided schizophrenia patients into two groups using normal serum 17β-estradiol reference ranges during the follicular phase of the menstrual cycle. Researchers found that women with low baseline levels of estradiol had diminished performance in verbal memory and executive function, compared to the group of women with higher baseline levels of 17β-estradiol [77].

Clinical research specifically concerning the influence of exogenous 17β-estradiol on cognition in schizophrenia patients is limited. Bergemann and colleagues [83] found that oral 17β-estradiol and adjunctive antipsychotic treatment for women with schizophrenia improved comprehension of metaphoric speech, but had no effect on verbal ability. Alternatively, using a transdermal method of administration of 17β-estradiol, Kulkarni et al. [2] found there were no significant differences between or within groups in cognitive domains including memory, language, constructional skills, and attention. Evidently, the effect of estrogen therapy on cognition differs between trials and within populations. Conflicting outcomes in the literature are likely due to a variety of inconsistent factors including dissimilar measures, variable treatment duration, additional pharmacotherapy, baseline endogenous hormone levels, method of treatment administration, and pharmacological and pharmacokinetic variations in estrogen.

The Effect of 17β-Estradiol on Cognition in Preclinical Studies

The beneficial effect of 17β-estradiol on cognition has been consistently replicated in animal studies. It should be noted that this section of the chapter only refers to studies of 17β-estradiol in rat cognition; see Gibbs [84] for research relevant to mice and non-human primates. In rats (Table 26.1), the beneficial effect of estrogen has been seen in learning and memory including spatial working, recognition, and reference memory domains [53, 73, 131, 135]. Luine et al. [73] examined spatial memory in OVX rats in an eight-arm radial maze; 3 days of 17β-estradiol treatment via subcutaneous implant did not enhance memory performance compared to the untreated OVX rats; however, 12 days of treatment significantly improved memory. Using the novel-object and placement-recognition paradigm, Luine et al. [53] demonstrated that 17β-estradiol treatment enhanced visual and

Table 26.1 Research investigating the effect of 17β-estradiol treatment on cognition in ovariectomised (OVX) female rats

		17β-estradiol			
Cognitive domain	Test	Method	Dose	Effects	Reference
Attention and impulsivity	5-choice serial reaction time task	Injection	Chronic	↑	Barnes et al. [193]
		Implant	Chronic	↑ Treatment at 17 months ∅ Treatment at 12 months	Bohacek and Daniel [90]
Learning and memory (classical and operant conditioning)	Active avoidance	Injection	Acute	↑ High dose ↓ Low and moderate dose	Diaz-Veliz et al. [96]
		Injection	Chronic	↑	Horvath et al. [116]
		Implant	Chronic	↑	Singh et al. [137]
	Fear conditioning	Injection	Chronic	↑ Contextual conditioning ∅ Cued fear conditioning	Barha et al. [87]
		Injection	Chronic	↑ Context discrimination ∅ Acquisition or extinction	Hoffman et al. [114]
	Inhibitory avoidance	Injection	Acute	↑	Rhodes and Frye [132]
		Injection	Acute	↑ Post-training ∅ 1–3 hours post-training	Rhodes and Frye [131]
		Injection	Chronic	∅	Horvath et al. [116]
		Injection	Chronic	↑	Frye and Rhodes [102]
		Implant	Chronic	↑	Frye and Rhodes [102]
Recognition memory	Novel object recognition	Injection	Acute	↑ Immediately post-training ∅ 1 hour post-training	Walf et al. [146]
		Injection	Acute	↑ Moderate dose ∅ Low and high doses	Inagaki et al. [54]
		Injection	Acute	↑ Prior to training ↑ Immediately post-training ∅ 2 hours post-training	Luine et al. [53]
		Injection	Acute	↑	Jacome et al. [117]
Spatial recognition memory	Y-maze	Injection	Acute	↑ Low dose ∅ Moderate or high doses	Hawley et al. [113]
	Novel object place	Injection	Acute	↑ Moderate doses ∅ Low and high doses	Inagaki et al. [54]
		Injection	Acute	↑ Prior to training ↑ Immediately post-training ∅ 2 hours post-training	Luine et al. [53]
		Injection	Acute	↑ High dose ∅ Low dose	McLaughlin et al. [127]
		Injection	Acute	↑ Immediately post-training ∅ 1.5 hour post-training	Frye et al. [103]
		Injection	Acute	↑	Jacome et al. [117]
		Implant	Chronic	↑	Walf et al. [147]

(continued)

Table 26.1 (continued)

		17β-estradiol			
Cognitive domain	Test	Method	Dose	Effects	Reference
Spatial learning and memory	Barnes maze	Injection	Chronic	↑	Ping et al. [130]
	Morris water maze	Injection	Acute	∅	Chesler and Juraska [91], McLaughlin et al. [127]
		Injection	Acute	↑	Sandstrom and Williams [135], Rhodes and Frye [132], Markham et al. [125]
		Injection	Acute	↑ Low dose ∅ High dose	McLaughlin et al. [127]
		Injection	Acute	↓ During acquisition	Frick et al. [101]
		Injection	Chronic	↑	El-Bakri et al. [97], Feng et al. [100], Bimonte-Nelson et al. [89]
		Implant	Chronic	↑	Bimonte-Nelson et al. [89], Markham et al. [125]
		Implant	Chronic	↓ During acquisition	Daniel and Lee [93]
		Implant	Chronic	∅	Singh et al. [137]
		Implant	Chronic	↑ Young and middle-aged ∅ Older	Talboom et al. [139]
		Implant	Chronic	↑ Middle-aged and older ∅ Young	Kiss et al. [118]
		Orally	Chronic	↑	Liu et al. [122]
		Orally	Chronic	↑ Continuous treatment ∅ Cycling treatment	Lowry et al. [123]
		Orally	Chronic	↑	Wu et al. [152]
	T-maze	Implant	Chronic	↑	Gibbs [105]
		Implant	Chronic	↑ Treatment 3 months post-OVX ∅ Treatment 10 months post-OVX	Gibbs [106]
		Mini-osmotic pump	Chronic	↑	Hammond et al. [112]
	Open-field tower maze	Injections	Chronic	↑ Cycling treatment ∅ Continuous treatment	Lipatova et al. [121]
		Implant	Chronic	↑	Lipatova and Toufexis [120]
	Plus maze	Injection	Acute	↑ Place learning ↓ Response learning	Korol and Kolo [119]
Spatial working memory	Delayed spatial alternation	Implant	Chronic	↓	Wang et al. [148, 149]
	Radial arm maze	Injection	Chronic	↓ Spatial working-reference memory, cued win-stay, conditioned place preference ∅ Delayed win-shift task	Galea et al. [104]

Table 26.1 (continued)

		17β-estradiol			
Cognitive domain	Test	Method	Dose	Effects	Reference
		Injection	Chronic	↑ Low dose ↓ High doses	Holmes et al. [115]
		Implant	Chronic	↑	Daniel et al. [92]
		Implant	Chronic	↑ Moderate dose ∅ Low dose	Bimonte and Denenberg [88]
		Implant	Chronic	↑ Immediately post-OVX ∅ Five months post-OVX	Daniel et al. [94]
		Implant	Chronic	↑ Place learning ↓ Response learning	Davis et al. [95]
		Implant	Chronic	↑ Working memory ∅ Reference memory	Fader et al. [99], Luine et al. [73], Gibbs and Johnson [108]
		Implant	Chronic	∅	Luine and Rodriguez [124]
	Y-maze	Injection	Acute	∅	McLaughlin et al. [127]
		Injection	Acute	↑	Velásquez-Zamora et al. [145]
Working memory	Non-spatial delayed alternation T-maze	Injection	Chronic	↑ Low dose, short delay ↓ High doses, long delay	Wide et al. [150]

Note ↓ impaired performance, ↑ facilitated performance, ∅ no effect or difference compared to control, Chronic >3 days treatment, OVX ovariectomy

place memory in OVX rats compared to controls. In addition, estrogen treatment enhanced memory when given prior to or immediately after the sample trial, but not, however, when administered 2 h later. Thus, it is theorised that 17β-estradiol treatment affects memory encoding or consolidation, rather than retrieval. Interestingly, authors also determined that compared to novel-object recognition, a different dose of 17β-estradiol for novel-place recognition was necessary to see a significant effect [53].

Dose-dependent effects of 17β-estradiol have also been demonstrated in reference memory [55]. In their 2010 review, Barha and Galea concluded that high levels of 17β-estradiol can impede working and reference memory, whereas low levels of 17β-estradiol have no significant effect on reference memory, however can facilitate working memory [55]. Similarly, contextual fear conditioning can be facilitated by a low dose of 17β-estradiol, however, a high dose can impair [55, 115]. This demonstrates the capacity of 17β-estradiol to differentially affect forms of memory, all of which are hippocampus-dependent. Collectively, the literature indicates an inverted U-shaped dose–response curve [54]; lower and higher doses of 17β-estradiol can often inhibit or impair cognition [56].

Numerous studies have shown that behavioural tasks employing the hippocampus can be altered by 17β-estradiol [88, 92, 99, 102, 107, 112]; however, fewer have investigated cortical-dependent tasks [29]. Wide et al. [150] examined the effect of 17β-estradiol in OVX rats in the non-spatial delayed alternation task, mediated by the integrity of the prefrontal cortex. A lower dose of 17β-estradiol was most effective for facilitation of non-spatial working memory; subjects receiving a high dose made significantly more errors compared to the controls, demonstrating that a task considered primarily prefrontal cortical-dependent can also be affected by 17β-estradiol [150].

Pharmacological Models of Schizophrenia

Pharmacologically disrupted prepulse inhibition is frequently used to model psychosis-like symptoms

in rodents. Prepulse inhibition, a measure of sensory gating, is considered to represent the interface of psychosis and cognition [156, 157]. In healthy subjects, the impact of a startle-inducing acoustic stimulus (a pulse) is successfully attenuated by a preceding stimulus (a prepulse), and consequently the magnitude of startle response is reduced. However, individuals with schizophrenia do not experience the same level of filtering [158]. Administering certain drug treatments in animals allows for observation of cognitive-behavioural and neurochemical changes similar to those seen in schizophrenia patients [157]. In studies of rat prepulse inhibition, 17β-estradiol can attenuate disruptions induced by the serotonin-1A receptor agonist 8-OH-DPAT, the NMDA receptor antagonist MK-801, and the dopamine $D_{1/2}$ receptor agonist, apomorphine [109–111, 142, 159].

There are a number of cross-species paradigms used to measure information processing; another method of measuring auditory sensory gating is the P50 event-related potential (ERP) suppression paradigm, measured using electroencephalography. Thwaites et al. [141] tested the effect of 17β-estradiol on sensory gating in OVX and intact rats. Similarly to the prepulse inhibition studies of Gogos et al. [111], Thwaites et al. induced deficits by administering dopaminergic and glutamatergic drugs. Subjects were injected acutely with apomorphine, amphetamine, and phencyclidine. Chronic estrogen treatment via subcutaneous implant successfully prevented apomorphine-induced sensory gating disruption in OVX rats, but had no effect on amphetamine or phencyclidine.

Latent inhibition is another cognitive-behavioural assay used to assess the neurobiological underpinning of schizophrenia. Disrupted latent inhibition reflects a deficit in selective attention, whereby the subject loses the ability to ignore an irrelevant stimulus. Similar to prepulse inhibition and P50 ERP, latent inhibition gauges the animal's ability to filter out unnecessary information. Arad and Weiner [86] tested the antipsychotic effect of 17β-estradiol in drug-induced disruption of latent inhibition. Intact female rats received pretreatment of estradiol prior to acute amphetamine administration, and disruption of latent inhibition was successfully reversed. Similarly, in OVX rats treated with MK-801, 17β-estradiol pre-treatment successfully reversed MK-801-induced latent inhibition persistence. Intriguingly, a low 17β-estradiol dose has been found to disrupt latent inhibition in both OVX and intact rats [85, 128]. It is theorised that a high dose of 17β-estradiol can exert an antipsychotic effect, while low doses exert a pro-psychotic effect [86, 128].

Preclinical research on 17β-estradiol and memory relevant to models of schizophrenia has primarily focused on recognition memory [134, 160]. Using an acute dose of the NMDA receptor antagonist phencyclidine in intact female rats, Sutcliffe et al. [160] found 17β-estradiol treatment attenuated drug-induced memory deficits in the novel-object recognition task. Using chronic 17β-estradiol and sub-chronic phencyclidine treatment, Roseman et al. [134] demonstrated comparable results to Sutcliffe et al., however, by using OVX instead of intact rats; 17β-estradiol alleviated deficits in recognition memory when administered either before or after phencyclidine.

Thus far, research specific to 17β-estradiol and preclinical pharmacological models of schizophrenia-like cognitive impairments is limited, and further experimentation is needed. Fortunately, due to the large volume of literature concerning 17β-estradiol and cognition in OVX rats (as outlined in Table 26.1), we have a greater understanding of the neuroprotective effects. It is important to note that despite various methods of treatment, doses, and timing regimes, 17β-estradiol has consistently shown positive effects in numerous measures of learning, memory, and information processing.

Selective Estrogen Receptor Modulators (SERMS)

SERMs are a promising alternative to 17β-estradiol due to their ability to exert mixed agonist/antagonist effects in different areas of the body and brain [162]. Raloxifene, approved for the treatment and prevention of osteoporosis, is an agonist in the bone and an antagonist in the endometrium, while

tamoxifen, used as an anti-estrogen in the treatment of breast cancer, is a partial agonist in endometrial tissue; both SERMs act as an antagonist in breast tissue [163]. Although raloxifene and tamoxifen display differing mechanisms of action within different brain areas [164], both SERMs have demonstrated effects in cognitive–behavioural tasks including facilitation of learning and memory [145], and reduction in anxious and depressive behaviours [165].

Raloxifene Treatment for the Clinical Symptoms of Schizophrenia

Over the past few years, a number of studies have trialled raloxifene as an adjunctive therapy to antipsychotic treatment in women and men with schizophrenia. The effect of tamoxifen has not been clinically tested in schizophrenia (see Kulkarni et al. (166) for a case study of tamoxifen treatment for schizoaffective disorder). However, an increased risk of endometrial cancer following tamoxifen therapy has been reported [167]; therefore, at this time raloxifene is a more suitable candidate for the treatment of schizophrenia.

To determine the most effective therapeutic dose of raloxifene, Kulkarni and colleagues [168] compared two groups of peri-and postmenopausal women with schizophrenia; participants were administered either 60 or 120 mg of raloxifene per day. Following 12 weeks of raloxifene plus antipsychotic treatment, a significant reduction in total and general positive and negative syndrome scale (PANSS) scores was seen in the 120 mg/day group. Similarly, Kianimehr et al. [169] demonstrated that 120 mg/day of raloxifene plus 6 mg/day of risperidone for the duration of 8 weeks had a beneficial effect in postmenopausal women with schizophrenia; however, effects were limited to only the positive symptoms. In contrast, Usall et al. [170] trialled a lower dose of raloxifene (60 mg/day) over a 12-week duration in conjunction with antipsychotic treatment, and discovered a reduction in both positive and negative symptoms in addition to general psychopathology. Usall et al. [171] expanded upon their previous findings by conducting a longer trial (24 weeks) inclusive of a larger sample. Compared to the antipsychotic-only group, negative symptoms and general psychopathological symptoms were improved in postmenopausal women with schizophrenia administered adjuvant raloxifene (60 mg/day). Interestingly, in contrast to their previous study, the authors did not find improved positive symptoms. Discrepancy between the two trials may be attributed to sample characteristics, specifically, focus on recruiting patients with prominent negative symptoms for the 2015 study. The inclusion criteria for Kulkarni et al. [168] and Kianimehr et al. [169] included a PANSS score of ≥60 (acute patients), however, the inclusion criteria in Usall et al. [170, 171] specifically noted non-acute patients with significant negative symptoms, indicating participants should have one or more negative symptom subscale scores of >4 on the PANSS. Hence, the disparity in trial outcomes may be due to the clinical characteristics of the samples.

Raloxifene trials predominantly include women with schizophrenia ≥ 45 years of age, however, Weickert et al. [172] recently trialled raloxifene treatment in men with schizophrenia. Six weeks of adjunctive raloxifene (120 mg/day) and antipsychotic treatment in young to middle-aged men and women with schizophrenia or schizoaffective disorder, produced no significant effects on the clinical symptoms (as measured by the PANSS). It is noteworthy that the aforementioned raloxifene trials included primarily samples of peri- or postmenopausal women with schizophrenia, indicating that endogenous hormone status, more specifically estrogen and progestin levels, in the younger sample of Weickert et al. [172] could potentially be a factor in response to raloxifene treatment [173, 174].

Overall, five separate studies from four different laboratories (and thus inclusive of four diverse populations) have demonstrated the beneficial effect of raloxifene for clinical symptoms including positive, negative, and general symptomatology in peri- and postmenopausal women, and in men with schizophrenia.

Raloxifene Treatment for the Cognitive Symptoms of Schizophrenia

Only in recent years have SERMs been trialled in patients with schizophrenia with the primary aim of monitoring changes in cognitive symptoms [172, 176]. Initial case studies in patients with schizophrenia have described raloxifene in conjunction with antipsychotic treatment to be beneficial for this cluster of symptoms; areas of improvement included verbal learning and memory, and psychomotor speeds [177–179]. Huerta-Ramos et al. [176] recently conducted the first randomized controlled trial examining the effect of raloxifene on neuropsychological functioning in women with schizophrenia. Similar to previous clinical trials examining SERMs and cognitive function [180–182], these authors used a sample of postmenopausal women; additionally, the participants remained on their antipsychotic regime during the experiment [176]. Following 12 weeks of antipsychotic and raloxifene treatment, significant improvements were found in verbal memory and executive functioning. Semantic memory, attention, and processing speed were near statistical significance, with the raloxifene group exhibiting a trend toward higher scores. A similar effect has been demonstrated in a randomised controlled trial sampling healthy postmenopausal women; Jacobsen et al. [181] found women receiving raloxifene daily for 12 months had significantly improved verbal memory compared to the placebo group. Weickert et al. [172] examined the effect of raloxifene in a more representative sample including both men and women with schizophrenia and schizoaffective disorder (ages 18–51). In a randomised, double-blind, crossover, placebo-controlled trial, raloxifene (120 mg/day for 6 weeks) in addition to antipsychotic treatment improved memory and attention/processing speed. Analysis by sex also found a beneficial effect of raloxifene on verbal fluency in females [172].

The effect of estrogen treatment in men with schizophrenia remains an underexplored area. Until recently, the effect of SERMs on cognitive symptoms had only been trialled in samples of postmenopausal women with schizophrenia. This is important to note, as preclinical data has found SERMs can change their effect dependent on the presence of endogenous 17β-estradiol [173, 174]. For example, in the hippocampus, a structure crucial for mediating verbal memory [183], raloxifene can exert a partial agonist effect in the absence of 17β-estradiol, and a mixed agonist/antagonist effect in its presence [174]. Further, evidence from basic science, randomised controlled trials, and observational studies suggests a 'critical period' for the benefits of hormone therapy on cognitive function, with research suggesting earlier intervention during the first phases of menopause, and following recent gonadectomy in animals, is optimal for the most benefit [64, 191]. Therefore, depending on sex, reproductive status, and hormone levels, the clinical efficacy of SERMs is likely to vary. Positive outcomes in the research of Weickert et al. [172], however, show the potential benefit of raloxifene treatment for both men and women. Thus far, trials have shown raloxifene to be tolerated well in patients of both sexes, with few experiencing adverse events [168, 170, 172]. Further research is required to determine the long-term effects of raloxifene and replicate the beneficial effects seen on the cognitive symptoms in schizophrenia patients thus far.

Facilitative Effect of SERMs in Preclinical Research

Preclinical research examining the effect of SERM treatment on cognition primarily concerns short-term spatial memory, and research thus far suggests both tamoxifen and raloxifene can have a facilitative effect. For example, Wu et al. [152] found chronic raloxifene treatment in female rats significantly reduced escape latency in the Morris water maze compared to controls. In male rats, Lagunas et al. [185] demonstrated a similar result; following gonadectomy raloxifene and tamoxifen treated rats displayed improved spatial memory acquisition compared to controls. Analogous to the effect seen with 17β-estradiol, the SERMs showed an inverted U-shaped dose response, with the 1 mg/kg exerting an effect, yet

not the 0.5 or 2 mg/kg dose. In contrast, Gibbs et al. [188] discovered that raloxifene did not significantly enhance acquisition of the delayed matching-to-position T-maze task in OVX rats; while in OVX non-human primates, Lacreuse et al. [189] found that raloxifene had no effect on cognitive tasks of spatial working memory and recognition memory. Velázquez-Zamora et al. [145], however, concluded that raloxifene and tamoxifen not only improved Y-maze performance in OVX rats, but they also significantly increased the density of dendritic spines in the prelimbic/infralimbic prefrontal cortical area compared to the controls [145]. Comparably, studies have found 17β-estradiol can enhance dendritic spine density in the prefrontal cortex [186], which is associated with improved prefrontal cognitive performance [187].

To our knowledge, only one study has explored the effect of SERMs on animal behaviour relevant to schizophrenia symptomatology. Gogos and van den Buuse [1] tested SERM treatment on prepulse inhibition in OVX rats; deficits in sensorimotor gating were induced by treatment with the dopamine D_1/D_2 receptor agonist apomorphine (0.1, 0.3 and 1 mg/kg). While tamoxifen treatment reversed the effect of all three doses of apomorphine, raloxifene reversed only the 1 mg/kg dose, demonstrating that both SERMs can mediate dopaminergic activity, yet with differing mechanisms. These findings have potential implications for the positive symptoms of schizophrenia.

Overall, while testing the effect of SERM treatment on rat cognition helps to elucidate the behavioural effects and potential mechanisms of action, further studies involving animal models of schizophrenia are necessary to provide clearer insight into the feasibility of these SERMs for the treatment of schizophrenia.

Summary and Future Directions

There is undeniable necessity for an alternative or adjunctive pharmacological treatment for schizophrenia. Current treatment for the disorder, antipsychotics, are associated with several side-effects, do not treat the entire array of schizophrenia symptoms, and have unpredictable efficacy, which in turn generates a difficult and lengthy trial-and-error treatment process [192]. The sex hormone, 17β-estradiol, has demonstrated putative effects in preclinical and clinical studies of positive symptoms and psychosis-like behaviour [2, 79, 111]. In contrast to its effect on the positive symptoms of schizophrenia, the effect of 17β-estradiol on the cognitive symptoms is inconclusive and has varied widely between and within cognitive domains [2, 75, 83]. Unfortunately, even if solely for the treatment of positive symptoms, the efficacious dose of 17β-estradiol is associated with potential health risks, and additional side-effects for men. The SERM, raloxifene, is another feasible treatment option for schizophrenia, having demonstrated an effect in all categories of schizophrenia symptoms, albeit inconsistently between laboratory groups [168–172, 176]. Further, the neurochemical mechanisms underlying the effect of SERMs on schizophrenia symptomatology are not entirely understood; knowledge of these mechanisms could further validate the viability of SERMs as a treatment option for schizophrenia.

Ultimately, despite the heterogeneous outcomes between samples, raloxifene has demonstrated promising effects, and notably in schizophrenia patients of both sexes. Thus, further research should attempt to clarify the effects of raloxifene on schizophrenia symptomatology, including investigation of its long-term effects and suitability for patients of varying ages and symptom severity, in an effort to validate the feasibility of estrogen analogue raloxifene as an adjunctive treatment for schizophrenia.

References

1. Gogos A, van den Buuse M. Comparing the effects of 17β-oestradiol and the selective oestrogen receptor modulators, raloxifene and tamoxifen, on prepulse inhibition in female rats. Schizophr Res. 2015;168(3):634–9.
2. Kulkarni J, Berk M, Gavrilidis E, Fitzgerald P, Wang W, Worsley R, et al. Estradiol for treatment-resistant schizophrenia: a large-scale randomized–controlled trial in women of child-bearing age. Mol Psychiatry. 2015;20(6):695–702.

3. Riecher-Rössler A. Estrogens and schizophrenia. In: Bergemann N, Riecher-Rössler PDA, editors. Estrogen effects in psychiatric disorders. Vienna: Springer; 2005.
4. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. PLoS Med. 2005;2(5):e141.
5. Mueser KT, McGurk SR. Schizophrenia. Lancet. 2004;363(9426):2063–72.
6. Bowie CR, Harvey PD. Cognitive deficits and functional outcome in schizophrenia. Neuropsychiatr Dis Treat. 2006;2(4):531–6.
7. Green MF. What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry. 1996;153(3):321–30.
8. Marder SR. The NIMH-MATRICS project for developing cognition-enhancing agents for schizophrenia. Dialogues Clin Neurosci. 2006;8(1):109–13.
9. Keefe RSE, Silva SG, Perkins DO, Lieberman JA. The effects of atypical antipsychotic drugs on neurocognitive impairment in schizophrenia: a review and meta-analysis. Schizophr Bull. 1999;25(2):201–22.
10. Keefe RSE. The longitudinal course of cognitive impairment in schizophrenia: an examination of data from premorbid through posttreatment phases of illness. J Clin Psychiatry. 2014;75(Suppl 2):8–13.
11. Stevenson A. Oxford dictionary of english. Oxford: Oxford University Press; 2010.
12. Forbes NF, Carrick LA, McIntosh AM, Lawrie SM. Working memory in schizophrenia: a meta-analysis. Psychol Med. 2009;39(6):889–905.
13. van Oel CJ, Sitskoorn MM, Cremer MPM, Kahn RS. School performance as a premorbid marker for schizophrenia: a twin study. Schizophr Bull. 2002;28(3):401–14.
14. Park S, Püschel J, Sauter BH, Rentsch M, Hell D. Spatial working memory deficits and clinical symptoms in schizophrenia: a 4-month follow-up study. Biol Psychiatry. 1999;46(3):392–400.
15. Marder SR, Fenton W. Measurement and treatment research to improve cognition in schizophrenia: NIMH MATRICS initiative to support the development of agents for improving cognition in schizophrenia. Schizophr Res. 2004;72(1):5–9.
16. Häfner H, Maurer K, Löffler W, Riecher-Rössler A. The influence of age and sex on the onset and early course of schizophrenia. Br J Psychiatry J Ment Sci. 1993;162:80–6.
17. Abel KM, Drake R, Goldstein JM. Sex differences in schizophrenia. Int Rev Psychiatry. 2010;22(5):417–28.
18. Canuso CM, Pandina G. Gender and schizophrenia. Psychopharmacol Bull. 2007;40(4):178–90.
19. Markham JA. Sex steroids and schizophrenia. Rev Endocr Metab Disord. 2012;13(3):187–207.
20. Brockington IF, Kelly A, Hall P, Deakin W. Premenstrual relapse of puerperal psychosis. J Affect Disord. 1988;14(3):287–92.
21. Kendell RE, Chalmers JC, Platz C. Epidemiology of puerperal psychoses. Br J Psychiatry. 1987;150(5):662–73.
22. Nott PN. Psychiatric illness following childbirth in Southampton: a case register study. Psychol Med. 1982;12(03):557–61.
23. Seeman M. Psychopathology in women and men: focus on female hormones. Am J Psychiatry. 1997;154(12):1641–7.
24. Dalton K. Menstruation and acute psychiatric illnesses. Br Med J. 1959;1(5115):148–9.
25. Halari R, Kumari V, Mehrotra R, Wheeler M, Hines M, Sharma T. The relationship of sex hormones and cortisol with cognitive functioning in schizophrenia. J Psychopharmacol (Oxf). 2004;18(3):366–74.
26. Hallonquist JD, Seeman M, Lang M, Rector NA. Variation in symptom severity over the menstrual cycle of schizophrenics. Biol Psychiatry. 1993;33(3):207–9.
27. Cohen RZ, Seeman M, Gotowiec A, Kopala L. Earlier puberty as a predictor of later onset of schizophrenia in women. Am J Psychiatry. 1999;156(7):1059–64.
28. Fischer B, Gleason C, Asthana S. Effects of hormone therapy on cognition and mood. Fertil Steril. 2014;101(4):898–904.
29. Srivastava DP, Woolfrey KM, Penzes P. Insights into rapid modulation of neuroplasticity by brain estrogens. Pharmacol Rev. 2013;65(4):1318–50.
30. Ross SM. Menstruation in its relationships to insanity. Br J Psychiatry. 1909;55(229):270–80.
31. Lønning PE, Taylor PD, Anker G, Iddon J, Wie L, Jørgensen L-M, et al. High-dose estrogen treatment in postmenopausal breast cancer patients heavily exposed to endocrine therapy. Breast Cancer Res Treat. 2001;67(2):111–6.
32. Oh WK. The evolving role of estrogen therapy in prostate cancer. Clin Prostate Cancer. 2002;1(2):81–9.
33. Lindsay R, Tohme JF. Estrogen treatment of patients with established postmenopausal osteoporosis. Obstet Gynecol. 1990;76(2):290–5.
34. Klaiber EL, Broverman DM, Vogel W, Kobayashi Y. Estrogen therapy for severe persistent depressions in women. Arch Gen Psychiatry. 1979;36(5):550–4.
35. Ahokas A, Kaukoranta J, Aito M. Estrogen deficiency in severe postpartum depression: successful treatment with sublingual physiologic 17beta-estradiol: a preliminary study. J Clin Psychiatry. 2001;62(5):332–6.
36. Schmidt PJ, Nieman L, Danaceau MA, Tobin MB, Roca CA, Murphy JH, et al. Estrogen replacement in perimenopause-related depression: a preliminary report. Am J Obstet Gynecol. 2000;183(2):414–20.
37. Kulkarni J, Garland KA, Scaffidi A, Headey B, Anderson R, de Castella A, et al. A pilot study of hormone modulation as a new treatment for mania in women with bipolar affective disorder. Psychoneuroendocrinology. 2006;31(4):543–7.
38. Rao ML, Kölsch H. Effects of estrogen on brain development and neuroprotection—implications for negative symptoms in schizophrenia. Psychoneuroendocrinology. 2003;28(Supplement 2):83–96.
39. Seeman M. The role of estrogen in schizophrenia. J Psychiatry Neurosci. 1996;21(2):123–7.
40. Diczfalusy E, Lauritzen C. Oestrogene beim Menschen. Berlin/Heidelberg: Springer; 1961.
41. Bergemann N, Riecher-Rössler A. Estrogen effects in psychiatric disorders. New York: Springer; 2005.

42. Montgomery J, Winterbottom E, Jessani M, Kohegyi E, Fulmer J, Seamonds B, et al. Prevalence of hyperprolactinemia in schizophrenia: association with typical and atypical antipsychotic treatment. J Clin Psychiatry. 2004;65(11):1491–8.
43. Bergemann N, Parzer P, Nagl I, Salbach B, Runnebaum B, Mundt C, et al. Acute psychiatric admission and menstrual cycle phase in women with schizophrenia. Arch Womens Ment Health. 2002;5(3):119–26.
44. Bergemann N, Mundt C, Parzer P, Jannakos I, Nagl I, Salbach B, et al. Plasma concentrations of estradiol in women suffering from schizophrenia treated with conventional versus atypical antipsychotics. Schizophr Res. 2005;73(2–3):357–66.
45. Zhang-Wong JH, Seeman MV. Antipsychotic drugs, menstrual regularity and osteoporosis risk. Arch Womens Ment Health. 2002;5(3):93–8.
46. Gogos A, Sbisa AM, Sun J, Gibbons A, Udawela M, Dean B. A role for estrogen in schizophrenia: clinical and preclinical findings. Int J Endocrinol. 2015;2015:615356.
47. Weickert CS, Miranda-Angulo AL, Wong J, Perlman WR, Ward SE, Radhakrishna V, et al. Variants in the estrogen receptor alpha gene and its mRNA contribute to risk for schizophrenia. Hum Mol Genet. 2008;17(15):2293–309.
48. Sayed Y, Taxel P. The use of estrogen therapy in men. Curr Opin Pharmacol. 2003;3(6):650–4.
49. Azcoitia I, Yague JG, Garcia-Segura LM. Estradiol synthesis within the human brain. Neuroscience. 2011;191:139–47.
50. Broverman DM, Vogel W, Klaiber EL, Majcher D, Shea D, Paul V. Changes in cognitive task performance across the menstrual cycle. J Comp Physiol Psychol. 1981;95(4):646–54.
51. Sherwin BB. Estrogen and cognitive functioning in women. Endocr Rev. 2003;24(2):133–51.
52. Joffe H, Hall JE, Gruber S, Sarmiento IA, Cohen LS, Yurgelun-Todd D, et al. Estrogen therapy selectively enhances prefrontal cognitive processes: a randomized, double-blind, placebo-controlled study with functional magnetic resonance imaging in perimenopausal and recently postmenopausal women. Menopause. 2006;13(3):411–22.
53. Luine VN, Jacome L, Maclusky N. Rapid enhancement of visual and place memory by estrogens in rats. Endocrinology. 2003;144(7):2836–44.
54. Inagaki T, Gautreaux C, Luine VN. Acute estrogen treatment facilitates recognition memory consolidation and alters monoamine levels in memory-related brain areas. Horm Behav. 2010;58(3):415–26.
55. Barha CK, Galea LAM. Influence of different estrogens on neuroplasticity and cognition in the hippocampus. Biochim Biophys Acta. 2010;1800(10):1056–67.
56. Luine VN. Estradiol and cognitive function: past, present and future. Horm Behav. 2014;66(4):602–18.
57. Wharton W, Baker LD, Gleason CE, Dowling M, Barnet JH, Johnson S, et al. Short-term hormone therapy with transdermal estradiol improves cognition for postmenopausal women with Alzheimer's disease: results of a randomized controlled trial. J Alzheimers Dis. 2011;26(3):495–505.
58. Sundström Poromaa I, Gingnell M. Menstrual cycle influence on cognitive function and emotion processing from a reproductive perspective. Front Neurosci. 2014;8:380.
59. Hausmann M, Slabbekoorn D, Van Goozen SHM, Cohen-Kettenis PT, Gunturkun O. Sex hormones affect spatial abilities during the menstrual cycle. Behav Neurosci. 2000;114(6):1245–50.
60. Sherwin BB. Estrogen and cognitive functioning in women: lessons we have learned. Behav Neurosci. 2012;126(1):123–7.
61. Hampson E. Variations in sex-related cognitive abilities across the menstrual cycle. Brain Cogn. 1990;14(1):26–43.
62. Gordon HW, Lee PA. No difference in cognitive performance between phases of the menstrual cycle. Psychoneuroendocrinology. 1993;18(7):521–31.
63. Jacobs E, D'Esposito M. Estrogen shapes dopamine-dependent cognitive processes: Implications for women's health. J Neurosci. 2011;31(14):5286–93.
64. Maki PM, Rich JB, Rosenbaum SR. Implicit memory varies across the menstrual cycle: estrogen effects in young women. Neuropsychologia. 2002;40(5):518–29.
65. Mordecai KL, Rubin LH, Maki PM. Effects of menstrual cycle phase and oral contraceptive use on verbal memory. Horm Behav. 2008;54(2):286–93.
66. Rosenberg L, Park S. Verbal and spatial functions across the menstrual cycle in healthy young women. Psychoneuroendocrinology. 2002;27(7):835–41.
67. Gogos A. Natural and synthetic sex hormones: effects on higher-order cognitive function and prepulse inhibition. Biol Psychol. 2013;93(1):17–23.
68. Gogos A, Wu YC, Williams AS, Byrne LK. The effects of ethinylestradiol and progestins ("the pill") on cognitive function in pre-menopausal women. Neurochem Res. 2014;39(12):2288–300.
69. Cyr M, Calon F, Morissette M, Di Paolo T. Estrogenic modulation of brain activity: implications for schizophrenia and Parkinson's disease. J Psychiatry Neurosci. 2002;27(1):12–27.
70. Nelson HD. Commonly used types of postmenopausal estrogen for treatment of hot flashes: scientific review. JAMA. 2004;291(13):1610–20.
71. Resnick SM, Maki PM, Rapp SR, Espeland MA, Brunner R, Coker LH, et al. Effects of combination estrogen plus progestin hormone treatment on cognition and affect. J Clin Endocrinol Metab. 2006;91(5):1802–10.
72. Engler-Chiurazzi EB, Talboom JS, Braden BB, Tsang CWS, Mennenga S, Andrews M, et al. Continuous estrone treatment impairs spatial memory and does not impact number of basal forebrain cholinergic neurons in the surgically menopausal middle-aged rat. Horm Behav. 2012;62(1):1–9.
73. Luine VN, Richards ST, Wu VY, Beck KD. Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. Horm Behav. 1998;34(2):149–62.
74. Akhondzadeh S, Nejatisafa AA, Amini H, Mohammadi MR, Larijani B, Kashani L, et al. Adjunctive estro-

gen treatment in women with chronic schizophrenia: a double-blind, randomized, and placebo-controlled trial. Prog Neuropsychopharmacol Biol Psychiatry. 2003;27(6):1007–12.
75. Kulkarni J, Riedel A, de Castella AR, Fitzgerald PB, Rolfe TJ, Taffe J, et al. Estrogen — a potential treatment for schizophrenia. Schizophr Res. 2001;48(1):137–44.
76. Kulkarni J, de Castella A, Smith D, Taffe J, Keks N, Copolov D. A clinical trial of the effects of estrogen in acutely psychotic women. Schizophr Res. 1996;20(3):247–52.
77. Ko Y-H, Joe S-H, Cho W, Park J-H, Lee J-J, Jung I-K, et al. Estrogen, cognitive function and negative symptoms in female schizophrenia. Neuropsychobiology. 2006;53(4):169–75.
78. Kulkarni J, Riedel A, de Castella AR, Fitzgerald PB, Rolfe TJ, Taffe J, et al. A clinical trial of adjunctive oestrogen treatment in women with schizophrenia. Arch Womens Ment Health. 2002;5(3):99–104.
79. Kulkarni J, de Castella A, Fitzgerald PB, Gurvich CT, Bailey M, Bartholomeusz C, et al. Estrogen in severe mental illness: a potential new treatment approach. Arch Gen Psychiatry. 2008;65(8):955–60.
80. Bergemann N, Mundt C, Parzer P, Pakrasi M, Eckstein-Mannsperger U, Haisch S, et al. Estrogen as an adjuvant therapy to antipsychotics does not prevent relapse in women suffering from schizophrenia: results of a placebo-controlled double-blind study. Schizophr Res. 2005;74(2–3):125–34.
81. Lindamer LA, Buse DC, Lohr JB, Jeste DV. Hormone replacement therapy in postmenopausal women with schizophrenia: positive effect on negative symptoms? Biol Psychiatry. 2001;49(1):47–51.
82. Hoff AL, Kremen WS, Wieneke MH, Lauriello J, et al. Association of estrogen levels with neuropsychological performance in women with schizophrenia. Am J Psychiatry. 2001;158(7):1134–9.
83. Bergemann N, Parzer P, Jaggy S, Auler B, Mundt C, Maier-Braunleder S. Estrogen and comprehension of metaphoric speech in women suffering from schizophrenia: results of a double-blind, placebo-controlled trial. Schizophr Bull. 2008;34(6):1172–81.
84. Gibbs RB. Estrogen therapy and cognition: a review of the cholinergic hypothesis. Endocr Rev. 2010;31(2):224–53.
85. Arad M, Weiner I. Contrasting effects of increased and decreased dopamine transmission on latent inhibition in ovariectomized rats and their modulation by 17β-estradiol: an animal model of menopausal psychosis? Neuropsychopharmacology. 2010;35(7):1570–82.
86. Arad M, Weiner I. Sex-dependent antipsychotic capacity of 17β-estradiol in the latent inhibition model: a typical antipsychotic drug in both sexes, atypical antipsychotic drug in males. Neuropsychopharmacology. 2010;35(11):2179–92.
87. Barha CK, Dalton GL, Galea LAM. Low doses of 17α-estradiol and 17β-estradiol facilitate, whereas higher doses of estrone and 17α- and 17β-estradiol impair, contextual fear conditioning in adult female rats. Neuropsychopharmacology. 2009;35(2):547–59.
88. Bimonte HA, Denenberg VH. Estradiol facilitates performance as working memory load increases. Psychoneuroendocrinology. 1999;24(2):161–73.
89. Bimonte-Nelson HA, Francis KR, Umphlet CD, Granholm A-C. Progesterone reverses the spatial memory enhancements initiated by tonic and cyclic oestrogen therapy in middle-aged ovariectomized female rats. Eur J Neurosci. 2006;24(1):229–42.
90. Bohacek J, Daniel JM. The beneficial effects of estradiol on attentional processes are dependent on timing of treatment initiation following ovariectomy in middle-aged rats. Psychoneuroendocrinology. 2010;35(5):694–705.
91. Chesler EJ, Juraska JM. Acute administration of estrogen and progesterone impairs the acquisition of the spatial Morris water maze in ovariectomized rats. Horm Behav. 2000;38(4):234–42.
92. Daniel JM, Fader AJ, Spencer AL, Dohanich GP. Estrogen enhances performance of female rats during acquisition of a radial arm maze. Horm Behav. 1997;32(3):217–25.
93. Daniel JM, Lee CD. Estrogen replacement in ovariectomized rats affects strategy selection in the Morris water maze. Neurobiol Learn Mem. 2004;82(2):142–9.
94. Daniel JM, Hulst JL, Berbling JL. Estradiol replacement enhances working memory in middle-aged rats when initiated immediately after ovariectomy but not after a long-term period of ovarian hormone deprivation. Endocrinology. 2006;147(1):607–14.
95. Davis DM, Jacobson TK, Aliakbari S, Mizumori SJY. Differential effects of estrogen on hippocampal- and striatal-dependent learning. Neurobiol Learn Mem. 2005;84(2):132–7.
96. Diaz-Veliz G, Urresta F, Dussaubat N, Mora S. Effects of estradiol replacement in ovariectomized rats on conditioned avoidance responses and other behaviors. Physiol Behav. 1991;50(1):61–5.
97. El-Bakri NK, Islam A, Zhu S, Elhassan A, Mohammed A, Winblad B, et al. Effects of estrogen and progesterone treatment on rat hippocampal NMDA receptors: relationship to Morris water maze performance. J Cell Mol Med. 2004;8(4):537–44.
98. Fader AJ, Hendricson AW, Dohanich GP. Estrogen improves performance of reinforced T-maze alternation and prevents the amnestic effects of scopolamine administered systemically or intrahippocampally. Neurobiol Learn Mem. 1998;69(3):225–40.
99. Fader AJ, Johnson PEM, Dohanich GP. Estrogen improves working but not reference memory and prevents amnestic effects of scopolamine on a radial-arm maze. Pharmacol Biochem Behav. 1999;62(4):711–7.
100. Feng Z, Cheng Y, Zhang J. Long-term effects of melatonin or 17β-estradiol on improving spatial memory performance in cognitively impaired, ovariectomized adult rats. J Pineal Res. 2004;37(3):198–206.
101. Frick KM, Fernandez SM, Bennett JC, Prange-Kiel J, MacLusky NJ, Leranth C. Behavioral training interferes with the ability of gonadal hormones to increase

CA1 spine synapse density in ovariectomized female rats. Eur J Neurosci. 2004;19(11):3026–32.
102. Frye CA, Rhodes ME. Enhancing effects of estrogen on inhibitory avoidance performance may be in part independent of intracellular estrogen receptors in the hippocampus. Brain Res. 2002;956(2):285–93.
103. Frye CA, Duffy CK, Walf AA. Estrogens and progestins enhance spatial learning of intact and ovariectomized rats in the object placement task. Neurobiol Learn Mem. 2007;88(2):208–16.
104. Galea LAM, Wide JK, Paine TA, Holmes MM, Ormerod BK, Floresco SB. High levels of estradiol disrupt conditioned place preference learning, stimulus response learning and reference memory but have limited effects on working memory. Behav Brain Res. 2001;126(1–2):115–26.
105. Gibbs RB. Estrogen replacement enhances acquisition of a spatial memory task and reduces deficits associated with hippocampal muscarinic receptor inhibition. Horm Behav. 1999;36(3):222–33.
106. Gibbs RB. Long-term treatment with estrogen and progesterone enhances acquisition of a spatial memory task by ovariectomized aged rats. Neurobiol Aging. 2000;21(1):107–16.
107. Gibbs RB. Estradiol enhances DMP acquisition via a mechanism not mediated by turning strategy but which requires intact basal forebrain cholinergic projections. Horm Behav. 2007;52(3):352–9.
108. Gibbs RB, Johnson DA. Sex-specific effects of gonadectomy and hormone treatment on acquisition of a 12-arm radial maze task by Sprague Dawley rats. Endocrinology. 2008;149(6):3176–83.
109. Gogos A, van den Buuse M. Estrogen and progesterone prevent disruption of prepulse inhibition by the serotonin-1a receptor agonist 8-hydroxy-2-dipropylaminotetralin. J Pharmacol Exp Ther. 2004;309(1):267–74.
110. Gogos A, Kwek P, Chavez C, van den Buuse M. Estrogen treatment blocks 8-Hydroxy-2-Dipropylaminotetralin- and apomorphine-induced disruptions of prepulse inhibition: involvement of dopamine D1 or D2 or serotonin 5-HT1A, 5-HT2A, or 5-HT7 receptors. J Pharmacol Exp Ther. 2010;333(1):218–27.
111. Gogos A, Kwek P, van den Buuse M. The role of estrogen and testosterone in female rats in behavioral models of relevance to schizophrenia. Psychopharmacology (Berl). 2012;219(1):213–24.
112. Hammond R, Mauk R, Ninaci D, Nelson D, Gibbs R. Chronic treatment with estrogen receptor agonists restores acquisition of a spatial learning task in young ovariectomized rats. Horm Behav. 2009;56(3):309–14.
113. Hawley WR, Grissom EM, Moody NM, Dohanich GP, Vasudevan N. Activation of G-protein-coupled receptor 30 is sufficient to enhance spatial recognition memory in ovariectomized rats. Behav Brain Res. 2014;262:68–73.
114. Hoffman AN, Armstrong CE, Hanna JJ, Conrad CD. Chronic stress, cyclic 17β-estradiol, and daily handling influences on fear conditioning in the female rat. Neurobiol Learn Mem. 2010;94(3):422–33.
115. Holmes MM, Wide JK, Galea LAM. Low levels of estradiol facilitate, whereas high levels of estradiol impair, working memory performance on the radial arm maze. Behav Neurosci. 2002;116(5):928–34.
116. Horvath KM, Hårtig W, Van der Veen R, Keijser JN, Mulder J, Ziegert M, et al. 17β-Estradiol enhances cortical cholinergic innervation and preserves synaptic density following excitotoxic lesions to the rat nucleus basalis magnocellularis. Neuroscience. 2002;110(3):489–504.
117. Jacome LF, Gautreaux C, Inagaki T, Mohan G, Alves S, Lubbers LS, et al. Estradiol and ERβ agonists enhance recognition memory, and DPN, an ERβ agonist, alters brain monoamines. Neurobiol Learn Mem. 2010;94(4):488–98.
118. Kiss A, Delattre AM, Pereira SIR, Carolino RG, Szawka RE, Anselmo-Franci JA, et al. 17β-Estradiol replacement in young, adult and middle-aged female ovariectomized rats promotes improvement of spatial reference memory and an antidepressant effect and alters monoamines and BDNF levels in memory- and depression-related brain areas. Behav Brain Res. 2012;227(1):100–8.
119. Korol DL, Kolo LL. Estrogen-induced changes in place and response learning in young adult female rats. Behav Neurosci. 2002;116(3):411–20.
120. Lipatova O, Toufexis DJ. Estrogen enhances the retention of spatial reference memory in the open field tower task, but disrupts the expression of spatial memory following a novel start position. Neurobiol Learn Mem. 2013;99:50–8.
121. Lipatova O, Byrd D, Green JT, Toufexis DJ. Effects of continuous vs. cycling estrogen replacement on the acquisition, retention and expression of place- and response-learning in the open-field tower maze. Neurobiol Learn Mem. 2014;114:81–9.
122. Liu J, Lin H, Huang Y, Liu Y, Wang B, Su F. Cognitive effects of long-term dydrogesterone treatment used alone or with estrogen on rat menopausal models of different ages. Neuroscience. 2015;290:103–14.
123. Lowry NC, Pardon LP, Yates MA, Juraska JM. Effects of long-term treatment with 17 β-estradiol and medroxyprogesterone acetate on water maze performance in middle aged female rats. Horm Behav. 2010;58(2):200–7.
124. Luine VN, Rodriguez M. Effects of estradiol on radial arm maze performance of young and aged rats. Behav Neural Biol. 1994;62(3):230–6.
125. Markham JA, Pych JC, Juraska JM. Ovarian hormone replacement to aged ovariectomized female rats benefits acquisition of the morris water maze. Horm Behav. 2002;42(3):284–93.
126. Markowska AL, Savonenko AV. Effectiveness of estrogen replacement in restoration of cognitive function after long-term estrogen withdrawal in aging rats. J Neurosci Off J Soc Neurosci. 2002;22(24):10985–95.

127. McLaughlin KJ, Bimonte-Nelson H, Neisewander JL, Conrad CD. Assessment of estradiol influence on spatial tasks and hippocampal CA1 spines: Evidence that the duration of hormone deprivation after ovariectomy compromises 17β-estradiol effectiveness in altering CA1 spines. Horm Behav. 2008;54(3):386–95.
128. Nofrey BS, Ben-Shahar OM, Brake WG. Estrogen abolishes latent inhibition in ovariectomized female rats. Brain Cogn. 2008;66(2):156–60.
129. Packard MG, Teather LA. Posttraining estradiol injections enhance memory in ovariectomized rats: cholinergic blockade and synergism. Neurobiol Learn Mem. 1997;68(2):172–88.
130. Ping SE, Trieu J, Wlodek ME, Barrett GL. Effects of estrogen on basal forebrain cholinergic neurons and spatial learning. J Neurosci Res. 2008;86(7):1588–98.
131. Rhodes ME, Frye CA. Estrogen has mnemonic-enhancing effects in the inhibitory avoidance task. Pharmacol Biochem Behav. 2004;78(3):551–8.
132. Rhodes ME, Frye CA. ERβ-selective SERMs produce mnemonic-enhancing effects in the inhibitory avoidance and water maze tasks. Neurobiol Learn Mem. 2006;85(2):183–91.
133. Rodgers SP, Bohacek J, Daniel JM. Transient estradiol exposure during middle age in ovariectomized rats exerts lasting effects on cognitive function and the hippocampus. Endocrinology. 2010;151(3):1194–203.
134. Roseman AS, McGregor C, Thornton JE. Estradiol attenuates the cognitive deficits in the novel object recognition task induced by sub-chronic phencyclidine in ovariectomized rats. Behav Brain Res. 2012;233(1):105–12.
135. Sandstrom NJ, Williams CL. Memory retention is modulated by acute estradiol and progesterone replacement. Behav Neurosci. 2001;115(2):384–93.
136. Savonenko AV, Markowska AL. The cognitive effects of ovariectomy and estrogen replacement are modulated by aging. Neuroscience. 2003;119(3):821–30.
137. Singh M, Meyer EM, Millard WJ, Simpkins JW. Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague–Dawley rats. Brain Res. 1994;644(2):305–12.
138. Sinopoli KJ, Floresco SB, Galea LAM. Systemic and local administration of estradiol into the prefrontal cortex or hippocampus differentially alters working memory. Neurobiol Learn Mem. 2006;86(3):293–304.
139. Talboom JS, Williams BJ, Baxley ER, West SG, Bimonte-Nelson HA. Higher levels of estradiol replacement correlate with better spatial memory in surgically menopausal young and middle-aged rats. Neurobiol Learn Mem. 2008;90(1):155–63.
140. Tanabe F, Miyasaka N, Kubota T, Aso T. Estrogen and progesterone improve scopolamine-induced impairment of spatial memory. J Med Dent Sci. 2004;51(1):89–98.
141. Thwaites SJ, van den Buuse M, Gogos A. Differential effects of estrogen and testosterone on auditory sensory gating in rats. Psychopharmacology (Berl). 2014;231(1):243–56.
142. Vaillancourt C, Cyr M, Rochford J, Boksa P, Di Paolo T. Effects of ovariectomy and estradiol on acoustic startle responses in rats. Pharmacol Biochem Behav. 2002;74(1):103–9.
143. van den Buuse M, Eikelis N. Estrogen increases prepulse inhibition of acoustic startle in rats. Eur J Pharmacol. 2001;425(1):33–41.
144. van den Buuse M, Mingon RL, Gogos A. Chronic estrogen and progesterone treatment inhibits ketamine-induced disruption of prepulse inhibition in rats. Neurosci Lett. 2015;607:72–6.
145. Velázquez-Zamora DA, Garcia-Segura LM, González-Burgos I. Effects of selective estrogen receptor modulators on allocentric working memory performance and on dendritic spines in medial prefrontal cortex pyramidal neurons of ovariectomized rats. Horm Behav. 2012;61(4):512–7.
146. Walf AA, Rhodes ME, Frye CA. Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. Neurobiol Learn Mem. 2006;86(1):35–46.
147. Walf AA, Paris JJ, Frye CA. Chronic estradiol replacement to aged female rats reduces anxiety-like and depression-like behavior and enhances cognitive performance. Psychoneuroendocrinology. 2009;34(6):909–16.
148. Wang VC, Sable HJK, Ju YH, Allred CD, Helferich WG, Korol DL, et al. Effects of chronic estradiol treatment on delayed spatial alternation and differential reinforcement of low rates of responding. Behav Neurosci. 2008;122(4):794–804.
149. Wang VC, Neese SL, Korol DL, Schantz SL. Chronic estradiol replacement impairs performance on an operant delayed spatial alternation task in young, middle-aged, and old rats. Horm Behav. 2009;56(4):382–90.
150. Wide JK, Hanratty K, Ting J, Galea LAM. High level estradiol impairs and low level estradiol facilitates non-spatial working memory. Behav Brain Res. 2004;155(1):45–53.
151. Witty CF, Gardella LP, Perez MC, Daniel JM. Short-term estradiol administration in aging ovariectomized rats provides lasting benefits for memory and the hippocampus: a role for insulin-like growth factor-I. Endocrinology. 2012;154(2):842–52.
152. Wu J, Zhu Y, Wu J. Effects of estrogen and estrogenic compounds on cognition in ovariectomized rats. Climacteric. 2008;11(3):212–20.
153. Zurkovsky L, Brown SL, Korol DL. Estrogen modulates place learning through estrogen receptors in the hippocampus. Neurobiol Learn Mem. 2006;86(3):336–43.
154. Zurkovsky L, Brown SL, Boyd SE, Fell JA, Korol DL. Estrogen modulates learning in female rats by acting directly at distinct memory systems. Neuroscience. 2007;144(1):26–37.
155. Ziegler DR, Gallagher M. Spatial memory in middle-aged female rats: assessment of estrogen replacement after ovariectomy. Brain Res. 2005;1052(2):163–73.

156. Desbonnet L, Waddington JL, O'Tuathaigh CMP. Mutant models for genes associated with schizophrenia. Biochem Soc Trans. 2009;37(1):308.
157. van den Buuse M. Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. Schizophr Bull. 2010;36(2):246–70.
158. Braff DL, Light GA. The use of neurophysiological endophenotypes to understand the genetic basis of schizophrenia. Dialogues Clin Neurosci. 2005;7(2):125–35.
159. Gogos A, van den Buuse M. Castration reduces the effect of Serotonin-1A receptor stimulation on prepulse inhibition in rats. Behav Neurosci. 2003;117(6):1407–15.
160. Sutcliffe JS, Rhaman F, Marshall KM, Neill JC. Oestradiol attenuates the cognitive deficit induced by acute phencyclidine treatment in mature female hooded-Lister rats. J Psychopharmacol (Oxf). 2008;22(8):918–22.
161. Sánchez MG, Bourque M, Morissette M, Di Paolo T. Steroids–dopamine interactions in the pathophysiology and treatment of CNS disorders. CNS Neurosci Ther. 2010;16(3):e43–71.
162. Dhingra K. Antiestrogens — tamoxifen, SERMs and beyond. Invest New Drugs. 1999;17(3):285–311.
163. Maximov PY, Lee TM, Jordan VC. The discovery and development of selective estrogen receptor modulators (SERMs) for clinical practice. Curr Clin Pharmacol. 2013;8(2):135–55.
164. Janicki SC, Schupf N. Hormonal influences on cognition and risk for alzheimer disease. Curr Neurol Neurosci Rep. 2010;10(5):359–66.
165. Calmarza-Font I, Lagunas N, Garcia-Segura LM. Antidepressive and anxiolytic activity of selective estrogen receptor modulators in ovariectomized mice submitted to chronic unpredictable stress. Behav Brain Res. 2012;227(1):287–90.
166. Legault C, Maki PM, Resnick SM, Coker L, Hogan P, Bevers TB, et al. Effects of tamoxifen and raloxifene on memory and other cognitive abilities: cognition in the study of tamoxifen and raloxifene. J Clin Oncol. 2009;27(31):5144–52.
167. Fisher B, Costantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM, et al. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst. 1994;86(7):527–37.
168. Kulkarni J, Gurvich C, Lee SJ, Gilbert H, Gavrilidis E, de Castella A, et al. Piloting the effective therapeutic dose of adjunctive selective estrogen receptor modulator treatment in postmenopausal women with schizophrenia. Psychoneuroendocrinology. 2010;35(8):1142–7.
169. Kianimehr G, Fatehi F, Hashempoor S, Khodaei-Ardakani M-R, Rezaei F, Nazari A, et al. Raloxifene adjunctive therapy for postmenopausal women suffering from chronic schizophrenia: a randomized double-blind and placebo controlled trial. DARU J Pharm Sci. 2014;22(1):55.
170. Usall J, Huerta-Ramos E, Iniesta R, Cobo J, Araya S, Roca M, et al. Raloxifene as an adjunctive treatment for postmenopausal women with schizophrenia: a double-blind, randomized, placebo-controlled trial. J Clin Psychiatry. 2011;72(11):1552.
171. Usall J, Huerta-Ramos E, Labad J, Cobo J, Núñez C, Creus M, et al. Raloxifene as an adjunctive treatment for postmenopausal women with schizophrenia: a 24-week double-blind, randomized, parallel, placebo-controlled trial. Schizophr Bull. 2016;46:309–17.
172. Weickert TW, Weinberg D, Lenroot R, Catts SV, Wells R, Vercammen A, et al. Adjunctive raloxifene treatment improves attention and memory in men and women with schizophrenia. Mol Psychiatry. 2015;20(6):685–94.
173. Buwalda B, Schagen SB. Is basic research providing answers if adjuvant anti-estrogen treatment of breast cancer can induce cognitive impairment? Life Sci. 2013;93(17):581–8.
174. O'Neill K, Chen S, Brinton RD. Impact of the selective estrogen receptor modulator, raloxifene, on neuronal survival and outgrowth following toxic insults associated with aging and Alzheimer's disease. Exp Neurol. 2004;185(1):63–80.
175. Khodaie-Ardakani M-R, Khosravi M, Zarinfard R, Nejati S, Mohsenian A, Tabrizi M, et al. A placebo-controlled study of raloxifene added to risperidone in men with chronic schizophrenia. Acta Med Iran. 2015;53(6):337–45.
176. Huerta-Ramos E, Iniesta R, Ochoa S, Cobo J, Miquel E, Roca M, et al. Effects of raloxifene on cognition in postmenopausal women with schizophrenia: a double-blind, randomized, placebo-controlled trial. Eur Neuropsychopharmacol. 2014;24(2):223–31.
177. Kulkarni J, Gurvich C, Gilbert H, Mehmedbegovic F, Mu L, Marston N, et al. Hormone modulation: a novel therapeutic approach for women with severe mental illness. Aust N Z J Psychiatry. 2008;42(1):83–8.
178. Sharma E, Raveendranathan D, Shivakumar V, Jayaram N, Rao NP, Venkatasubramanian G. Beneficial effects of add-on raloxifene in schizophrenia. Arch Womens Ment Health. 2012;15(2):147–8.
179. Wong J, Seeman M, Shapiro H. Case report — raloxifene in postmenopausal women with psychosis: preliminary findings. Am J Geriatr Psychiatry Off J Am Assoc Geriatr Psychiatry. 2003;11(6):697–8.
180. Espeland MA, Shumaker SA, Limacher M, Rapp SR, Bevers TB, Barad DH, et al. Relative effects of tamoxifen, raloxifene, and conjugated equine estrogens on cognition. J Womens Health. 2010;19(3):371–9.
181. Jacobsen DE, Samson MM, Emmelot-Vonk MH, Verhaar HJJ. Raloxifene improves verbal memory in late postmenopausal women: a randomized, double-blind, placebo-controlled trial. Menopause. 2010;17(2):309–14.
182. Yaffe K, Krueger K, Cummings SR, Blackwell T, Henderson VW, Sarkar S, et al. Effect of raloxifene on prevention of dementia and cognitive impairment in older women: the multiple outcomes of raloxifene evaluation (MORE) randomized trial. Am J Psychiatry. 2005;162(4):683–90.

183. Cabeza R, Nyberg L. Imaging Cognition II: an empirical review of 275 PET and fMRI studies. J Cogn Neurosci. 2000;12(1):1–47.
184. Kulkarni J, de Castella A, Headey B, Marston N, Sinclair K, Lee S, et al. Estrogens and men with schizophrenia: is there a case for adjunctive therapy? Schizophr Res. 2011;125(2–3):278–83.
185. Lagunas N, Calmarza-Font I, Grassi D, Garcia-Segura LM. Estrogen receptor ligands counteract cognitive deficits caused by androgen deprivation in male rats. Horm Behav. 2011;59(4):581–4.
186. Hao J, Rapp PR, Leffler AE, Leffler SR, Janssen WGM, Lou W, et al. Estrogen alters spine number and morphology in prefrontal cortex of aged female rhesus monkeys. J Neurosci. 2006;26(9): 2571–8.
187. Rapp PR, Morrison JH, Roberts JA. Cyclic estrogen replacement improves cognitive function in aged ovariectomized Rhesus monkeys. J Neurosci. 2003;23(13):5708–14.
188. Gibbs RB, Gabor R, Cox T, Johnson DA. Effects of raloxifene and estradiol on hippocampal acetylcholine release and spatial learning in the rat. Psychoneuroendocrinology. 2004;29(6):741–8.
189. Lacreuse A, Wilson ME, Herndon JG. Estradiol, but not raloxifene, improves aspects of spatial working memory in aged ovariectomized rhesus monkeys. Neurobiol Aging. 2002;23(4):589–600.
190. Vasudevan N, Pfaff DW. Non-genomic actions of estrogens and their interaction with genomic actions in the brain. Front Neuroendocrinol. 2008;29(2):238–57.
191. Morrison JH, Brinton RD, Schmidt PJ, Gore AC. Estrogen, menopause, and the aging brain: how basic neuroscience can inform hormone therapy in women. J Neurosci. 2006;26(41):10332–48.
192. Seeman P. Atypical antipsychotics: mechanism of action. FOCUS J Lifelong Learn Psychiatry. 2004;2(1):48–58.
193. Barnes P, Staal V, Muir J, Good MA. 17-β estradiol administration attenuates deficits in sustained and divided attention in young ovariectomized rats and aged acyclic female rats. Behavioral Neuroscience. 2006;120(6):1225–34. https://doi.org/10.1037/0735-7044.120.6.1225

27 Neuropsychiatric Symptoms Related to Cholinergic Deficits in Parkinson's Disease

Santiago Perez-Lloret, María Cecilia Peralta, and Francisco J. Barrantes

Abstract

Given its ability to explain the most frequent motor symptoms of Parkinson's disease (PD), degeneration of dopaminergic neurons has been considered one of the disease's main pathophysiological features. Several studies have shown that neurodegeneration also affects noradrenergic, serotoninergic, cholinergic, and other monoaminergic neuronal populations. In this work, the characteristic contribution of cholinergic deficits to cognitive dysfunction, psychosis, and sleep disturbances in PD and their treatment are explored. Important neurophysiological processes at the root of several motor and cognitive functions remit to cholinergic neurotransmission at the synaptic pathway and circuital levels. The bulk of evidence highlights the link between cholinergic alterations and the aforementioned symptoms. The pathophysiology of these symptoms is related to degeneration of cholinergic nuclei, most importantly the nucleus basalis magnocellularis and the pedunculopontine nucleus. Rivastigmine, a drug that increases cholinergic tone by inhibiting the enzyme cholinesterase, is effective for dementia, whereas the use of donepezil is still in the realm of investigation. Evidence on the clinical effects of these drugs for psychosis and rapid eye movement sleep disturbances is still weak. Anticholinergic drugs should be used with caution in PD, as they may aggravate these cholinergic symptoms.

S. Perez-Lloret, MD, PhD, CPI (✉)
Institute of Cardiology Research,
University of Buenos Aires,
National Research Council (CONICET-ININCA),
Marcelo T. de Alvear 2270, C1122AAJ Buenos Aires,
Argentina
e-mail: santiagopl@conicet.gov.ar

M.C. Peralta
Parkinson's disease and Movement Disorders Clinic,
Neurology Department, CEMIC University Hospital,
Elias Galvan 4102, C1431 Buenos Aires, Argentina

F.J. Barrantes
Laboratory of Molecular Neurobiology,
Institute for Biomedical Research, UCA-CONICET,
Faculty of Medical Sciences,
Av. Alicia Moreau de Justo 1600, C1107AFF Buenos Aires, Argentina

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_27

Keywords
Parkinson's disease • Acetylcholine • Neurodegeneration • Treatment • Pathophysiology

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting about one in every 1,000 people in their fifth decade and 19 of every 1,000 in their eighth decade or older [1]. Its principal epiphenomenological clinical symptoms are abnormal involuntary movements, bradykinesia, rigidity, and tremor. Patients also frequently display non-motor symptoms, including cognitive impairment, mood disorders, sleep alterations, dysautonomia, and hallucinations, among other symptoms [2].

Histopathological changes are mainly, but not exclusively, characterized by the progressive loss of the nigrostriatal dopaminergic pathway and of the source dopaminergic neurons in the substantia nigra pars compacta, which explains the most typical motor symptoms [3]. Administration of levodopa to parkinsonian patients has been considered the most effective symptomatic treatment for the past 40 years [4].

At the cellular and molecular levels, neuronal death may be preceded by a series of dysfunctional states, including loss of redox control, alteration of lysosomal activity, abnormal protein control mechanisms in the endoplasmic reticulum (ER), and perturbation of the ER–Golgi trafficking mechanisms. These pathologies are closely intertwined with one of the hallmarks of the disease, namely the abnormal accumulation of misfolded protein aggregates [5]. Lewy bodies constitute a characteristic pathological finding, second only to the neurofibrillary tangles in Alzheimer's disease (AD). Early work identified the immunoreactivity of the Lewy bodies with antibodies against the presynaptic protein α-synuclein [6]. One major target of α-synuclein is Rab1, a key component of the ER–Golgi trafficking pathway [7]. ER stress has been invoked as a possible major disruptive mechanism, leading to an adaptive reaction known as the unfolded protein response [8]. This response may be cytoprotective when activated to a moderate level, but is deleterious at a higher level, triggering in turn the apoptotic death of the damaged neuron [9, 10]. PD may also be considered a synaptopathy, that is, abnormal synaptic connectivity compromising nigrostriatal pathways and intra-striatal interneuronal connections, presumably most apparent at the initial stages of the disease. Mutations in the α-synuclein gene cause familial forms of PD and dementia with Lewy bodies. Synaptic accumulation of α-synuclein is accompanied by the redistribution of the synaptic SNARE proteins SNAP-25, syntaxin-1, and synaptobrevin-2, as well as by an age-dependent reduction in dopamine release [11].

The striatum is the most important input nucleus of the basal ganglia. The principal source of afferents is layer 5 of the cerebral cortex, conveying glutamatergic (Glu) excitatory synapses. Motor areas (4 and 6 plus the supplementary motor area) together with the primary somatosensory cortex follow, also with Glu neurotransmission. The second major striatal input is dopaminergic acid (DA), stemming from the substantia nigra A9 cell group.

Many features of PD are unresponsive to levodopa, such as gait disorders and cognitive impairment or dementia, indicating the involvement of other neurotransmitter systems [12]. In this regard, recent evidence suggests degeneration of adrenergic, serotoninergic, and cholinergic neurons, among others [12].

The importance of cholinergic dysfunction in the physiopathology of many PD features cannot be overemphasized. For example, in a recent study in 137 PD patients, cholinergic denervation could be related to rapid eye movement (REM) behavior disorder, fall history, gait disorders, psychosis, and

cognitive dysfunction [13, 14]. In this chapter, we review cholinergic neurotransmission in health as well as the pathophysiology and treatment of neuropsychiatric symptoms originating from cholinergic degeneration, namely dementia, psychosis, and sleep disturbances in PD.

Bibliographical references were searched in PubMed by the following string: (acetylcholine OR cholinergic) AND Parkinson's disease. Articles in English, Spanish, and French were retrieved. Reference sections from retrieved papers were also explored for data-base enrichment.

Cholinergic Neurotransmission in Health

Acetylcholine (ACh) is an ubiquitous, small molecular weight neurotransmitter which plays a pivotal role in chemical neurotransmission in the central (CNS) and peripheral nervous system (PNS). In the brain, ACh mediates distant signaling through projection neurons and local signaling via interneurons. The type of message conveyed by ACh depends on a variety of factors, including site of release, the localization of the target neurons, the target receptor subtypes [15], and the status of the target cells at the time of release. Furthermore, ACh signaling may be circumscribed to the synapse or result from the delocalized diffusion of the neurotransmitter in the extracellular milieu and binding to non-synaptic sites [16, 17]. In terms of gross anatomical brain regions, it is safe to state that ACh affects the brain in its entirety. A recent review [18] meticulously dissects current knowledge on the anatomy of cholinergic projections, summarized in two main tracks: (i) the brainstem; and (ii) the magnocellular basal forebrain– cholinergic systems. The former, as worked out by immunochemical techniques [19], involves neuronal soma located in the pedunculopontine tegmental nucleus (PPN) and the laterodorsal pontine tegmentum (LDT) and projecting to the thalamus, basal ganglia, the basal forebrain and to a much lesser extent, the cerebral cortex. The basal forebrain cholinergic system comprises neurons located in the medial septal nucleus (MS), the vertical and horizontal limbs of the diagonal band of Broca, and the nucleus basalis magnocellularis (NBM, the nucleus basalis of Meynert in humans), which send projections to neocortex, entorhinal cortex, limbic cortices, cingulate cortex, and hippocampus. Cholinergic fibers in cortex appear not to be associated with postsynaptic densities, a fact that has been linked with the hypothesis that cholinergic transmission may not be synaptic but may involve modulation of target neurons via diffusion, so-called volume transmission [16].

From the standpoint of the target receptors, ACh neurotransmission is mediated through two entirely different types of receptor proteins and ensuing molecular mechanisms, i.e., the metabotropic 7-transmembrane domain (TM) muscarinic AChRs and the ionotropic pentameric nicotinic nAChRs (See Table 27.1 for a summary). The former are members of the G protein-coupled superfamily of receptors, which possess 7-transmembrane segments and mediate intracellular signals associated with metabolic cascades. The nAChRs, on the other hand, are members of the superfamily of pentameric ligand-gated ion channels (pLGIC), a collection of neurotransmitter receptors which also includes γ-amino butyric acid (GABA-A, GABA-C), glycine, serotonin (5-HT3), and bacterial homologs [25, 26].

The metabotropic mAChRs are coupled to different types of G proteins, e.g., $G_{i/o}$ type (M2 and M4 subtypes of mAChRs) of G proteins that negatively couple to adenylate cyclase or G_q proteins (M1, M3 and M5 subtypes of mAChRs), which convert the cholinergic signal into metabolic cascades [20]. Presynaptic mAChRs (M2, M4 subtypes) are largely inhibitory, and perform this function partly as inhibitory autoreceptors on cholinergic terminals [27]. The M2 subtype is the predominant autoreceptor in the hippocampus and cerebral cortex, whereas M4 is the main subtype in the striatum [15, 20, 21]. Postsynaptic mAChRs can be either inhibitory (M2, M4) or excitatory (M1, M3, M5) [15, 21].

Table 27.1 Most abundant acetylcholine receptors expressed in the CNS

Pharmacol. type	Metabotropic		Ionotropic[a]		
Common denomination	Muscarinic		Nicotinic		
Subtype	M2, M4	M1 (50–60% of the total mAChRs), M3, M5	Hetero-pentameric (α4 and β2 subunits)	Homo-pentameric receptor (only α7 subunits)[b]	Hetero-pentameric (α6 and β2 subunits)
Second messengers	$G_{i/o}$ protein, inhibition of AC	G_q protein, metabolic cascades	Increased Na^+ and K^+ permeability	Increased Ca^{2+}, Na^+ and K^+ permeability	Increased Na^+ and K^+ permeability
Localization	Presynaptic (M2/4). hippocampus and cerebral cortex, pedunculopontine and laterodorsal tegmental nuclei of the mesopontine tegmentum (M2), striatum (M4), co-localized with dopamine receptors	Predominantly extrasynaptic in forebrain, hippocampus, cerebral cortex, striatum, thalamus (M1). Hypothalamus and various other brain regions (M3). Pars compacta of the substantia nigra, ventral tegmental area (M5)	90% of the high-affinity nAChR in brain: localized in cortex, hippocampus, striatum, thalamus, superior colliculus and mesencephalon	Involved in classic excitatory neurotransmission in some brain regions where the release of neurotransmitters, neurite outgrowth and neuronal survival is also modulated	Mesostriatal pathway, substantia nigra, ventral tegmental area, nucleus accumbens, caudate–putamen, visual pathways

For further reference see Refs. [15, 20–24]
Subtypes listed correspond to the most frequently found combination of subunits
AC adenylate cyclase
[a]Only neuronal-type receptors present in the CNS are included
[b]See the particular case of the heteromeric α7β2 in the text

The ionotropic, fast-signaling nAChRs are composed of five polypeptide subunits organized pseudo-symmetrically around a central pore [22]. Each subunit contains an extracellular domain, four hydrophobic transmembrane segments arranged in the form of three concentric rings around the pore, and a short extracellular carboxy-terminal domain. nAChRs are characteristically involved in the rapid "phasic" effects of ACh under conditions of brief release/high local concentration of the neurotransmitter, but they also operate under the low, tonic ACh release or mimicking systemically applied cholinergic drugs [16], a condition which may be particularly relevant to cholinergic neurotransmission — and/or its modulation — in the striatum.

Muscle-type nAChRs are expressed in the PNS and neuronal-type nAChRs in both PNS and the CNS as well as in other non-neural tissues such as immune cells, lymphocytes, lung epithelium, and other tissues [28]. In the CNS, the nAChR is present in various combinations of subunits (α4, α5, α6, α7, α9, α10, and β2 [29]), the two most abundant ones being the hetero-pentameric receptor formed by α4 and β2 subunits and the homo-pentameric receptor formed exclusively by α7 subunits. The deficit of some of the nAChR subunits in PD has been explicitly explored [30]. The two predominant forms of the nAChR, the α4β2 and the α7 oligomers, are also strongly expressed in the striatum, accompanied by the α6β2 form [31]. It is not clear whether other subunits are present in the heteromeric nAChRs. The α4β2 and α6β2 nAChRs in the striatum are localized at the dopaminergic terminal, the predominant target undergoing degeneration in PD. The α4β2 nAChR is also found in striatal GABAergic inhibitory interneurons [32].

The α7nAChR exhibits certain functional properties that distinguish it from other nicotinic receptors: (a) fast desensitization kinetics, (b) unusually high Ca^{2+} permeability, and (c) high affinity for binding α-bungarotoxin [29, 33]. In most regions of the brain, the α7 nAChR is found presynaptically, where it modulates enhanced neurotransmitter release of various other neurotransmitters, including DA, 5-HT, glutamate, and GABA; and postsynaptically, where it generates postsynaptic currents [23, 24]. In addition, the perisynaptic presence of the receptor has also been demonstrated, where it modulates neuronal activity, presumably by an unconventional mechanism involving diffusion of the natural neurotransmitter and binding to non-synaptic sites [16]. In the striatum, the α7 nAChRs are found in cortical glutamatergic excitatory afferents [23].

Cholinergic mechanisms are intimately linked to cognitive functions associated with cortical and hippocampal brain anatomical regions. Working memory, spatial and episodic memory acquisition, storage, maintenance and retrieval, attention, and other neurophysiological processes at the root of neural information and cognitive functions remit to ACh neurotransmission at synaptic, pathway, and circuital levels.

Neuropsychiatric Symptoms Resulting from Cholinergic Degeneration in PD

A summary of the brain cholinergic nuclei exhibiting signs of denervation in PD and their neuropsychiatric correlates is presented in Table 27.2. In this section, the contributions of cholinergic degeneration to the physiopathology of cognitive impairment, psychosis, and sleep disturbances in PD will be summarized.

Table 27.2 Sources of cholinergic dysfunction in PD and its main clinical correlates

PD feature	Pathological basis
Cognitive impairment	Degeneration of the NBM
REM sleep behavior disorder	Degeneration of the PPN
Psychosis	Reduced cholinergic tone (maybe PPN)

NBM nucleus basalis magnocellularis (Meynert's nucleus), *PPN* pedunculopontine nucleus

Cognitive Impairment and Mood Disorders

The physiopathology of PD dementia (PDD) is complex and involves severe dopaminergic and cholinergic deficits, the main pathological drivers of cognitive decline being a synergistic effect between α-synuclein and AD pathology [34]. Only cholinergic deficits will be reviewed in this section.

Alteration in Cholinergic Receptors Expression or Function in Cognitive Impairment in PD

Because of their distribution in brain anatomical regions associated with cognitive processes, various subtypes of nAChR have been invoked as being associated with abnormal cognitive processes. The α7 nAChR is highly expressed in the hippocampus, a region particularly affected in cognitive disorders [29, 35–37], as recently reviewed in [38], whereas a massive loss in cerebral cortex of the other most abundant type of CNS nAChRs, the α4β2-type, accompanies the cognitive decline observed in AD [39, 40]. Not surprisingly, alterations in memory and cognition associated with nAChRs have also been reported in pathological states other than AD, such as schizophrenia [41]. The various functions afflicted in PD have been associated with nAChR dysfunction of different brain nAChR oligomeric forms [42–44]. α7 nAChR ligands are a subject of intense research in diseases affecting cognitive functions, especially the subclass of ligands termed positive allosteric modulators (PAMs, see reviews in [45]). This is a group of compounds that enhance recognition memory and cognitive improvement in animal models (e.g., [46, 47]).

Muscarinic receptors are also implicated in cognitive disturbances. Antagonists such as scopolamine perturb the performance of cognitive tasks in animal models [48] and even lead to extreme cognitive disturbances with delirium at higher doses [49]. This condition has also been reported in children after application of postsurgical transdermal patches to ameliorate nausea and motion sickness [50], and in the elderly, who are particularly vulnerable to even modest levels of antimuscarinic drugs due to their cumulative effects [51].

Several lines of evidence link brain nicotinic nAChRs, the α7 in particular, with the development of neurodegenerative disease with cognitive impairments, such as AD [45]. The greater the depletion of cholinergic neurons and associated cholinergic pathways in cognitive-associated brain areas such as the neocortex and hippocampus, the more severe the associated dementia, suggesting a relationship between the clinical manifestations and the level of cholinergic decline [52, 53]. Cholinergic pathways are associated with the processes of learning and memory, and nicotinic agonists and cholinomimetics in general have been used as therapeutic agents, providing symptomatic improvements in cognitive impairment [54–57]. This constitutes the basis of therapeutic approaches aiming at α7 AChR activation with selective agonists.

Data from Studies in PD Patients

The involvement of ACh pathways in PD is further exemplified by the results of a recent trial by Park and colleagues [58]. White matter hyperintensities in the cholinergic pathways were assessed by means of the Cholinergic Pathways Hyperintensities Scale (CHIPS) using 3.0 Tesla magnetic resonance. Patients with AD (n = 20), PDD (n = 21) and dementia with Lewy bodies (DLB, n = 17) were compared with a group of 20 healthy controls. Results showed that the CHIPS score was correlated with MMSE, SOB scores of the Clinical Dementia Rating, and verbal and visuospatial memory domains in demented patients.

Degeneration of the NBM appears to be highly correlated with PDD [59]. A recent study showed that PD patients with mild cognitive impairments (PD-MCI) who would develop PDD during follow-up had greater degeneration of the substantia innominata, where the NBM is located [60]. In this study, 51 PD-MCI were followed for a minimum of 2 years, during which PDD was diagnosed in 15 cases. Greater grey matter loss in the prefrontal area was also observed in subjects developing PDD. Loss of neurons in the substantia innominata was observed in early stages of the disease, and was further accentuated in PDD.

Recent results in post-mortem analyses of brains from demented and non-demented PD

patients confirmed these results. In the study by Hall and colleagues, stereological analyses of the A9 and A10 dopaminergic neurons and Ch1, Ch2 and Ch4 cholinergic neurons located in the basal forebrain, along with an assessment of α-synuclein pathology in these regions and in the hippocampus, were performed in six demented and five non-demented PD patients and five age-matched control individuals with no signs of neurological disease [61]. Choline acetyltransferase (ChAT) activity in the hippocampus and frontal cortex was also measured in a different set of eight demented and eight non-demented PD patients, as well as in the same areas of eight age-matched controls. Stereological analyses showed a significant 54% reduction in the NMB of PDD compared to controls and a non-significant reduction of 30% in non-demented PD. No differences were observed in other cholinergic regions. Furthermore, the density of ACh neurons in the NBM correlated inversely with the severity of dementia. ChAT activity, a measure of the presence of cholinergic terminals in a given brain region, was reduced in the hippocampus of PD with dementia compared to non-demented patients and controls. Interestingly, neocortical ChAT activity was reduced in the neocortex of both demented and non-demented PD compared to controls. Finally, α-synuclein pathology and Lewy-body deposition in the basal forebrain of patients with PDD were more severe than in non-demented patients, thus suggesting the possible role of α-synuclein aggregation in the development of cortical and hippocampal cholinergic dysfunction.

The diminution in the density of the α4β2 nAChR in the CNS has been recently correlated with cognitive impairments in non-demented PD patients [62]. Previous studies had revealed reduced binding to these receptors in PD brains, and some preliminary findings suggest that the lower density of these receptors might correlate with cognitive impairments. In this study, 25 non-demented PD patients underwent a 5-[123I] iodo-3-[2(S)-2-azetidinylmethoxy] pyridine (5-I-A-85380) SPECT to visualize α4β2 nAChRs, and cognitive testing with the CERAD (Consortium to Establish a Registry for Alzheimer's Disease) battery to identify domains of cognitive dysfunction [62]. Results showed significant correlations between performance of the CERAD subtests Boston Naming Test (a specific test for visual perception and for detection of word-finding difficulties) and Word List Intrusions (a specific test for learning capacity and memory for language information) with the density of α4β2 nAChRs at the right superior parietal lobe cortex and the left thalamus, and left and right posterior subcortical regions.

An interesting question is whether the alteration of the NBM is the same as that found in AD. NBM degeneration is comparable or even more intense in PD compared to the latter, yet the clinical characteristics of the two dementias differ significantly. Some authors have suggested that the divergence may be connected to possible differences in the degree to which subsections of the NBM are affected [63], but this hypothesis remains to be studied.

Depression can precede dementia, or at least depressed patients are at greater risk of developing PDD [64]. In a recent study, neocortical cholinergic innervation was assessed in 12 non-demented PD patients, six PDD and ten normal control patients [65] by means of dynamic PET scanning of previously injected [^{11}C]methyl-4-piperidinyl propionate radioligand, a selective substrate for the enzyme AChE. Pooled analyses demonstrated a significant inverse correlation between cortical AChE activity and Cornell Scale for Depression in Dementia scores (r = 0.5, p = 0.007). The correlation remained significant when only PD patients were assessed, in whom AChE activity also correlated with the MMSE score. Recent evidence suggests that the early involvement of the posterior neocortex and visuoperceptual impairment may be risk factors for the rapid symptomatic progression and dementia in PD [66].

Cognitive Dysfunction as a Side-Effect of Cholinergic Drugs

In the light of the evidence reviewed above, it is not surprising that drugs interfering with cholinergic function have profound effects on cognitive function in PD. Muscarinic receptor blockers can cause

acute confusion, dementia, and chronic intellectual impairment [67]. In a study with trihexyphenidyl, an oral anticholinergic agent, clinical disability, cognitive assessment, and measurements of cerebral blood flow (rCBF) and oxygen metabolic rate (rCMRO) were performed in six PD patients before and after administration of the drug for 7 weeks at 6 mg/day [68]. Results showed improvements in motor symptoms without evident changes in cognitive function. Cortical and striatal rCBF and rCMRO2 were significantly decreased, a typical finding in PDD [69].

Psychosis and Delirium

Visual hallucinations (VHs) are frequently reported by PD patients [2]. Besides the effect of dopaminergic medication, anticholinergics are associated with VHs even in patients without PD.

Data from Studies in PD Patients

In a recent study, inhibitory cholinergic activity in the CNS was measured by means of the short-latency afferent inhibition (SAI) technique in ten non-demented PD patients with VHs, in 12 non-demented PD patients without VHs, and in 11 age-matched healthy controls [70]. Results showed reduced SAI in patients with VHs, which was otherwise normal in patients without hallucinations. In addition, patients with VHs showed more frequent MCI and had reduced values in some cognitive function tests. The authors speculated that these results might be related to diminished neocortical cholinergic input from the NBM.

Delirium

Characterized by an acute and fluctuating disturbance in attention and awareness accompanied by an additional disturbance in cognition, delirium is more frequent in PD than in the general population [71]. Cholinergic deficiency is one of the most frequently found abnormalities in delirium. In a recent study, the association between exposure to anticholinergic drugs and delirium was studied in a database of more than 16,000 PD patients [72]. Results showed that 57.8% of PD patients were prescribed non-PD medications with moderate to very strong anticholinergic potential. Subjects exposed to anticholinergic polypharmacy had increased risk of delirium (adjusted OR: 1.61, 95% CI: 1.08–2.40).

Sleep Disturbances

Sleep disturbances are common disabling non-motor features of PD that have a detrimental effect on health-related quality of life [2]. Activation of the PPN is capable of inducing REM sleep [73] and degeneration of cholinergic neurons in the basal forebrain and brainstem is one of the factors resulting in a reduction in REM sleep and REM-sleep behavior disorder (RBD) [74]. RBD is characterized by a loss of normal muscle atonia during REM sleep and dream-enacting behavior. RBD occurs in 0.5% of the general population, but is considered to be a risk factor for synucleinopathies and even as a pre-motor sign of PD [75].

Data from Studies on Animal Models

The effects on REM sleep of drugs acting on different monoaminergic systems have been explored in the MPTP mouse model of PD [76]. The objective of the study was to assess the effects of these drugs on sleep/wakefulness patterns, measuring the amount of REM sleep (or paradoxical sleep -PS-). Arecoline, a muscarinic agonist, increased the amount of PS in the MPTP-treated mice but not in the controls, probably reflecting supersensitivity in the former.

Data from Studies in PD Patients

Cholinergic function has been recently evaluated in PD patients with or without RBD by means of short-latency afferent inhibition (SAI), a transcranial magnetic stimulation protocol able to test an inhibitory cholinergic circuit in the human brain [77]. In this study, ten PD patients with RBD diagnosis by polysomnography, 13 patients without the disorder, and ten healthy controls were enrolled. In addition to SAI, neuropsychological examination was also performed. SAI was reduced in PD patients with RBD compared to

unaffected PD and healthy controls. Interestingly, MCI was more frequent in the former, and cognitive parameters correlated with SAI. These findings indicate that cholinergic dysfunction may play an important role in RBD in PD.

Symptomatic Treatment of Cholinergic Deficits in PD

The idea that enhancing cholinergic tone might be a first-line therapeutic strategy for cholinergic symptoms is logical and appealing. In this section, clinical use of cholinergic tone manipulation at the brain level by pharmacological or neurosurgical approaches will be explored. Other non-cholinergic treatments will be (briefly) mentioned for the sake of completeness.

Cognitive Impairment

A recent systematic review and meta-analysis suggested that inhibitors of cholinesterase are effective in the treatment of cognitive impairment in patients with PD [78]. The systematic search yielded three studies involving donepezil and one involving rivastigmine. The EXPRESS study included 541 patients with PDD who were randomly administered 12 mg rivastigmine or placebo and followed up for a mean of 24 weeks [79]. The study by Dubois et al. included 355 patients with PDD receiving 5 mg donepezil, 10 mg donepezil, or placebo, followed up for a mean duration of 24 weeks [80]. The study by Ravina et al. was a crossover study in which 22 patients with PDD were randomized to receive either 10 mg/day donepezil followed by placebo, or placebo followed by 10 mg/day donepezil for a mean follow-up of 10 weeks [81]. Results showed that these drugs significantly slowed MMSE decline (MD = −1.123, 95% CI = −1.638 to −0.608; p = 0.001; I2 = 44.6%), and ADAS-cog (SMD = −0.266, 95% CI −0.399 to −0.133; $p < 0.0001$; I2 = 0%). Interestingly, the death rate was lower in treated patients than in those receiving a placebo (OR = 0.295, 95% CI 0.108 to 0.806; p = 0.017; I2 = 0%). Tremor and adverse drug reactions in general were more frequent with cholinesterase inhibitors. A summary of these studies is presented in Table 27.3.

Long-term safety of rivastigmine was studied in a 76-week, prospective, open-label, randomized study of 583 PD patients aged 50–85 years old [83]. Patients were randomly assigned rivastigmine 12 mg/d capsules or

Table 27.3 Studies with cholinesterase inhibitors for cognitive impairment in PD

Author & Year	Drug/ Procedure	Design	Sample	Main results
Emre 2004 [79]	Rivastigmine	Randomized, double-blind, placebo-controlled trial	541 demented PD patients	Improvement of 2.1 points in the ADAS-cog with rivastigmine vs 0.7-point worsening with placebo ($p < 0.001$)
Ravina 2005 [81]	Donepezil	Randomized, double-blind, placebo-controlled trial	22 demented PD	There was a 1.9 point trend toward better scores on the ADAS-cog on donepezil vs placebo (p = NS)
Dubois 2012 [80]	Donepezil	Randomized, double-blind, placebo-controlled trial	355 demented PD	In a post-hoc analysis, donepezil was better than placebo
Mamikonyan 2015 [82]	Rivastigmine	Randomized, double-blind, placebo-controlled trial	28 PD patients with minimal cognitive impairment	Non-significant benefits with rivastigmine

PPN DBS deep brain stimulation of the pedunculopontine tegmental nuclei

9.5 mg/24 h patches. Primary outcomes included incidence of, and discontinuation due to, predefined adverse events (AEs) potentially arising from worsening of PD. Incidence of predefined AEs was 36.1% for capsules vs 31.9% for patch. Discontinuation due to worsening of motor symptoms was observed in 4.4% and 2.4% for capsule/patch respectively, and tremor in 24.5% vs 9.7%. Authors argued that these figures were in the range expected due to the natural progression of Parkinson's disease. Rivastigmine is considered to be "Clinically Useful" for the treatment of dementia in PD according to the latest review of the Movement Disorder Society Evidence-Based Medicine Task Force [84]. Use of donepezil is considered "Investigational" [84].

The efficacy and safety of rivastigmine for the treatment of minimal cognitive impairment in PD (PD-MCI) have been explored in a recent study [82]. Patients with PD-MCI (n = 28) were enrolled in a 24-week, randomized, double-blind, placebo-controlled, crossover, single-site study of the rivastigmine transdermal patch. The primary outcome measure was the Alzheimer's Disease Cooperative Study — Clinical Global Impression of Change (ADCS-CGIC). Twenty-six participants (92.9%) completed both study phase assessments, and 23 (82.1%) completed both phases on study medication. The CGIC response rate demonstrated a non-significant difference favoring rivastigmine.

In a recent study, patients with PDD or PD-MCI were compared to controls and studied before and after a 3-month therapy with rivastigmine patch [85]. At baseline, patients showed reduced spontaneous brain activity in regions important for motor control (e.g., caudate, supplementary motor area, precentral gyrus, thalamus), attention and executive functions (e.g., lateral prefrontal cortex), and episodic memory (e.g., precuneus, angular gyrus, hippocampus). Spontaneous brain activity deficits in the left premotor cortex, inferior frontal gyrus, and supplementary motor area were restored such that the activity was increased post-treatment compared with baseline and was no longer different from controls. These results are in line with a potential restoration of cortical cholinergic tone from the NBM induced by the drug.

Antimuscarinic drugs have a well-known deleterious effect on cognitive function [67], which has been confirmed in PD by a study measuring cerebral blood flow and oxygen metabolic rate after treatment with trihexyphenidyl [68]. These parameters are markers for dementia in PD [69]. Therefore, antimuscarinic drugs should be avoided in patients with cognitive impairments.

Memantine, a channel blocker of the N-methyl-d-aspartate (NMDA) type of glutamate receptors, has been prescribed for Alzheimer's disease patients, and could possibly be used for the treatment of PDD who cannot tolerate cholinesterase inhibitors, but its efficacy has been poorly documented [86].

Psychosis, Delirium and Sleep Disturbances

The management of psychosis should start by withdrawal of potential offending drugs, anticholinergics and tricyclic antidepressant in particular [86]. Quetiapine in small doses might also be used if drug withdrawal fails [86]. Anecdotal evidence suggests interesting clinical effects with donepezil [87], and there is an ongoing randomized, double-blind, placebo-controlled trial with donepezil for this indication [88].

Withdrawal of anticholinergic drugs is also the first therapeutic measure for delirium. Cholinesterase inhibitors do not appear to be effective for delirium in older adults [89]; however, there are no studies in PD patients.

RBD symptoms were improved by rivastigmine in two small case series of patients with DLB [90]. Further results in PD are awaited. The most commonly used RBD treatments include low-dose clonazepam or high-dose melatonin taken orally at bedtime [91].

The potential effects of PPN DBS on sleep and somnolence were explored in a pilot study involving two PD subjects with intractable gait dysfunction [92]. Low-frequency stimulation of the PPN area increased alertness, whereas high-frequency stimulation induced non-REM sleep.

In addition, the sudden withdrawal of the low-frequency stimulation was consistently followed by REM sleep episodes in one of the patients.

Discussion

Cholinergic deficits are common in PD and contribute to motor disturbances including gait dysfunction and non-motor symptoms like cognitive impairment, dementia, mood abnormalities, psychosis, and sleep disorders [14, 93]. It is therefore logical to hypothesize that increasing cholinergic tone might be a first-line strategy for the treatment of these symptoms. The bulk of evidence suggests that administering inhibitors of the enzyme cholinesterase and/or withdrawing drugs with cholinergic antagonizing effects, could be effective for treating cognitive impairment, psychosis, and sleep disturbances in PD. Interestingly, cholinesterase inhibitors have also been found to be effective for gait impairment and falls [94, 95], which are also related to cholinergic degeneration [96, 97].

Several pieces of evidence suggest that rivastigmine may be considered pivotal for the treatment of PDD [78]. The evidence is weaker for PD patients with minimal cognitive impairment [82]. These patients might obtain some benefit from this type of drug, but further evidence is needed before any concrete claims can be made in this connection.

Withdrawing anticholinergic drugs is the first step in the management of psychosis and delirium in PD [86]. If further treatment is needed, cholinesterase inhibitors might also offer some benefit in the former, as suggested by anecdotal evidence. A randomized, double-blind, placebo-controlled trial with donepezil is underway. Delirium might not respond to these drugs, but no study on PD is available. Case reports also suggest that these drugs might be beneficial for RBD, at least in patients with Lewy body dementia [90]. Trials in PD are still lacking.

There are, however, certain issues that will need to be addressed by future studies. Firstly, the safety of cholinesterase inhibitors should be more thoroughly explored. Theoretically, these drugs could worsen parkinsonian motor symptoms by further misbalancing ACh-DA tone in the striatum. Although short-term trials have not disclosed any such effects, and one 1.5-year study showed results that could be considered in line with normal disease progression [83], further studies comparing disease progression in patients under these treatments with untreated controls are needed to dispel any doubts on the matter.

Psychosis and related sleep disturbances might also benefit from cholinesterase inhibitors, as suggested by low-quality evidence [86] or by evidence from other diseases [98]. Studies are urgently needed for these domains, as current treatments either have low efficacy or are unsafe.

Conflict of interests The authors declare no conflict of interests to disclose.

References

1. Pringsheim T, Jette N, Frolkis A, et al. The prevalence of Parkinson's disease: a systematic review and meta-analysis. Mov Disord. 2014;29:1583–90.
2. Chaudhuri KR, Schapira AH. Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. Lancet Neurol. 2009;8:464–74.
3. Hornykiewicz O. Dopamine (3-hydroxytyramine) and brain function. Pharmacol Rev. 1966;18:925–64.
4. Fahn S. The history of dopamine and levodopa in the treatment of Parkinson's disease. Mov Disord. 2008;23(Suppl 3):S497–508.
5. Soto C. Unfolding the role of protein misfolding in neurodegenerative diseases. Nat Rev Neurosci. 2003;4:49–60.
6. Spillantini MG, Schmidt ML, Lee VM, et al. Alpha-synuclein in Lewy bodies. Nature. 1997;388:839–40.
7. Cooper AA, Gitler AD, Cashikar A, et al. α-synuclein blocks ER-golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science. 2006;313:324–8.
8. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007;8:519–29.
9. Mercado G, Valdes P, Hetz C. An ERcentric view of Parkinson's disease. Trends Mol Med. 2013;19:165–75.
10. Hetz C, Mollereau B. Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases. Nat Rev Neurosci. 2014;15:233–49.
11. Garcia-Reitbock P, Anichtchik O, Bellucci A, et al. SNARE protein redistribution and synaptic failure in a transgenic mouse model of Parkinson's disease. Brain. 2010;133:2032–44.

12. Lim SY, Fox SH, Lang AE. Overview of the extranigral aspects of Parkinson disease. Arch Neurol. 2009;66:167–72.
13. Muller ML, Bohnen NI, Kotagal V, et al. Clinical markers for identifying cholinergic deficits in Parkinson's disease. Mov Disord. 2015;30:269–73.
14. Perez-Lloret S, Barrantes FJ. Deficits in cholinergic neurotransmission and their clinical correlates in Parkinson's disease. npj Parkinson's Disease. 2016;2:16001–12.
15. Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron. 2012;76:116–29.
16. Descarries L, Gisiger V, Steriade M. Diffuse transmission by acetylcholine in the CNS. Prog Neurobiol. 1997;53:603–25.
17. Descarries L, Parent M. Chapter 14. Asynaptic and synaptic innervation by acetylcholine neurons of the central nervous system. In: Pickel V, Segal M, editors. The synapse. Boston: Academic Press; 2014. p. 447–66.
18. Newman EL, Gupta K, Climer JR, et al. Cholinergic modulation of cognitive processing: insights drawn from computational models. Front Behav Neurosci. 2012;6:24.
19. Mesulam MM, Mufson EJ, Wainer BH, et al. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience. 1983;10:1185–201.
20. Wess J. Novel insights into muscarinic acetylcholine receptor function using gene targeting technology. Trends Pharmacol Sci. 2003;24:414–20.
21. Wess J, Duttaroy A, Zhang W, et al. M1-M5 muscarinic receptor knockout mice as novel tools to study the physiological roles of the muscarinic cholinergic system. Recept Channels. 2003;9:279–90.
22. Karlin A. Emerging structure of the nicotinic acetylcholine receptors. Nat Rev Neurosci. 2002;3:102–14.
23. Kaiser S, Wonnacott S. alpha-bungarotoxin-sensitive nicotinic receptors indirectly modulate [(3)H]dopamine release in rat striatal slices via glutamate release. Mol Pharmacol. 2000;58:312–8.
24. Alkondon M, Pereira EFR, Barbosa CTF, et al. Neuronal nicotinic acetylcholine receptor activation modulates g-aminobutyric acid release from CA1 neurons of rat hippocampal slices. J Pharmacol Exp Ther. 1997;283:1396–411.
25. Nys M, Kesters D, Ulens C. Structural insights into Cys-loop receptor function and ligand recognition. Biochem Pharmacol. 2013;86:1042–53.
26. Barrantes FJ. Phylogenetic conservation of protein–lipid motifs in pentameric ligand-gated ion channels. Biochim Biophys Acta Biomembr. 2015;1848:1796–805.
27. Raiteri M, Leardi R, Marchi M. Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in the rat brain. J Pharmacol Exp Ther. 1984;228:209–14.
28. Cecchini M, Changeux J-P. The nicotinic acetylcholine receptor and its prokaryotic homologues: structure, conformational transitions & allosteric modulation. Neuropharmacol. 2015;96, Part B:137–49.
29. Gotti C, Riganti L, Vailati S, et al. Brain neuronal nicotinic receptors as new targets for drug discovery. Curr Pharm Des. 2006;12:407–28.
30. Gotti C, Moretti M, Bohr I, et al. Selective nicotinic acetylcholine receptor subunit deficits identified in Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies by immunoprecipitation. Neurobiol Dis. 2006;23:481–9.
31. Bordia T, Mcgregor M, Mcintosh JM, et al. Evidence for a role for alpha6(*) nAChRs in l-dopa-induced dyskinesias using Parkinsonian alpha6(*) nAChR gain-of-function mice. Neuroscience. 2015;295:187–97.
32. Luo R, Janssen MJ, Partridge JG, et al. Direct and GABA-mediated indirect effects of nicotinic ACh receptor agonists on striatal neurones. J Physiol. 2013;591:203–17.
33. Zoli M, Pistillo F, Gotti C. Diversity of native nicotinic receptor subtypes in mammalian brain. Neuropharmacol. 2015;96, Part B:302–11.
34. Halliday GM, Leverenz JB, Schneider JS, et al. The neurobiological basis of cognitive impairment in Parkinson's disease. Mov Disord. 2014;29:634–50.
35. Hasselmo ME, Wyble BP, Wallenstein GV. Encoding and retrieval of episodic memories: role of cholinergic and GABAergic modulation in the hippocampus. Hippocampus. 1996;6:693–708.
36. Palma E, Conti L, Roseti C, et al. Novel approaches to study the involvement of alpha7-nAChR in human diseases. Curr Drug Targets. 2012;13:579–86.
37. Wallace TL, Porter RH. Targeting the nicotinic alpha7 acetylcholine receptor to enhance cognition in disease. Biochem Pharmacol. 2011;82:891–903.
38. Quik M, Zhang D, Mcgregor M, et al. Alpha7 nicotinic receptors as therapeutic targets for Parkinson's disease. Biochem Pharmacol. 2015;97(4):399–407.
39. Flynn DD, Mash DC. Characterization of L-[3H]nicotine binding in human cerebral cortex: comparison between Alzheimer's disease and the normal. J Neurochem. 1986;47:1948–54.
40. Burghaus L, Schutz U, Krempel U, et al. Quantitative assessment of nicotinic acetylcholine receptor proteins in the cerebral cortex of Alzheimer patients. Brain Res Mol Brain Res. 2000;76:385–8.
41. Freedman R, Olincy A, Buchanan RW, et al. Initial phase 2 trial of a nicotinic agonist in schizophrenia. Am J Psychiatry. 2008;165:1040–7.
42. Barrantes FJ. Molecular pathology of the nicotinic acetylcholine receptor. In: Barrantes FJ, editor. The nicotinic acetylcholine receptor: current views and future trends. Austin: Landes Bioscience; 1998. p. 175–213.
43. Aubert I, Araujo DM, Cécyre D, et al. Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. J Neurochem. 1992;58:529–41.
44. Banerjee C, Nyengaard JR, Wevers A, et al. Cellular expression of alpha7 nicotinic acetylcholine receptor

protein in the temporal cortex in Alzheimer's and Parkinson's disease — a stereological approach. Neurobiol Dis. 2000;7:666–72.
45. Valles AS, Borroni MV, Barrantes FJ. Targeting brain alpha7 nicotinic acetylcholine receptors in Alzheimer's disease: rationale and current status. CNS Drugs. 2014;28:975–87.
46. Nikiforuk A, Kos T, Potasiewicz A, et al. Positive allosteric modulation of alpha 7 nicotinic acetylcholine receptors enhances recognition memory and cognitive flexibility in rats. Eur Neuropsychopharmacol. 2015;25:1300–13.
47. Sadigh-Eteghad S, Talebi M, Mahmoudi J, et al. Selective activation of alpha7 nicotinic acetylcholine receptor by PHA-543613 improves Abeta25-35-mediated cognitive deficits in mice. Neuroscience. 2015;298:81–93.
48. Beatty WW, Butters N, Janowsky DS. Patterns of memory failure after scopolamine treatment: implications for cholinergic hypotheses of dementia. Behav Neural Biol. 1986;45:196–211.
49. Ostfeld AM, Aruguete A. Central nervous system effects of hyoscine in man. J Pharmacol Exp Ther. 1962;137:133–9.
50. Lin CH, Lung HL, Li ST, et al. Delirium after transdermal scopolamine patch in two children. J Neuropsychiatr Clin Neurosci. 2014;26:E01–2.
51. Gerretsen P, Pollock BG. Drugs with anticholinergic properties: a current perspective on use and safety. Expert Opin Drug Saf. 2011;10:751–65.
52. Pappas BA, Bayley PJ, Bui BK, et al. Choline acetyltransferase activity and cognitive domain scores of Alzheimer's patients. Neurobiol Aging. 2000;21:11–7.
53. Minger SL, Esiri MM, Mcdonald B, et al. Cholinergic deficits contribute to behavioral disturbance in patients with dementia. Neurology. 2000;55:1460–7.
54. Francis PT, Palmer AM, Snape M, et al. The cholinergic hypothesis of Alzheimer's disease: a review of progress. J Neurol Neurosurg Psychiatry. 1999;66:137–47.
55. Babic T. The cholinergic hypothesis of Alzheimer's disease: a review of progress. J Neurol Neurosurg Psychiatry. 1999;67:558.
56. Barage SH, Sonawane KD. Amyloid cascade hypothesis: Pathogenesis and therapeutic strategies in Alzheimer's disease. Neuropeptides. 2015;52:1–18.
57. Kar S, Slowikowski SP, Westaway D, et al. Interactions between beta-amyloid and central cholinergic neurons: implications for Alzheimer's disease. J Psychiatry Neurosci. 2004;29:427–41.
58. Park HE, Park IS, Oh YS, et al. Subcortical whiter matter hyperintensities within the cholinergic pathways of patients with dementia and parkinsonism. J Neurol Sci. 2015;353:44–8.
59. Gaspar P, Gray F. Dementia in idiopathic Parkinson's disease. A neuropathological study of 32 cases. Acta Neuropathol. 1984;64:43–52.
60. Lee JE, Cho KH, Song SK, et al. Exploratory analysis of neuropsychological and neuroanatomical correlates of progressive mild cognitive impairment in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2014;85:7–16.
61. Hall H, Reyes S, Landeck N, et al. Hippocampal Lewy pathology and cholinergic dysfunction are associated with dementia in Parkinson's disease. Brain. 2014;137:2493–508.
62. Lorenz R, Samnick S, Dillmann U, et al. Nicotinic alpha4beta2 acetylcholine receptors and cognitive function in Parkinson's disease. Acta Neurol Scand. 2014;130:164–71.
63. Liu AK, Chang RC, Pearce RK, et al. Nucleus basalis of Meynert revisited: anatomy, history and differential involvement in Alzheimer's and Parkinson's disease. Acta Neuropathol. 2015;129:527–40.
64. Lieberman A. Are dementia and depression in Parkinson's disease related? J Neurol Sci. 2006;248:138–42.
65. Bohnen NI, Kaufer DI, Hendrickson R, et al. Cortical cholinergic denervation is associated with depressive symptoms in Parkinson's disease and parkinsonian dementia. J Neurol Neurosurg Psychiatry. 2007;78:641–3.
66. Shoji Y, Nishio Y, Baba T, et al. Neural substrates of cognitive subtypes in Parkinson's disease: a 3-year longitudinal study. PLoS One. 2014;9:e110547.
67. Yarnall A, Rochester L, Burn DJ. The interplay of cholinergic function, attention, and falls in Parkinson's disease. Mov Disord. 2011;26:2496–503.
68. Takahashi S, Tohgi H, Yonezawa H, et al. The effect of trihexyphenidyl, an anticholinergic agent, on regional cerebral blood flow and oxygen metabolism in patients with Parkinson's disease. J Neurol Sci. 1999;167:56–61.
69. Lenzi GL, Jones T, Reid JL, et al. Regional impairment of cerebral oxidative metabolism in Parkinson's disease. J Neurol Neurosurg Psychiatry. 1979;42:59–62.
70. Manganelli F, Vitale C, Santangelo G, et al. Functional involvement of central cholinergic circuits and visual hallucinations in Parkinson's disease. Brain. 2009;132:2350–5.
71. Vardy ER, Teodorczuk A, Yarnall AJ. Review of delirium in patients with Parkinson's disease. J Neurol. 2015;262(11):2401–10.
72. Crispo JA, Willis AW, Thibault DP, et al. Associations between anticholinergic burden and adverse health outcomes in Parkinson disease. PLoS ONE. 2016;11:e0150621.
73. Van Dort CJ, Zachs DP, Kenny JD, et al. Optogenetic activation of cholinergic neurons in the PPT or LDT induces REM sleep. Proc Natl Acad Sci U S A. 2015;112:584–9.
74. Suzuki K, Miyamoto M, Miyamoto T, et al. Parkinson's disease and sleep/wake disturbances. Curr Neurol Neurosci Rep. 2015;15:8.
75. Postuma RB, Lang AE, Massicotte-Marquez J, et al. Potential early markers of Parkinson disease in idiopathic REM sleep behavior disorder. Neurology. 2006;66:845–51.

76. Laloux C, Derambure P, Jacquesson JM, et al. The effects of serotoninergic, noradrenergic, cholinergic and dopaminergic drugs on vigilance states in MPTP-treated mice. Brain Res. 2007;1161:79–87.
77. Nardone R, Bergmann J, Brigo F, et al. Functional evaluation of central cholinergic circuits in patients with Parkinson's disease and REM sleep behavior disorder: a TMS study. J Neural Transm. 2013;120:413–22.
78. Pagano G, Rengo G, Pasqualetti G, et al. Cholinesterase inhibitors for Parkinson's disease: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2014;86(7):767–73.
79. Emre M, Aarsland D, Albanese A, et al. Rivastigmine for dementia associated with Parkinson's disease. N Engl J Med. 2004;351:2509–18.
80. Dubois B, Tolosa E, Katzenschlager R, et al. Donepezil in Parkinson's disease dementia: a randomized, double-blind efficacy and safety study. Mov Disord. 2012;27:1230–8.
81. Ravina B, Putt M, Siderowf A, et al. Donepezil for dementia in Parkinson's disease: a randomised, double blind, placebo controlled, crossover study. J Neurol Neurosurg Psychiatry. 2005;76:934–9.
82. Mamikonyan E, Xie SX, Melvin E, et al. Rivastigmine for mild cognitive impairment in Parkinson disease: a placebo-controlled study. Mov Disord. 2015;30:912–8.
83. Emre M, Poewe W, De Deyn PP, et al. Long-term safety of rivastigmine in Parkinson disease dementia: an open-label, randomized study. Clin Neuropharmacol. 2014;37:9–16.
84. Seppi K, Weintraub D, Coelho M, et al. The movement disorder society evidence-based medicine review update: treatments for the non-motor symptoms of Parkinson's disease. Mov Disord. 2011;26(Suppl 3):S42–80.
85. Possin KL, Kang GA, Guo C, et al. Rivastigmine is associated with restoration of left frontal brain activity in Parkinson's disease. Mov Disord. 2013;28:1384–90.
86. Hindle JV. The practical management of cognitive impairment and psychosis in the older Parkinson's disease patient. J Neural Transm (Vienna). 2013;120:649–53.
87. Bergman J, Lerner V. Successful use of donepezil for the treatment of psychotic symptoms in patients with Parkinson's disease. Clin Neuropharmacol. 2002;25:107–10.
88. Schrag A, Sauerbier A, Chaudhuri KR. New clinical trials for nonmotor manifestations of Parkinson's disease. Mov Disord. 2015;30:1490–504.
89. Tampi RR, Tampi DJ, Ghori AK. Acetylcholinesterase Inhibitors for delirium in older adults. Am J Alzheimers Dis Other Demen. 2016;31:305–10.
90. Aurora RN, Zak RS, Maganti RK, et al. Best practice guide for the treatment of REM sleep behavior disorder (RBD). J Clin Sleep Med. 2010;6:85–95.
91. Howell MJ, Schenck CH. Rapid eye movement sleep behavior disorder and neurodegenerative disease. JAMA Neurol. 2015;72:707–12.
92. Arnulf I, Ferraye M, Fraix V, et al. Sleep induced by stimulation in the human pedunculopontine nucleus area. Ann Neurol. 2010;67:546–9.
93. Bohnen NI, Albin RL. The cholinergic system and Parkinson disease. Behav Brain Res. 2011;221:564–73.
94. Chung KA, Lobb BM, Nutt JG, et al. Effects of a central cholinesterase inhibitor on reducing falls in Parkinson disease. Neurology. 2010;75:1263–9.
95. Henderson EJ, Lord SR, Brodie MA, et al. Rivastigmine for gait stability in patients with Parkinson's disease (ReSPonD): a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Neurol. 2016;15:249–58.
96. Bohnen NI, Frey KA, Studenski S, et al. Gait speed in Parkinson disease correlates with cholinergic degeneration. Neurology. 2013;81:1611–6.
97. Bohnen NI, Muller ML, Koeppe RA, et al. History of falls in Parkinson disease is associated with reduced cholinergic activity. Neurology. 2009;73:1670–6.
98. Zahodne LB, Fernandez HH. Pathophysiology and treatment of psychosis in Parkinson's disease: a review. Drugs Aging. 2008;25:665–82.

28

Pharmacotherapy Through the Inhibition of Glycine Transporters: An Update on and Beyond Schizophrenia

Philipp Singer and Benjamin K. Yee

Abstract

The glycine reuptake inhibitor (GRI) bitopertin (also known as RG1678 or RO-4917838), invented by Hoffmann–La Roche, was poised to make an impact on the pharmacotherapy of schizophrenia, but hope was finally dashed by the disappointing outcomes of the recently completed multi-centre phase III clinical trials. Against this backdrop, this review aims to survey the rationale and potential of GRIs to treat neuropsychiatric conditions beyond schizophrenia. Indeed, although the development of bitopertin as an anti-schizophrenia drug has since been shelved, it is still being pursued by Roche as a potential adjunctive medication for the treatment of obsessive–compulsive disorder. Several lines of research have independently indicated that the pharmacological inhibition of glycine reuptake may be relevant to the treatment of diverse clinical conditions, including depression, anxiety disorders, alcohol dependence, epilepsy, and pain. In each case, the rationale emphasizes the physiological impact of glycine reuptake inhibition on either the inhibitory glycinergic neurotransmission or the excitatory *N*-methyl-D-aspartate receptor–dependent glutamatergic neurotransmission. None of the proposed clinical applications, however, can readily incorporate and integrate, *a priori*, both expected neuropharmacological effects of GRIs. The dual action of glycine in the nervous system may be the Achilles heel in precisely predicting the outcome of the systemic effects

P. Singer, PhD
Roche Diagnostics International Ltd.,
CH-6343 Rotkreuz, Switzerland

B.K. Yee, DPhil (Oxon) (✉)
Department of Rehabilitation Sciences, The Hong Kong Polytechnic University,
Hung Hom, Hong Kong
e-mail: benjamin.yee@polyu.edu.hk

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_28

of GRIs, which may explain why none of these approaches has yet yielded any clinic-ready GRI drug. A better understanding at the circuitry level implicated in the respective disorders would be needed to overcome this roadblock to drug discovery.

Keywords
Glutamate • Glycine transporter • NMDA receptor • Schizophrenia • Addiction • Pain • Obsessive–compulsive disorder

Introduction

The inhibition of glycine transporters is a novel pharmacological strategy to inhibit as well as potentiate neuronal excitation in the central nervous system through an interference with the homeostatic regulation of extracellular glycine availability at the relevant synapses [1, 2]. Drugs or genetic disruptions that inhibit glycine reuptake have been shown to (i) potentiate glycinergic neurotransmission, which is invariably inhibitory in nature in the adult mammalian central nervous system, and (ii) facilitate the neurotransmission at the *N*-methyl-D-aspartate (NMDA) receptors, where the glycine-B site occupancy (by glycine or D-serine) essentially gates the activation of NMDA receptors. Since glycine-B site occupancy is normally not saturated at physiological conditions, increasing the availability of glycine in the vicinity of NMDA receptors by inhibition of glycine reuptake via glycine transporter 1 (GlyT1) is effective in potentiating NMDA receptor excitability [3]. Moreover, this pharmacological strategy is expected to stimulate NMDA receptors in a use-dependent manner, which minimizes excitotoxic effects associated with direct NMDA receptor agonists. This has raised hope for a new generation of anti-psychotic medication targeting the negative and cognitive symptoms of schizophrenia, of which no effective therapy is available. It is because these symptoms are believed to stem from the deficiency of NMDA receptor function. This was the focus of our review in 2015 [4], and we begin here with an update on the major development.

Update on Bitopertin as a Potential Antipsychotic Drug

Bitopertin (also known as RG1678 or RO-4917838) is the only selective GlyT1-inhibiting drug that had reached phase 3 clinical trials as an adjunctive medication for schizophrenia. However, its development in this direction has been halted, and the decision is primarily due to the consistent lack of efficacy across six independent clinical trials. Three trials were designed to focus on negative and cognitive symptoms in patients with persistent, predominantly negative symptoms of schizophrenia, whereas the other three trials focused on persistent positive symptoms that are sub-optimally controlled [4]. Bitopertin was always administered as an add-on medication to current atypical antipsychotic drugs other than clozapine (because of known adverse interaction). The equal emphasis on negative as well as positive symptoms in this large-scale multi-centre study (code name 'SearchLyte') with a sample size exceeding 2,500 patients reflected that Roche had taken into consideration evidence from early trials suggesting that significant improvement in positive symptoms had been observed in some patients treated with glycine or the naturally occurring GlyT1 inhibitor sarcosine [5].

As revealed by Bugarski-Kirola et al. [6–8], however, none of the six multi-centre trials had yielded evidence that adjunctive bitopertin could produce significantly superior efficacy in comparison with placebo add-on in terms of negative symptoms and cognitive functioning. The only favourable finding was a significant improvement in positive symptom factor scores observed at one specific dose (10 mg/kg/day) in the trial code

named 'NightLyte' (ClinicalTrials.gov Identifier: NCT01235559), which comprised a sample size of 200 patients [8]. The negative outcome of the 'SearchLyte' clinical study has been surprising, given that earlier data emerging from the phase 2 trials had indicated significant improvement in overall clinical outcomes, including negative symptom factor scores and social and cognitive functioning [9–11]. Hindsight suggests that the concerns expressed by Lin and colleagues [12] were well founded, especially when they were casting doubt on whether the low effect size (>0.4) obtained in phase 2 trials could be successfully translated into a broader examination.

At this time, not much could be read from Roche's 'SearchLyte' study with regard to the pharmacological mechanisms that had led to the apparent failure, but two relevant points may warrant consideration. First, although the reported efficacy against positive symptoms may be restricted to a particular subset of patients, the possibility that positive symptoms, as opposed to negative symptoms, might benefit from bitopertin suggests that one ought to re-focus on the emphasis of targeting negative/cognitive symptoms as a separate cluster that warrants a distinct pharmacological approach. Indeed, it reminds one of the suggestion that the emergence positive symptoms may stem from a underlying glutamatergic dysfunction (e.g., [13]). Second, we saw a shift of dosage from 10~30 mg/kg/day in phase 2 to 5~20 mg/kg/day in phase 3 — a move that seems to help improve tolerance and side-effects. However, this is accompanied by a clear lack of efficacy against negative and cognitive symptoms in schizophrenia patients. Hence, one may speculate that side-effects at higher doses may turn out to be a significant road block to developing GlyT1-inhibiting drugs as a new class of antipsychotics (personal communication, Dr Ronan Depoortère).

Nonetheless, one should not read the commercial decision to shelf the development of bitopertin as a final blow. A separate phase 2/3 trial of a smaller scale by Roche (NCT01234779, code name 'CandleLyte') had examined bitopertin as a monotherapy in patients with acute exacerbation of schizophrenia. The results showed that bitopertin was comparable to the positive control drug, olanzapine, in reducing overall illness severity as measured by the clinical global impression–severity scale (CGI-S), even though the improvement achieved by both drugs fell short of statistical significance ($p = 0.098$ and $p = 0.126$, respectively) relative to placebo. However, in agreement with the 'NightLyte' trial (10 mg/kg/day), a significant improvement in the positive symptom factor scores was observed in this monotherapy trial with bitopertin administered at 30 mg/kg/day. Taken together, it is apparent that GlyT1-inhibition therapy need not be marketed as an add-on medication targeting solely negative and cognitive symptoms — an impression that is very much in line with preclinical models in animals [14].

Depression

Since schizophrenia negative symptoms resemble some of the critical clinical signs of depression, it is not surprising that GlyT1 inhibition may also benefit people with clinical depression. However, evidence is limited to preclinical studies of two selective GlyT1 inhibitors, SSR504734 and SSR1038900, which have been withdrawn from development by Sanofi. SSR504734 showed protective effects in a chronic mild stress model of depression [15], while SSR1038900 yielded a dose-dependent effect in the Porsolt forced swim test of anti-depressant drugs [16]. Clinical investigation is limited to the naturally occurring GlyT1 inhibitor, sarcosine, which showed superior efficacy than the SSRI, citalopram [17]. Positive outcomes have also been reported with the glycine-B site partial agonists, D-cycloserine and GLYX-13 [18, 19]. Deletion of GlyT1 in forebrain neurons is associated with an increase in hippocampal neural proliferation, an effect that is common to many anti-depressant drugs [20]. However, attention in the field is currently focusing on the NMDA receptor blocker, ketamine [21] — an approach that is conceptually not compatible with glycine augmentation, which is expected to augment NMDA receptor function. An on-going NIH-sponsored phase 2 trial (NCT02484456) is examining the efficacy of a glycine-B site blocker, AV-101 (L-4-chlorokyurenine or 4-CI-KYN), for the treatment of major depression. This pharmacological approach may yield less side effects compared with ketamine.

Glycine Reuptake Inhibition in Obsessive–Compulsive Disorder

Obsessive–compulsive disorder (OCD) has a lifetime prevalence of 2.3% [22–24]. OCD is characterized by obsessive thoughts that are uncontrollable and persistent and can cause extreme anxiety and disruptive behaviour [25]. Such behaviour often takes the form of compulsive acts, which are ritualistic and stereotypic in nature. Performance of these acts temporally eases the anxiety and stress produced by the persistent obsessive thoughts in people with OCD, but they can be functionally disruptive and impairing. The aetiology of OCD is largely unknown. Both environmental and genetic factors are believed to play a role [26]. First-line treatments for OCD are antidepressants and exposure-based psychotherapy (see Table 28.1). However, about 30% of OCD patients do not respond well to these treatment options, and they continue to suffer from a diminished quality of life [36]. Hence, there is a need for improved therapeutics for this subpopulation of OCD patients.

Table 28.1 Current treatment available for OCD

Pharmacotherapy
Primary drugs
Antidepressants are used as first-line drugs to reduce OCD symptoms [27]
The tricyclic antidepressant risperidone,
Serotonin–norepinephrine reuptake inhibitors (SNRIs)
Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, fluvoxamine, and sertraline
Secondary drugs
If antidepressants are ineffective, low doses of antipsychotics are used including risperidone, quetiapine, or olanzapine [28, 29]
Antipsychotics are used as add-on to the base antidepressant medication [30]
Antipsychotics have been found to be beneficial in OCD patients with a history of tics or Tourette syndrome [31]
Psychotherapy
Cognitive–behavioural therapy (CBT) and related therapies (e.g., habit reversal training) effectively reduce OCD symptoms in many individuals – even in patients that do not respond to pharmacotherapy [32]
Other alternative procedures
Electroconvulsive therapy in patients with refractory symptoms [33]
Cingulate cortex lesions in severe cases [34]
Deep brain stimulation [35]

One emerging new pharmacological target is the NMDA receptor. Several findings suggest that a dysfunction of the glutamate pathway may contribute to the aetiology of OCD [37–40], although the direction of change remains uncertain. Reduction of OCD symptoms has been reported following treatment designed to augment or inhibit glutamatergic signalling [39, 40]. While such contradictory findings may point to the involvement of multiple, most likely regionally and temporally distinct, mechanisms that remain to be delineated, they also indicate that a consensual glutamate theory of OCD is still out of reach.

Against this backdrop, two exploratory studies have evaluated the efficacy of the NMDA receptor glycine-B site agonist, glycine, and the GlyT1 inhibitor, sarcosine, in patients with refractory OCD symptoms (see Table 28.2). Glycine adjunctive to standard medication with selective serotonin reuptake inhibitors (SSRIs) led to a stronger reduction in OCD symptoms compared with placebo add-on [42]. The study was admittedly limited in scale. Only 14 of the 26 participants completed the study, and only two out of five subjects in the glycine + SSRI arm showed substantial improvement. The efficacy of sarcosine monotherapy and add-on treatment to SSRI was evaluated in an open-label study [41]. Sarcosine treatment achieved a fast improvement of OCD symptoms, particularly in drug-free subjects who had discontinued SSRI treatment for at least 8 weeks before study entry. Interpretation of the results remains rather speculative, as the mechanistic explanations for the drugs' beneficial effects were made ad hoc. Nonetheless, the potential of glycine augmentation therapy via GlyT1 inhibition as a new treatment option for OCD has prompted F. Hoffman–La Roche to initiate a multi-centre phase 2 trial (NCT01674361) to evaluate the efficacy and safety of bitopertin as add-on medication to SSRIs in patients with OCD (see Table 28.2). At present, the status of the trial is unknown since its last update in 2013, and Roche has not released any information since

Table 28.2 Outcomes and study design of selected clinical trials with glycine transporter inhibitor (sarcosine, bitopertin) or glycine-B site ligands (glycine or D-cycoloserine) in patients with obsessive-compulsive disorder

Compounds	Study design	Published Outcomes	References
Sarcosine	A 10-week open-label study evaluating the efficacy of sarcosine (500–2,000 mg/day) on OCD symptoms in patients with inadequate response to SSRI. Drug-naive ($n = 8$), subjects discontinued from SSRI treatment receiving sarcosine monotherapy ($n = 6$), and sarcosine + SSRI treatment ($n = 12$)	Sarcosine reduced OCD symptoms in all three groups, and the most pronounced symptoms reduction was observed in the sarcosine monotherapy group	[41]
Sarcosine	A 10-week open-label trial evaluated the efficacy and safety of sarcosine on OCD symptoms in drug-free subjects at study entry. An escalating dose regime was used: started from 500 mg/kg, and increased by 500 mg biweekly up to 2,000 mg/day	Completed without published data	ClinicalTrials.gov identifier: NCT01031927
Bitopertin	A multi-centre, randomized, double-blind, parallel-group, placebo-controlled phase 2/3 study evaluating efficacy and safety of bitopertin as add-on medication to standard SSRI treatment in patients with OCD for 16 weeks; 99 patients were randomly allocated to 30 mg/kg/day, 10 mg/kg/day bitopertin or placebo control on top of their background SSRI medication	Unknown status	ClinicalTrials.gov identifier: NCT1674361
Glycine	A double-blind, placebo-controlled trial designed to evaluate the efficacy of glycine as add-on treatment (60 g/day for 12 weeks) to standard SSRI medication. Only 14 patients with OCD completed the trials. Sarcosine add-on: $n = 5$, placebo add-on: $n = 9$	Glycine add-on led to greater reduction of OCD symptoms compared with placebo add-on	[42]
Glycine	Single case study of high-dose glycine treatment (0.8 mg/kg for 5 years) in one adult male subject suffering from refractory OCD and body dysmorphic disorder	Reduction of OCD symptoms and improved social life	[43]
D-cycloserine	A placebo-control, double-blind study designed to evaluate the efficacy of D-cycloserine to facilitate extinction-based exposure therapy in OCD patients. 125 mg of D-cycloserine was administered 2 h before each exposure session	D-cycloserine facilitated exposure therapy and led to a transient relief from obsession-related distress	[44]
D-cycloserine	A randomized, double-blind, placebo-controlled trial to study the efficacy of D-cycloserine to augment behavioural therapy in 23 OCD patients, comprised two arms: D-cycloserine ($n = 10$) and placebo ($n = 13$). D-cycloserine ($n = 10$) and placebo were administered 1 h before each behavioural therapy session	D-cycloserine produced greater reduction of symptoms than placebo after five therapy sessions	[45]
D-cycloserine	A randomized, double-blind, placebo-controlled trial to study the efficacy of D-cycloserine to augment cognitive-behavioural therapy in OCD patients: Five cognitive therapy sessions and seven exposure and response prevention sessions were conducted with all patients, who received either D-cycloserine ($n = 15$) and placebo ($n = 15$) 1 h before each and every session	D-cycloserine produced a non-significant trend towards greater reduction of symptoms than placebo	[46]

then. Likewise, another open-label trial with sarcosine (NCT01031927) has been completed but no data have been published so far.

Another therapeutic approach in OCD is the use of pharmacological agents to assist or facilitate exposure-based psychotherapy. The rationale is to assist extinction learning that occurs during exposure treatment and its subsequent consolidation, in order to achieve sustained reduction in obsessive thoughts [47]. NMDA receptor-dependent neuroplasticity in the amygdala is believed to play a key role in fear extinction learning [47]. Evidence that augmentation of NMDA receptor activity could promote the efficacy of such exposure therapy was first obtained with D-cycloserine — a partial agonist at the NMDA receptor glycine-B site. The synergism between D-cycloserine and behavioural exposure therapy has been seen in various anxiety disorders including phobia [48], panic disorder [49], social anxiety [50, 51], and OCD (see Table 28.2). On the basis of these positive findings and the hypothesized mechanisms, one may readily predict that GlyT1 inhibition would also possess superior efficacy, since GlyT1 inhibitors are more efficacious than D-cycloserine in enhancing glycine-B site occupancy. Nations and colleagues had tested this prediction with compound ORG25935 [52] in conjunction with cognitive behavioural therapy in patients with panic disorder, but failed to find any evidence to support it. Despite this initial setback, it is still not known if ORG25935 may yield positive synergism with psychotherapy in individuals with OCD or other anxiety disorders, and thus further clinical trials are clearly necessary.

Inhibition of GlyT1 Exerts Anxiolytic Effects Via Stimulation of Strychnine-Sensitive Glycine Receptor

A separate line of evidence suggesting that GRI could regulate anxiety comes from a recent preclinical model of anxiety based on maternal-induced ultrasonic vocalisation [53]. Compounds SSR504734 and ALX5407 (also known as NFPS, *N*-[3-(4′-Fluorophenyl)-3-(4′-phenylphenoxy) propyl]sarcosine) both produced an anxiolytic effect in this animal model. These findings add to an earlier study in which SSR504734 exerted anxiolytic efficacy in two stress-related anxiety tests [54]. Importantly, Komatsu and colleagues showed that the dose-dependent anxiolytic effects of SSR504734 and ALX5407 could be reversed by strychnine, which blocks strychnine-sensitive glycine receptors (GlyRs), but not by L-687,414 — a compound that blocks NMDA receptors [53]. They therefore concluded that the anxiolytic effect of GlyT1 inhibitors stem primarily from the potentiation of the neuronal inhibition mediated by GlyRs rather than the facilitation of neuronal excitation mediated via NMDA receptors. Furthermore, the anxiolysis attributed to the potentiation of GlyRs is distinct from that seen following benzodiazepine (which potentiates the neuronal inhibition mediated by GABA-A receptors) or SSRIs (which potentiate serotoninergic stimulation) because their anxiolytic effects could not be reversed by strychnine. It follows that strychnine-sensitive inhibitory GlyR may represent a new target for anxiolytic drugs, and that GlyT1 inhibition is a feasible pharmacological strategy to augment GlyR-mediated neuronal inhibition.

Epilepsy

Epilepsy is a neurological condition with a population prevalence of approximately 1.65% in the United States [www.epilepsy.com/learn/epilepsy-statistics]. It is caused by a disruption of the homeostatic balance between neuronal excitation and inhibition in the brain [55, 56]. A variety of anti-epileptic drugs used in the clinic are designed with an aim to restore the equilibrium between excitatory (glutamate) and inhibitory (GABA) signalling [57, 58]. However, current anti-epileptic drugs are unsatisfactory due to a poor responsiveness, limited tolerability, and severe cognitive side-effects [59–61]. As epilepsy is also associated with cognitive dysfunction as the disease progresses [62], new types of more effective drugs combining anti-epileptic

and pro-cognitive properties would represent a significant advance in the management of refractory epilepsy.

Since glycine exerts both excitatory and inhibitory effects through its action at the glycine-B site of the NMDA receptor and the glycine-A site of the strychnine-sensitive GlyR, respectively, the modulation of ambient glycine levels may offer a suitable strategy for normalizing network homeostasis in the epileptic brain. Indeed, studies in animals have long suggested that glycine possesses anti-epileptic properties and can improve the efficacy of anti-epileptic drugs [63–68]. The anticonvulsive potential of several GlyT1 inhibitors have been demonstrated in standard acute seizure models in rodents (Table 28.3). In a more clinically relevant mouse model of temporal lobe epilepsy (TLE), the GlyT1 inhibitor LY2365109 has recently been shown to suppress chronic seizures [72] suggesting that GlyT1 inhibition can be effective for seizure control in chronic epilepsy. Interestingly, a significant increase of GlyT1 expression has been observed in the hippocampal formation of epileptic subjects in two mechanistically distinct rodent models of TLE. The increase mirrors a similar overexpression of hippocampal GlyT1 detected in post-mortem materials from epileptic patients [72]. This raises the possibility that GlyT1 overexpression is linked to the development of chronic epilepsy. Intriguingly, the early stages of epileptogenesis are associated with an initial down-regulation of GlyT1 expression. One speculation suggests that the transition from subnormal to supranormal levels of GlyT1 expression may underlie the transition from the latent phase to the chronic phase of epilepsy [72]. Given that changes in GlyT1 expression can modulate both inhibitory and excitatory activities in the hippocampus, it is important to delineate which direc-

Table 28.3 Summary of key preclinical tests in rodents of the anticonvulsive potential of inhibitors selective for GlyT1

Selective inhibitor of glyt1	Study design	Published outcomes	References
ALX-5407, SSR504734, LuAA21279, Org25935, SB710622, GSK931145	GlyT1 inhibitors were evaluated in the maximal electro shock threshold (MEST) test in rats. Sprague Dawley rats (n = 12 per group) were pre-treated with a GlyT1 inhibitor or vehicle before receiving an electric shock delivered via the corneal electrodes	All tested GlyT1 inhibitors elevated seizure threshold in a dose-dependent manner, suggestive of anticonvulsant activity	[69]
Sarcosine	The effect of sarcosine (100, 400, or 800 mg/kg) on seizure threshold was evaluated in two seizure models in mice: the timed intravenous injection of pentylenetetrazol (PTZ) infusion test, and the MEST test	Sarcosine did not elevated seizure threshold in the PTZ test, but it was effect at the two higher doses in the MEST, suggestive of weak anticonvulsive properties	[70]
M22	The PTZ and MEST tests were used to evaluate the anticonvulsive potential of compound M22 (10, 20, or 40 mg/kg) in C57BL/6 J mice	Significant elevation of seizure threshold was seen in the MEST test. The elevation could be reversed by strychnine indicating a contribution of GlyR-dependent mechanism	[71]
LY2365109	The anti-convulsive potential of 10 mg/kg LY2365109 was assessed in the intrahippocampalkainic acid model of temporal lobe epilepsy in mice and PTZ test in rats. A more extended dose range (10–60 mg/kg) was examined using the MEST test in rats	LY2365109 showed robust anti-convulsive effects in all three rodent tests	[72]

tion of altered GlyT1 expression may be pathologically relevant, and which may reflect compensatory plasticity. Appropriate pharmacological interventions may then be developed to halt the transition into chronic epilepsy.

The up-regulation of GlyT1 reported in the epileptic brain may present a potential mechanistic link to the emergence of cognitive deficits in chronic epilepsy. The up-regulation is expected to reduce the levels of extra-cellular glycine available in glutamatergic synapses, and thus undermines the excitability of NMDA receptors in these synapses. Hence, not only may GlyT1 inhibition produce anti-epileptic effects, but it may also improve cognitive performance in epileptic patients by normalizing the occupancy of the glycine-B site at NMDA receptors. The dual effects have yet to be demonstrated within a single animal model, although the latter precognitive potential of GlyT1 inhibitors has been reported with SSR504734 [54, 73].

Another step towards delineating the relative contributions of the glycinergic mechanisms at inhibitory GlyRs and excitatory NMDA receptors to the anti-epileptic versus the pro-cognitive effects of GlyT1 inhibition is the recent finding that strychnine (at a subconvulsive dose) can reverse the anti-convulsive effect of the GlyT1 inhibitor, M22 [71]. This study has provided the first pharmacological evidence for an involvement of GlyR stimulation in anti-convulsion attributed to GlyT1 inhibition. The contribution of NMDA receptor-mediated neuronal excitation, by contrast, seems less clear because both glycine-B site agonists and antagonists have exhibited anti-epileptic effects in several animal models [69, 74, 75]. The findings that glycine and D-serine, two endogenous glycine-B site agonists, could potentiate the action of existing anticonvulsants [67, 76] suggest that increased occupancy of the NMDA receptor's glycine-B site is potentially anti-convulsive and underlies the benefits of GlyT1 inhibition. Such drug–drug interactions are especially relevant to the development of polytherapy for improved therapeutic efficacy [77]. Further basic research is needed to shed light on the intricate neurophysiological mechanisms regulated by the co-activation of glycine-A and glycine-B site at the network level when glycine reuptake is disrupted by GlyT1-inhibiting drugs. Only then could the full potential of GlyT1 as a drug target to normalize the balance between inhibitory and excitatory neurotransmission in the epileptic brain be realized. In this regard, it is worth pointing out that one limitation of systemic GlyT1 inhibitor treatment in epilepsy might be a relatively narrow therapeutic dose range [72]. It is essential to avoid excessive inhibition of GlyT1 in the brain stem as it would probably increase the risk of sudden death in epilepsy — a significant cause of mortality in people with epilepsy, believed to stem from cardiac and respiratory complications [78, 79]. It may become necessary to develop local glycine augmentation therapy in order to restrict pharmacological intervention within the epileptic focus. Preclinical gene and cell therapies allowing the local delivery of anti-epileptic agents have been developed and the proof-of-concept demonstrated [80, 81].

Alcohol Dependence Therapy

Another relevant area of development based on blockade of glycine reuptake is the treatment of alcohol addiction. The potential synergism between extinction behavioural therapy and GlyT1 inhibition has been discussed with respect to the treatment of OCD and anxiety disorders. This is also of obvious relevance to rehabilitation of addicts in preventing relapse and to support long-term abstinence from potential drugs of abuse. In particular, suggestions have been made regarding the relevance of GlyT1 inhibition to the distinct mechanisms underlying both pathological alcohol consumption and relapse drinking [82, 83].

The efficacy of GlyT1-selective inhibitors, Org 25,935 and Org 24,598, in reducing relapse-like compulsive drinking alcohol preference has been shown in rats [84–86], although clinical trials with Org 25,935 had not been successful [87]. The efficacy of Org 25,935 in rodent models has been localized to the nucleus accumbens because intra-accumbens infusion of Org 25,935 is suffi-

cient to attenuate alcohol-induced dopamine release [84, 85]. Perfusion of glycine directly into the nucleus accumbens produced a similar decrease in alcohol preference and intake [86], suggesting that Org 25,935-induced local elevation of extracellular glycine levels is mechanistically relevant. Since strychnine can block the effects of Org 25,935 or glycine, it is further concluded that the activation of inhibitory glycine receptors within the nucleus accumbens is crucial. Increasing glycinergic inhibition in the nucleus accumbens suppresses the dopamine-dependent hedonic value of alcohol and thereby reduces alcohol consumption, whereas blockade of nucleus accumbens glycinergic neuronal inhibition by strychnine increases alcohol consumption [88]. The rationale of GlyT1 inhibition therapy is to reduce the reinforcing effect of alcohol and therefore similar to nalmefene, an opioid receptor antagonist approved by the European Medicines Agency for the treatment of alcohol dependence. And, like nalmefene, Org 25,935 and Org 24,598 are less susceptible to the development of drug tolerance [89, 90].

A separate line of evidence further suggests that the anti-alcohol effects of GlyT1 inhibitors RO4543338 and Org 24,598 [91, 92] may involve additional modifications to NMDA receptor density [93] or composition [94] that limit the NMDA receptor-mediated plasticity induced by alcohol consumption. The effective doses of Org 25,935 are associated with a 50–80% increase in extracellular glycine levels in the striatum of rats [84] — a level that may be sufficient to induce internalization of NMDA receptors [93]. In this respect, GlyT1 inhibitors may resemble another approved drug against alcohol dependence, acamprosate, which is thought to reverse the persistent NMDA receptor-dependent neuroplastic changes that precipitate relapses [95–97].

Despite the initial disappointment with Org 25,935 [87], clinical trials with other forms of glycine augmentation are still being explored. One on-going study specifically evaluates glycine in a population of schizophrenia patients with co-morbid alcoholism (NCT00338598) with an attempt to demonstrate the multiple benefits of glycine augmentation therapy.

Pain

The potential of glycine augmentation therapy for pain control has emphasized not only inhibition of glycine reuptake via GlyT1 but also GlyT2 [98]. Evidence has been derived from a variety of animal models ranging from the sciatic nerve injury model [99], the Freund's adjuvant-induced peripheral inflammation model [100], the formalin-induced pain model [99], acetic acid-induced writhing syndrome [98] and intravesical resinifera toxin-induced pain [101]. In the dorsal horn, the ascending pain pathway destined for the thalamus is tightly regulated by neuronal inhibition mediated by GlyRs containing the α3 subunit (GlyRα3). Their activation is known to suppress the transmission of the pain signals to the brain. Intracerebroventricular or intrathecal injection of glycine can produce analgesic effects in mice [102]. GlyT1 inhibitors, Org 25,935, and sarcosine, are also highly effective against allodynic pain in rodent, regardless of whether the drugs were administered via the oral, intravenous, or intrathecal route of delivery. Mechanistically, stimulation of GlyRα3 in the spinal cord underlies the anti-nociceptive action of the non-competitive GlyT1 inhibitors Org 25,935, sarcosine [100] as well as ALX5407 [99], since strychnine or genetic deletion of GlyRα3 nullifies the anti-nociceptive effects of these compounds [100].

It was first thought that inhibition of GlyT2 in the dorsal horn would not be effective in suppressing pain but might instead exacerbate pain perception, because GlyT2-mediated glycine reuptake is considered essential for vesicle refilling in presynaptic glycinergic nerve terminals and therefore should impair glycinergic neurotransmission. Yet, GlyT2-specific inhibitors, Org 25,543 and ALX 1393, are effective against acute thermal, mechanical, and chemical pain [100, 103, 104]. They may even be more effective than GlyT1 inhibitors for urological pain [101] and herpetic or post-herpetic pain [105]. Indeed, GlyT2 inhibitors are reported to be faster-acting and effective even at low doses [98]. The apparent superior action of GlyT2 inhibitors has been linked to the more restrictive and yet higher

expression of GlyT2 in the dorsal horn. By comparison, the distribution of GlyT1 is more diffused within the spinal cord. Similar to GlyT1 inhibitors, the anti-nociception produced by GlyT2-inhibition is also GlyR-dependent, as strychnine can abolish it [100].

In contrast, whether enhanced NMDA receptor-signalling by GlyT1 inhibition may potentiate or suppress transmission of the pain signals in the spinal cord is still unclear. Theoretically, neural excitation mediated by NMDA receptor activation ought to potentiate the pain signals and therefore sensitises nociception. At least, pain suppression has been demonstrated with the glycine-B site antagonist, GV196771, in patients with neuropathic pain [106]. Direct blockade of NMDA receptors by 2-amino-5-phosphonopentanoate (AP5) can also synergistically enhance the anti-nociception produced by intrathecal glycine in rats [107]. Nonetheless, no evidence so far has supported the possibility that GlyT1 inhibition is associated with any hyperalgesia.

One speculation is that NMDA receptor internalisation induced by chronic occupation of the glycine-B site [93] might contribute, at least partially, to the reduction of pain signals, when sufficiently high doses are used over an extended period [98]. Another suggestion is that GlyT1 inhibition may interfere with pain perception through an action in the higher cortical areas. Sarcosine infused directly into the medial prefrontal cortex has been reported to reduce pain sensitivity in a rat model of neuropathic pain [108]. This is in line with suggestions that inhibition of brain GlyT1 may also benefit the cognitive and behavioural abnormalities in patients with chronic pain [99, 109].

Attempts to translate the largely positive preclinical data into effective clinical tools, however, have not been met with much success. A proof-of-concept study of intrathecal administration of glycine (32 mg/day for 4 weeks) to relieve complex regional pain syndrome did not show an efficacy above placebo [110]. An on-going phase 2 clinical trial (NCT02489526) is exploring the potential of a mixed GlyT2/5HT$_{2A}$ receptor antagonist, VVZ-149, in the control of post-operative pain following colorectal surgery. This approach may represent a trend towards designing drugs with multiple targets to maximize efficacy.

Concluding Remarks

This chapter summarizes some suggested applications of glycine augmentation therapy through inhibition of glycine reuptake in addition to schizophrenia. This is not meant to be an exhaustive list. We emphasize instead areas with clear evidence at least at the preclinical stage of investigation. Although none of the areas highlighted has yet yielded any clinic-ready pharmacotherapy, it would be too early to dismiss entirely their clinical potential at this stage. Near the time of the disappointing news from the phase 3 trials of bitopertin also came the report that oral D-serine (60 mg/kg/day) was effective in treating the prodromal symptoms of schizophrenia in a double-blind, placebo-controlled pilot trial (NCT0082620) [111]. This paves the way for further drug studies targeting the glycine-B site designed to limit or even prevent development into full-blown psychosis. A focus on D-serine/glycine-B site interaction as such may offer a more selective intervention, as inhibition of glycine re-uptake necessarily produces dual effects on both inhibitory and excitatory activities as a result of the elevation in extracellular glycine. We also wish to point out that the rationale underpinning the suggested applications of GRI reviewed here are invariably based solely on the expected effects of GRI on either the NMDA receptor or the GlyR. Few have incorporated both, and if so, the rationalization often appears ad hoc. Indeed, one difficulty in consistently predicting the overall effect of the systemic GRI stems precisely from our inability to gauge the balance between the dual actions of glycine in the CNS. In particular, the role of glycinergic neurotransmission in the regulation of higher cortical function has been largely ignored, and so very little is known compared with our current appreciation of NMDA receptors functions in the brain. The knowledge gap would need to be filled before significant advance in GRI-based drug discovery can be made.

References

1. Eulenburg V, Armsen W, Betz H, Gomeza J. Glycine transporters: essential regulators of neurotransmission. Trends Biochem Sci. 2005;30(6):325–33.
2. Harvey RJ, Topf M, Harvey K, Rees MI. The genetics of hyperekplexia: more than startle! Trends Genet. 2008;24(9):439–47.
3. Danysz W, Parsons CG. Glycine and N-methyld-aspartate receptors: physiological significance and possible therapeutic applications. Pharmacol Rev. 1998;50(4):597–664.
4. Singer P, Dubroqua S, Yee BK. Inhibition of glycine transporter 1: the yellow brick road to new schizophrenia therapy? Curr Pharm Des. 2015;21(26):3771–87.
5. Singh SP, Singh V. Meta-analysis of the efficacy of adjunctive NMDA receptor modulators in chronic schizophrenia. CNS Drugs. 2011;25(10):859–85.
6. Bugarski-Kirola D, Iwata N, Sameljak S, et al. Efficacy and safety of adjunctive bitopertin versus placebo in patients with suboptimally controlled symptoms of schizophrenia treated with antipsychotics — results: from the SearchLyte clinical trial. Neuropsychopharmacology. 2014;39:S291–472, T121.
7. Bugarski-Kirola D, Arango C, Fleischhacker WW, Bressan R, Nasrallah H, Lawrie S, Blaettler T, Garibaldi G, Reid C, Marder SE. Efficacy and safety of adjunctive bitopertin versus placebo in subjects with persistent predominant negative symptoms of schizophrenia treated with antipsychotics — update from the SearchLyte programme. Schizophr Res. 2014;153(Suppl 1):29.
8. Bugarski-Kirola D, Fleischhacker WW, Blaettler T, Edgar CJ, Milosavljevic-Ristic S, Lamour F, Sun S, Kapur S. Efficacy and safety of adjunctive bitopertin (10 and 20 mg) versus placebo in subjects with suboptimally controlled symptoms of schizophrenia treated with antipsychotics — results from the Phase III TwiLyte study. Int J Neuropsychopharmacol. 2014;17(Suppl 1):65.
9. Umbricht D, Yoo K, Youssef E, Dorflinger E, Martin-Facklam M, Bausch A, Arrowsmith R, Alberati D, Marder SR, Santarelli L, editors. Glycine transporter type 1 (GLYT1) inhibitor RG1678: positive results of the proof-of-concept study for the treatment of negative symptoms in schizophrenia. Neuropsychopharmacol. 2010;35:s320–1.
10. Umbricht D, Martin-Facklam M, Pizzagalli E, Youssef E, Yoo K, Doerflinger E, Bausch A, Arrowsmith R, Alberati D, Santarelli L. Glycine transporter type 1 (GLYT1) inhibition RG1678: results of the proof-of-concept study for the treatment of negative symptoms in schizophrenia. Schizophr Bull. 2011;37 Suppl 1:324.
11. Umbricht D, Alberati D, Martin-Facklam M, Borroni E, Youssef EA, Ostland M, Wallace TL, Knoflach F, Dorflinger E, Wettstein JG, Bausch A, Garibaldi G, Santarelli L. Effect of bitopertin, a glycine reuptake inhibitor, on negative symptoms of schizophrenia: a randomized, double-blind, proof-of-concept study. JAMA Psychiatry. 2014;71:637–46.
12. Lin CH, Lane HY, Tsai GE. Glutamate signaling in the pathophysiology and therapy of schizophrenia. Pharmacol Biochem Behav. 2012;100(4):665–77.
13. Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, Carlsson ML. Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. Annu Rev Pharmacol Toxicol. 2001;41:237–60.
14. Möhler H, Boison D, Singer P, Feldon J, Pauly-Evers M, Yee BK. Glycine transporter 1 as a potential therapeutic target for schizophrenia-related symptoms: evidence from genetically modified mouse models and pharmacological inhibition. Biochem Pharmacol. 2011;81(9):1065–77.
15. Depoortere R, Dargazanli G, Estenne-Bouhtou G, Coste A, Lanneau C, Desvignes C, et al. Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. Neuropsychopharmacology. 2005;30(11):1963–85.
16. Boulay D, Pichat P, Dargazanli G, Estenne-Bouhtou G, Terranova JP, Rogacki N, et al. Characterization of SSR103800, a selective inhibitor of the glycine transporter-1 in models predictive of therapeutic activity in schizophrenia. Pharmacol Biochem Behav. 2008;91(1):47–58.
17. Huang CC, Wei IH, Huang CL, Chen KT, Tsai MH, Tsai P, et al. Inhibition of glycine transporter-I as a novel mechanism for the treatment of depression. Biol Psychiatry. 2013;74(10):734–41.
18. Heresco-Levy U, Gelfin G, Bloch B, Levin R, Edelman S, Javitt DC, Kremer I. A randomized add-on trial of high-dose d-cycloserine for treatment-resistant depression. Int J Neuropsychopharmacol. 2013;16(3):501–6.
19. Burgdorf J, Zhang XL, Nicholson KL, Balster RL, Leander JD, Stanton PK, Gross AL, Kroes RA, Moskal JR. GLYX-13, a NMDA receptor glycine-site functional partial agonist, induces antidepressant-like effects without ketamine-like side effects. Neuropsychopharmacology. 2013;38(5):729–42.
20. Dubroqua S, Singer P, Boison D, Feldon J, Möhler H, Yee BK. Impacts of forebrain neuronal glycine transporter 1 disruption in the senescent brain: evidence for age-dependent phenotypes in Pavlovian learning. Behav Neurosci. 2010;124(6):839–50.
21. Williams NR, Schatzberg AF. NMDA antagonist treatment of depression. Curr Opin Neurobiol. 2016;36:112–7.
22. Kessler RC, Petukhova M, Sampson NA, Zaslavsky AM, Wittchen HU. Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. Int J Methods Psychiatr Res. 2012;21(3):169–84.
23. Ruscio AM, Stein DJ, Chiu WT, Kessler RC. The epidemiology of obsessive-compulsive disorder in

the National Comorbidity Survey Replication. Mol Psychiatry. 2010;15(1):53–63.
24. Goodman WK, Grice DE, Lapidus KA, Coffey BJ. Obsessive–compulsive disorder. Psychiatr Clin N Am. 2014;37(3):257–67.
25. Markarian Y, Larson MJ, Aldea MA, Baldwin SA, Good D, Berkeljon A, Murphy TK, Storch EA, McKay D. Multiple pathways to functional impairment in obsessive–compulsive disorder. Clin Psychol Rev. 2010;30(1):78–88.
26. Brander G, Vigil AP, Larsson H, Mataix-Cols D. Systematic review of environmental risk factors for obsessive–compulsive disorder: a proposed roadmap from association to causation. Neurosci Biobehav Rev. 2016;S0149-7634(15):30229–3.
27. Grant JE. Clinical practice: obsessive–compulsive disorder. N Engl J Med. 2014;371(7):646–53.
28. Decloedt EH, Stein DJ. Current trends in drug treatment of obsessive–compulsive disorder. Neuropsychiatr Dis Treat. 2010;6:233–42.
29. Komossa K, Depping AM, Meyer M, Kissling W, Leucht S. Second-generation antipsychotics for obsessive compulsive disorder. Cochrane Database Syst Rev. 2010;12:CD008141.
30. Veale D, Miles S, Smallcombe N, Ghezai H, Goldacre B, Hodsoll J. Atypical antipsychotic augmentation in SSRI treatment refractory obsessive–compulsive disorder: a systematic review and meta-analysis. BMC Psychiatry. 2014;14:317.
31. Bloch MH, Landeros-Weisenberger A, Kelmendi B, Coric V, Bracken MB, Leckman JF. A systematic review: antipsychotic augmentation with treatment refractory obsessive–compulsive disorder. Mol Psychiatry. 2006;11(7):622–32.
32. Beck JS. Cognitive behavior therapy: basics and beyond. 2nd ed. New York: The Guilford Press; 2011. p. 19–20.
33. Cybulska EM. Obsessive compulsive disorder, the brain and electroconvulsive therapy. Br J Hosp Med (Lond). 2006;67(2):77–81.
34. Barlow DH, Durand VM. Essentials of abnormal psychology. Belmont: Thomson Wadsworth; 2006.
35. Hartmann CJ, Lujan JL, Chaturvedi A, Goodman WK, Okun MS, McIntyre CC, Haq IU. Tractography activation patterns in dorsolateral prefrontal cortex suggest better clinical responses in OCD DBS. Front Neurosci. 2016;9:519.
36. Koran LM, Hanna GL, Hollander E, Nestadt G, Simpson HB. American psychiatric association. Practice guideline for the treatment of patients with obsessive–compulsive disorder. Am J Psychiatry. 2007;164(7 Suppl):5–53.
37. Ting JT, Feng G. Glutamatergic synaptic dysfunction and obsessive–compulsive disorder. Curr Chem Genomics. 2008;2:62–75.
38. Wu P-L, Lane H-Y, Tang H-S, Tsai GE. Glutamate theory in developing novel pharmacotherapies for obsessive compulsive disorder: focusing on N-methyl- D-aspartate signaling. Biomedicine. 2012;2:75–9.
39. Grados MA, Specht MW, Sung HM, Fortune D. Glutamate drugs and pharmacogenetics of OCD: a pathway-based exploratory approach. Expert Opin Drug Discovery. 2013;8(12):1515–27.
40. Pittenger C. Glutamate modulators in the treatment of obsessive–compulsive disorder. Psychiatr Ann. 2015;45(6):308–15.
41. Wu PL, Tang HS, Lane HY, Tsai CA, Tsai GE. Sarcosine therapy for obsessive compulsive disorder: a prospective, open-label study. J Clin Psychopharmacol. 2011;31(3):369–74.
42. Greenberg WM, Benedict MM, Doerfer J, Perrin M, Panek L, Cleveland WL, Javitt DC. Adjunctive glycine in the treatment of obsessive–compulsive disorder in adults. J Psychiatr Res. 2009;43(6):664–70.
43. Cleveland WL, DeLaPaz RL, Fawwaz RA, Challop RS. High-dose glycine treatment of refractory obsessive–compulsive disorder and body dysmorphic disorder in a 5-year period. Neural Plast. 2009;2009:768398.
44. Kushner MG, Kim SW, Donahue C, Thuras P, Adson D, Kotlyar M, McCabe J, Peterson J, Foa EB. D-cycloserine augmented exposure therapy for obsessive–compulsive disorder. Biol Psychiatry. 2007;62(8):835–8.
45. Wilhelm S, Buhlmann U, Tolin DF, Meunier SA, Pearlson GD, Reese HE, Cannistraro P, Jenike MA, Rauch SL. Augmentation of behavior therapy with D-cycloserine for obsessive–compulsive disorder. Am J Psychiatry. 2008;165(3):335–41.
46. Storch EA, Murphy TK, Goodman WK, Geffken GR, Lewin AB, Henin A, Micco JA, Sprich S, Wilhelm S, Bengtson M, Geller DA. A preliminary study of D-cycloserine augmentation of cognitive-behavioral therapy in pediatric obsessive–compulsive disorder. Biol Psychiatry. 2010;68(11):1073–6.
47. Foa E, Franklin ME, Moser J. Context in the clinic: how well do cognitivebehavioral therapies and medications work in combination? Biol Psychiatry. 2002;52(10):987–97.
48. Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, Hodges L, Davis M. Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. Arch Gen Psychiatry. 2004;61(11):1136–44.
49. Otto MW, Tolin DF, Simon NM, Pearlson GD, Basden S, Meunier SA, Hofmann SG, Eisenmenger K, Krystal JH, Pollack MH. Efficacy of D-cycloserine for enhancing response to cognitive–behavior therapy for panic disorder. Biol Psychiatry. 2010;67(4):365–70.
50. Guastella AJ, Richardson R, Lovibond PF, Rapee RM, Gaston JE, Mitchell P, Dadds MR. A randomized controlled trial of D-cycloserine enhancement of exposure therapy for social anxiety disorder. Biol Psychiatry. 2008;63(6):544–9.
51. Hofmann SG, Meuret AE, Smits JA, Simon NM, Pollack MH, Eisenmenger K, Shiekh M, Otto MW. Augmentation of exposure therapy for social

anxiety disorder with D-Cycloserine. Arch Gen Psychiatry. 2006;63(3):298–304.
52. Nations KR, Smits JA, Tolin DF, Rothbaum BO, Hofmann SG, Tart CD, Lee A, Schipper J, Sjogren M, Xue D, Szegedi A, Otto MW. Evaluation of the glycine transporter inhibitor Org25935 as augmentation to cognitive–behavioral therapy for panic disorder: a multicenter, randomized, double-blind, placebo-controlled trial. J Clin Psychol. 2012;73(5):647–53.
53. Komatsu H, Furuya Y, Sawada K, Asada T. Involvement of the strychnine-sensitive glycine receptor in the anxiolytic effects of GlyT1 inhibitors on maternal separation-induced ultrasonic vocalization in rat pups. Eur J Pharmacol. 2015;746:252–7.
54. Depoortère R, Dargazanli G, Estenne-Bouhtou G, Coste A, Lanneau C, Desvignes C, et al. Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. Neuropsychopharmacology. 2005;30(11):1963–85.
55. Shorvon SD. The epidemiology and treatment of chronic and refractory epilepsy. Epilepsia. 1996;37(Suppl. 2):S1–3.
56. Ben-Ari Y. Seizures beget seizures: the quest for GABA as a key player. Crit Rev Neurobiol. 2006;18(1–2):135–44.
57. Schuele SU, Luders HO. Intractable epilepsy: management and therapeutic alternatives. Lancet Neurol. 2008;7(6):514–24.
58. Stafstrom CE, Carmant L. Seizures and epilepsy: an overview for neuroscientists, vol. 5. New York: Cold Spring Harbor; 2015.
59. Hirsch E, Schmitz B, Carreno M. Epilepsy, antiepileptic drugs (AEDs) and cognition. Acta Neurol Scand Suppl. 2003;180:23–32.
60. Arif H, Buchsbaum R, Weintraub D, Pierro J, Resor Jr SR, Hirsch LJ. Patient-reported cognitive side effects of antiepileptic drugs: predictors and comparison of all commonly used antiepileptic drugs. Epilepsy Behav. 2009;1491:202–9.
61. Loscher W, Schmidt D. Modern antiepileptic drug development has failed to deliver: ways out of the current dilemma. Epilepsia. 2011;52(4):657–78.
62. Kanner AM. Management of psychiatric and neurological comorbidities in epilepsy. Nat Rev Neurol. 2016;12(2):106–16.
63. Lapin IP. Antagonism of L-glycine to seizures induced by L-kynurenine, quinolinic acid and strychnine in mice. Eur J Pharmacol. 1981;71(4):495–8.
64. Seiler N, Sarhan S. Synergistic anticonvulsant effects of a GABA agonist and glycine. Gen Pharmacol. 1984;15(4):367–9.
65. Halsey MJ, Little HJ, Wardley-Smith B. Systemically administered glycine protects against strychnine convulsions, but not the behavioural effects of high pressure, in mice. J Physiol. 1989;408:431–41.
66. Toth E, Lajtha A, Sarhan S, Seiler N. Anticonvulsant effects of some inhibitory neurotransmitter amino acids. Neurochem Res. 1983;8(3):291–302.
67. Peterson SL. Glycine potentiates the anticonvulsant action of diazepam and phenobarbital in kindled amygdaloid seizures of rats. Neuropharmacology. 1986;25(12):1359–63.
68. Peterson SL, Frye GD. Glycine potentiates diazepam anticonvulsant activity in electroshock seizures of rats: possible sites of interaction in the brainstem. Brain Res Bull. 1987;18(6):715–21.
69. Kalinichev M, Starr KR, Teague S, Bradford AM, Porter RA, Herdon HJ. Glycine transporter 1 (GlyT1) inhibitors exhibit anticonvulsant properties in the rat maximal electroshock threshold (MEST) test. Brain Res. 2010;1331:105–13.
70. Socała K, Nieoczym D, Rundfeldt C, Wlaź P. Effects of sarcosine, a glycine transporter type 1 inhibitor, in two mouse seizure models. Pharmacol Rep. 2010;62(2):392–7.
71. Zhao J, Tao H, Xian W, Cai Y, Cheng W, Yin M, Liang G, Li K, Cui L, Zhao B. A highly selective inhibitor of glycine transporter-1 elevates the threshold for maximal electroshock-induced tonic seizure in mice. Biol Pharm Bull. 2016;39(2):174–80.
72. Shen HY, van Vliet EA, Bright KA, Hanthorn M, Lytle NK, Gorter J, Aronica E, Boison D. Glycine transporter 1 is a target for the treatment of epilepsy. Neuropharmacology. 2015;99:554–65.
73. Singer P, Feldon J, Yee BK. The glycine transporter 1 inhibitor SSR504734 enhances working memory performance in a continuous delayed alternation task in C57BL/6 mice. Psychopharmacology. 2009;202(1–3):371–84.
74. Croucher MJ, Bradford HF. 7-Chlorokynurenic acid, a strychnine-insensitive glycine receptor antagonist, inhibits limbic seizure kindling. Neurosci Lett. 1990;118(1):29–32.
75. Croucher MJ, Bradford HF. The influence of strychnine-insensitive glycine receptor agonists and antagonists on generalized seizure thresholds. Brain Res. 1991;543:91–6.
76. Peterson SL. Anticonvulsant drug potentiation by glycine in maximal electroshock seizures is mimicked by D-serine and antagonized by 7-chlorokynurenic acid. Eur J Pharmacol. 1991;199(3):341–8.
77. Brodie MJ, Sills GJ. Combining antiepileptic drugs — rational polytherapy? Seizure. 2011;20(5):369–75.
78. So EL. What is known about the mechanisms underlying SUDEP? Epilepsia. 2008;49(suppl 9):93–8.
79. Richerson GB, Buchanan GF. The serotonin axis: shared mechanisms in seizures, depression, and SUDEP. Epilepsia. 2011;52(Suppl. 1):28–38.
80. Boison D. Cell and gene therapies for refractory epilepsy. Curr Neuropharmacol. 2007;5(2):115–25.
81. Shetty AK, Hattiangady B. Concise review: prospects of stem cell therapy for temporal lobe epilepsy. Stem Cells. 2007;25:2396–407.
82. Soderpalm B, Ericson M. Neurocircuitry involved in the development of alcohol addiction: the dopamine

system and its access points. Curr Top Behav Neurosci. 2013;13:127–61.
83. Spanagel R, Vengeliene V. New pharmacological treatment strategies for relapse prevention. Curr Top Behav Neurosci. 2013;13:583–609.
84. Lidö HH, Stomberg R, Fagerberg A, Ericson M, Soderpalm B. The glycine reuptake inhibitor org 25935 interacts with basal and ethanol-induced dopamine release in rat nucleus accumbens. Alcohol Clin Exp Res. 2009;33(7):1151–7.
85. Lidö HH, Ericson M, Marston H, Söderpalm B. A role for accumbal glycine receptors in modulation of dopamine release by the glycine transporter-1 inhibitor Org25935. Front Psychol. 2011;2:8.
86. Molander A, Lidö HH, Löf E, Ericson M, Söderpalm B. The glycine reuptake inhibitor Org25935 decreases ethanol intake and preference in male Wistar rats. Alcohol Alcohol. 2007;42(1):11–8.
87. Szegedi A, de Bejczy A, Nations KR, Ruwe F, Soderpalm B, Michelson D, Gold L. Evaluation of glycine transporter inhibitor Org 25935 for the prevention of relapse in alcohol-dependent patients: a multisite, randomized, double-blind, placebo-controlled trial. Neuropsychopharmacology. 2012;38:S314–446.
88. Molander A, Lof E, Stomberg R, Ericson M, Soderpalm B. Involvement of accumbal glycine receptors in the regulation of voluntary ethanol intake in the rat. Alcohol Clin Exp Res. 2005;29(1):38–45.
89. Spanagel R, Bartsch D, Brors B, Dahmen N, Deussing J, Eils R, et al. An integrated genome research network for studying the genetics of alcohol addiction. Addict Biol. 2010;15(4):369–79.
90. Lidö HH, Marston H, Ericson M, Soderpalm B. The glycine reuptake inhibitor Org24598 and acamprosate reduce ethanol intake in the rat; tolerance development to acamprosate but not to Org24598. Addict Biol. 2012;17(5):897–907.
91. Achat-Mendes C, Dhonnchadha BA, Platt DM, Kantak K, Spealman RD. Glycine transporter-1 inhibition preceding extinction training inhibits reacquisition of cocaine seeking. Neuropsychopharmacology. 2012;37(13):2837–45.
92. NicDhonnchadha BA, Pinard E, Alberati D, Wettstein JG, Spealman RD, Kantak KM. Inhibiting glycine transporter-1 facilitates cocaine-cue extinction an attenuates reacquisition of cocaine-seeking behavior. Drug Alcohol Depend. 2012;122(1–2):119–26.
93. Nong Y, Huang YQ, Ju W, Kalia LV, Ahmadian G, Wang YT, et al. Glycine binding primes NMDA receptor internalization. Nature. 2003;422(6929):302–7.
94. Balu DT, Coyle JT. Glutamate receptor composition of the post-synaptic density is altered in genetic mouse models of NMDA receptor hypo- and hyperfunction. Brain Res. 2011;1392:1–7.
95. Vengeliene V, Bachteler D, Danysz W, Spanagel R. The role of the NMDA receptor in alcohol relapse: a pharmacological mapping study using the alcohol deprivation effect. Neuropharmacology. 2005;48(6):822–9.
96. Gass JT, Olive MF. Glutamatergic substrates of drug addiction and alcoholism. Biochem Pharmacol. 2008;75(1):218–65.
97. Sesack SR, Grace AA. Cortico-basal ganglia reward network: microcircuitry. Neuropsychopharmacology. 2010;35(1):27–47.
98. Dohi T, Morita K, Kitayama T, Motoyama N, Morioka N. Glycine transporter inhibitors as a novel drug discovery strategy for neuropathic pain. Pharmacol Ther. 2009;123(1):54–79.
99. Tanabe M, Takasu K, Yamaguchi S, Kodama D, Ono H. Glycine transporter inhibitors as a potential therapeutic strategy for chronic pain with memory impairment. Anesthesiology. 2008;108(5):929–37.
100. Morita K. Spinal antiallodynia action of glycine transporter inhibitors in neuropathic pain models in mice. Spinal antiallodynia action of glycine transporter inhibitors in neuropathic pain models in mice. J Pharmacol ExpTher. 2008;326(2):633–45.
101. Yoshikawa S, Oguchi T, Funahashi Y, de Groat WC, Yoshimura N. Glycine transporter type 2 (GlyT2) inhibitor ameliorates bladder overactivity and nociceptive behavior in rats. Eur Urol. 2012;62(4):704–12.
102. Cheng W, Yin Q, Cheng MY, Chen HS, Wang S, Feng T, Zeng YM, Liu GJ. Intracerebroventricular or intrathecal injection of glycine produces analgesia in thermal nociception and chemical nociception via glycine receptors. Eur J Pharmacol. 2009;614(1–3):44–9.
103. Hermanns H, Muth-Selbach U, Williams R, Krug S, Lipfert P, Werdehausen R, Braun S, Bauer I. Differential effects of spinally applied glycine transporter inhibitors on nociception in a rat model of neuropathic pain. Neurosci Lett. 2008;445(3):214–9.
104. Haranishi Y, Hara K, Terada T, Nakamura S, Sata T. The antinociceptive effect of intrathecal administration of glycine transporter-2 inhibitor ALX1393 in a rat acute pain model. Anesth Analg. 2010;110(2):615–21.
105. Nishikawa Y, Sasaki A, Kuraishi Y. Blockade of glycine transporter (GlyT) 2, but not GlyT1, ameliorates dynamic and static mechanical allodynia in mice with herpetic or postherpetic pain. J Pharmacol Sci. 2010;112(3):352–60.
106. Wallace MS, Rowbotham MC, Katz NP, Dworkin RH, Dotson RM, Galer BS, Rauck RL, Backonja MM, Quessy SN, Meisner PD. A randomized, double-blind, placebo-controlled trial of a glycine antagonist in neuropathic pain. Neurology. 2002;59(11):1694–700.
107. Beyer C, Komisaruk BR, Lopez-Colome AM, Caba M. Administration of AP5, a glutamate antagonist, unmasks glycine analgesic actions in the rat. Pharmacol Biochem Behav. 1992;42(2):229–32.
108. Centeno MV, Mutso A, Millecamps M, Apkarian AV. Prefrontal cortex and spinal cord mediated anti-

neuropathy and analgesia induced by sarcosine, a glycine-T1 transporter inhibitor. Pain. 2009;145(1–2):176–83.
109. Kodama D, Ono H, Tanabe M. Increased hippocampal glycine uptake and cognitive dysfunction after peripheral nerve injury. Pain. 2011;152(4):809–17.
110. Munts AG, van der Plas AA, Voormolen JH, Marinus J, Teepe-Twiss IM, Onkenhout W, van Gerven JM, van Hilten JJ. Intrathecal glycine for pain and dystonia in complex regional pain syndrome. Pain. 2009;146(1–2):199–204.
111. Kantrowitz JT, Woods SW, Petkova E, Cornblatt B, Corcoran CM, Chen H, Silipo G, Javitt DC. D-serine for the treatment of negative symptoms in individuals at clinical high risk of schizophrenia: a pilot, double-blind, placebo-controlled, randomised parallel group mechanistic proof-of-concept trial. Lancet Psychiatry. 2015;2(5):403–12.

29 The Adenosinergic System in the Neurobiology of Schizophrenia: Prospective Adenosine Receptor–Based Pharmacotherapy

Francisco Ciruela, Víctor Fernández-Dueñas, Xavier Altafaj, Fernando Contreras, Antoni Vallano, José Manuel Menchón, and Marta Valle-León

Abstract

The pharmacotherapy of schizophrenia relies on restoring a dysregulated striatal dopamine and prefrontal cortex glutamate neurotransmission. However, these treatments are usually insufficient to fully cover all the disease symptomatology (i.e., negative and cognitive symptoms). Thus, the search for alternative and/or complementary neurotransmitter systems involved in the etiology of schizophrenia constitutes a big challenge in psychiatry these days. Adenosine, a well known neuromodulator in the central nervous system, has been highlighted because its relationship with both dopaminergic and glutamatergic neurotransmission. Indeed, the disruption of adenosine homeostasis in the adult brain has multiple consequences in the circuitry implicated in the pathophysiology of schizophrenia. Consequently, the "adenosine hypothesis of schizophrenia" foresees that the disruption of adenosine homeostasis within certain brain areas has behavioral consequences resembling schizophrenia symptoms. Thus, it has been postulated that restoring adenosine concentration within the

F. Ciruela (✉) • V. Fernández-Dueñas • X. Altafaj
Francisco Ciruela Pharmacology Unit, Department of Pharmacology, Faculty of Medicine, University of Barcelona, Government Pavilion, Llobregat Hospital, Barcelona, Spain
e-mail: fciruela@ub.edu

Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain

F. Contreras • J.M. Menchón
Servei de Psiquitria, Hospital Universitari de Bellvitge-IDIBELL, Universitat de Barcelona, CIBERSAM, L'Hospitalet de Llobregat, 08907 Barcelona, Spain

A. Vallano
Unitat de Farmacologia, Departament Patologia i Terapèutica Experimental, Facultat de Medicina, IDIBELL, Universitat de Barcelona, L'Hospitalet de Llobregat, 08907 Barcelona, Spain

Servei de Farmacologia Clínica, Hospital Universitari de Bellvitge-IDIBELL, L'Hospitalet de Llobregat, 08907 Barcelona, Spain

M. Valle-León
Unitat de Farmacologia, Departament Patologia i Terapèutica Experimental, Facultat de Medicina, IDIBELL, Universitat de Barcelona, L'Hospitalet de Llobregat, 08907 Barcelona, Spain

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_29

schizophrenia-related brain areas might have beneficial antipsychotic properties. Overall, as adenosine dysfunction can trigger endophenotypes of schizophrenia, the development of drugs targeting the adenosinergic system will definitely constitute a new opportunity for therapeutic intervention in schizophrenia.

Keywords
Adenosinergic system • Adenosine receptors • Schizophrenia • Adenosine–dopamine–glutamate interactions

Introduction

In the 1920s, Drury and Szent-Györgyi [1] demonstrated that adenosine modulated kidney functioning as well as promoting profound hypotension and bradycardia. Since that time, the physiological role and potential therapeutic use of adenosine have been fundamentally assessed [2, 3]. Certainly, a deficit in endogenous nucleosides, mostly adenosine, has been eventually associated with multiple neurological diseases and neuropsychiatric conditions including epilepsy, chronic pain, and schizophrenia [4]. Thus, increasing adenosinergic function either by inhibiting adenosine metabolism or by activating adenosine receptors seems to be a rational therapeutic strategy for these adenosine-related diseases. Accordingly, within this chapter we highlight both the role of adenosine function and dysfunction in physiological and pathophysiological conditions, and the potential use of adenosine-based drugs as a new pharmacotherapeutic opportunity for schizophrenia.

Adenosine Metabolism

The purinergic transmission system involves two main extracellular effectors within the central nervous system (CNS), namely adenosine and adenosine 5′-triphosphate (ATP) [5] (Fig. 29.1). Thus, while ATP is considered a canonical neurotransmitter, adenosine technically behaves as a neuromodulator. Therefore, despite the initial opposition to considering ATP as a selective extracellular signaling molecule, the existence of potent physiological effects and extracellular enzymes regulating the amount of ATP available quickly provided support for ATP as a neurotransmitter, and thus for the existence of a purinergic neurotransmission system [5]. Afterward, ATP was identified as a co-transmitter in peripheral nerves and subsequently as a co-transmitter with glutamate, noradrenaline, GABA, acetylcholine, and dopamine in the CNS [6]. Indeed, extracellular ATP is promptly hydrolyzed into adenosine 5′-diphosphate, adenosine 5′-monophosphae (AMP), and adenosine plus inorganic phosphate, through the action of the ectonucleoside triphosphate diphosphohydrolase CD39 and the 5′-nucleotidase CD73 [7] (Fig. 29.1), thus constituting the main mechanism behind high extracellular adenosine levels.

Adenosine consists of a purine base (adenine) attached to the 1′ carbon atom of ribose (Fig. 29.1). As mentioned above, this ribonucleoside is mostly produced by the catabolism of ATP, both at the intra- and extracellular levels (Fig. 29.1), although to a lesser extent it can also be generated by S-adenosyl-L-homocysteine (SAH) metabolism (Fig. 29.1). Adenosine, once synthesized, can be either released through Na^+-dependent transporters or intracellularly phosphorylated to form AMP by the action of adenosine kinase (Fig. 29.1). In addition, adenosine can react with L-homocysteine to form SAH (Fig. 29.1). Finally, adenosine can be deaminated to form inosine by the action of intra- and extracellular adenosine deaminase (Fig. 29.1).

Adenosine has been historically considered a retaliatory metabolite that “increases oxygen supply and decreases oxygen consumption” [8], thus modulating a large array of physiological processes.

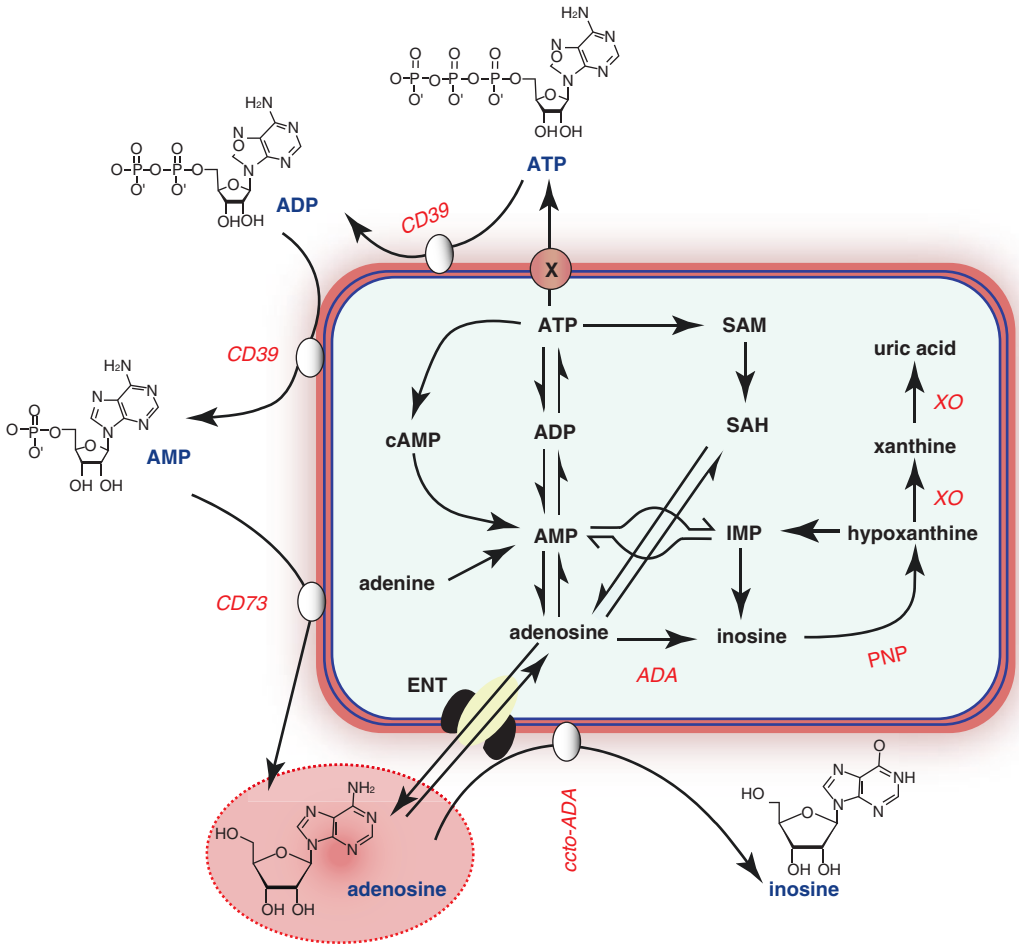


Fig. 29.1 Schematic representation of purine metabolism and different sources of extracellular adenosine**.** The intracellular ATP could be catabolized into adenosine, which can be further metabolized into inosine and hypoxanthine by intracellular adenosine deaminase (*ADA*) and purine nucleoside phosphorylase (*PNP*) respectively. Hypoxanthine can be either salvaged into inosine monophosphate (*IMP*), or further metabolized to xanthine and uric acid by xanthine oxidase (*XO*). In addition, adenosine could also be generated intracellularly through the hydrolysis of the S-adenosyl-homo-cysteine (*SAH*) by an SAH hydrolase. Interestingly, the intracellularly generated adenosine and ATP can be released to the extracellular milieu through an equilibrative nucleoside transporter (*ENT*) or open-ended systems (i.e., nucleotide-permeable channels, exocytosis, injury or lysis, transport vesicles, lysosomes), respectively. Subsequently, the ATP is dephosphorylated into adenosine by the ectonucleoside triphosphate diphosphohydrolase CD39 and the 5′-nucleotidase CD73, thus constituting the main mechanism behind high extracellular adenosine levels. Finally, extracellular adenosine can be either transported back into the cell through ENTs or transformed into inosine through ecto-adenosine deaminase ADA

Therefore, adenosine participates in the control of respiratory function [9], neural activity [10], platelet aggregation [11], neutrophil function [12], lymphocyte differentiation [13], and vascular tone [14]. Also, adenosine is able to provoke both dilatation of coronary arteries and contraction of kidney blood vessels, thus reducing renal filtration [15]. In addition, it exerts a negative chronotropic and dromotropic effect on the heart [16], as well as mediating the inhibition of neurotransmitter release [17] and lipolysis [18]. Accordingly, adenosine has been postulated as a mediator of metabolic distress, thus having a considerable impact on homeostatic cellular functioning.

Interestingly, within the CNS adenosine has been shown to play a key regulatory role, thus

acting as a presynaptic, postsynaptic and/or non-synaptic neuromodulator [19]. Extracellular adenosine levels in the brain range high nM concentration at basal conditions and correlate to the intracellular concentration of adenosine and nucleotides, such as ATP, AMP, and cAMP [20]. Indeed, the intracellular adenosine concentration is related to the rate of breakdown and synthesis of ATP [20]. Thus, adenosine is released as a neuromodulator [21] by the effector cells in response to an increased metabolic demand [22]. Interestingly, it has been postulated that the main source of extracellular adenosine in the striatum comes from intracellular cAMP [23], which is metabolized to AMP by means of phosphodiesterases and then to adenosine by the ecto-nucleotidases (Fig. 29.1). Overall, since cAMP can only be generated by the action of the enzyme adenylyl cyclase, striatal extracellular adenosine would mostly reflect an increased activation of receptors positively linked to adenylyl cyclase.

Adenosine Receptors in the Brain

Early in the 1970s, it was shown that the electrical stimulation of brain slices promoted adenosine release [24]. Interestingly, this stimulated release of endogenous adenosine concomitantly produced cAMP intracellular accumulation, a fact that was blocked by methylxanthine (i.e., caffeine and theophylline) incubation [25]. Moreover, this phenomenon was observed in other tissues (i.e., heart) [26]. Together, these observations constituted the first evidence suggesting that extracellular adenosine exerted its effects through specific plasma membrane receptors. Subsequently, it was demonstrated that the adenosine-mediated antilipolytic effect on fat cells took place with a concomitant reduction in cAMP [27]. Thus, this dual effect of adenosine on cAMP accumulation was further supported when it was confirmed that adenosine could either inhibit or stimulate adenylyl cyclase. Collectively, these observations ended with the first sub-classification of plasma membrane adenosine receptors into R_i and R_a [28], or alternatively, A_1 and A_2 adenosine receptors [29].

Currently, it is well established that adenosine mediates its actions by activating specific G protein-coupled adenosine receptors (AR), for which four subtypes (A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R) have been identified so far. These ARs have a distinguishable pharmacological profile, tissue distribution, and effector coupling [30], and their functioning have been largely studied in the CNS (Table 29.1). ARs belong to the rhodopsin family or class A of G protein-coupled receptors (GPCRs) [52], thus sharing some common molecular signatures. For instance, within their sequence all adenosine receptors contain the widely conserved NPxxY(x)5,6F and the DRY motifs [53, 54]. Thus, adenosine-mediated AR conformational rearrangement determines the binding and activation of specific G proteins (Table 29.1), which are responsible for activation of different intracellular signaling pathways associated with adenosine function (Table 29.1).

A_1Rs and $A_{2A}Rs$ are primarily responsible for the CNS effects of adenosine (Table 29.1) [55]. The most abundant and homogeneously distributed AR within the brain is the A_1R, which couples to members of the pertussis toxin-sensitive G proteins (G_{i1}, G_{i2}, G_{i3} and G_o), and whose activation regulates several intracellular effector molecules such us adenylyl cyclase (AC), Ca^{2+} channels, K^+ channels, and phospholipase C (PLC) (Table 29.1) [56]. On the other hand, $A_{2A}R$ is expressed at high levels only in some specific brain regions, for instance striatum, olfactory tubercle, and nucleus accumbens [23, 57]. $A_{2A}Rs$ are mainly coupled to G_s/G_{olf} proteins [58], thus activating AC and increasing intracellular cAMP levels (Table 29.1). Interestingly, $A_{2A}R$ may also signal through a G-protein independent pathway eventually associated to mitogen-activated protein kinase (MAPK) signaling cascade activation [59]. Next, the $A_{2B}R$ is positively coupled to AC and PLC through a G_s and G_q protein, respectively (Table 29.1) [2]. $A_{2B}R$ is thought to be fairly ubiquitous in the brain, and the association of this receptor to specific physiological or behavioral responses remains quite scarce, since the $A_{2B}R$-specific pharmacological tools still are under development [60]. Finally, the A_3R has been shown to be coupled to $G_{i/o}$ proteins, thus inhibiting AC and also stimulating PLC (Table 29.1) [2].

Table 29.1 Adenosine receptors

Receptor	Adenosine affinity (nM)	G protein	Transduction mechanisms[b]	Physiological actions
A_1	~70	$G_{i/o}$[a] $G_{q/11}$ G_s	*Inhibits*: AC[a] *Activates*: PLC, AC	Vasoconstriction [31]; hypothermia and sedation [32]; analgesia [33]; neurotransmitter release [34, 35]; chemotaxis [36]; Neuroprotection [37]
A_{2A}	~150	G_s[a] G_{olf} $G_{15,16}$[§]	*Activates*: AC[a], PLC *Inhibits*: Ca^{2+} channels	Platelet aggregation inhibition [38]; vasodilation [39]; neurotransmitter release [40]; regulation of sensorimotor integration in basal ganglia [41]; sleep promotion [42]
A_{2B}	~5,000	G_s[a] $G_{q/11}$	*Activates*: AC[a], PLC	Vasodilation [43]; vasoconstriction [44]; cytokine production [45]; inhibition of cell proliferation [46]
A_3	~6,500	$G_{i/o}$[a]	*Inhibits*: AC[a] *Activates*: PLC	Mast cell activation [47]; preconditioning [48]; coronary vasodilation [49]; regulation of intraocular pressure [50]; hypotension [51]

[a] Main mechanism of coupling
[b] *AC* adenylyl cyclase, *PLC* phospholipase C, *PLA2* phospholipase A2, *PLD* phospholipase D, *GIRKs* G protein-dependent inwardly rectifying K^+ channels

The Adenosine Hypothesis of Schizophrenia

Schizophrenia is a serious mental disorder which comprises a heterogeneous group of syndromes of unknown etiology. It affects up to 1% of the population worldwide, and usually arises at late adolescence and early adulthood (i.e., median age onset is about 23 years in men and 28 years in women) [61]. Importantly, the definition of schizophrenia has evolved through the six editions of the Diagnostic and Statistical Manual of Mental Disorders (DSM) published by the American Psychiatric Association. Thus, for instance, in the DSM-IV version published in 1994, schizophrenia was defined as a mental disorder involving a range of cognitive and emotional dysfunctions that include perception, inferential thinking, language and communication, behavioral monitoring, affect, fluency and productivity of thought and speech, hedonic capacity, volition and drive, and attention. The diagnosis involved the recognition of a constellation of signs and symptoms associated with impaired occupational or social functioning: and no one symptom was pathognomonic of the disorder. In addition, in this version the pathology had very high diagnostic stability, with 80–90% of individuals receiving an initial diagnosis of schizophrenia retaining that diagnosis at 1–10 years [62, 63]. On the other hand, in the current DSM-5 version, five characteristic symptoms for the diagnosis of schizophrenia are established, with the requirement that at least two of these symptoms have to be present for a month [64]. Three changes with respect the previous version have been made, which include the elimination of the special treatment of bizarre delusions and Schneiderian "first-rank" hallucinations, clarification of the definition of negative symptoms, and the addition of a requirement that at least one of the minimum two requisite characteristic symptoms must be delusions, hallucinations, or disorganized speech [64]. It should be noted that the present classification seeks to incorporate the new information about the nature of the disorder accumulated over the past two decades. Thus, the disease is now considered to be characterized by positive, negative, and cognitive symptoms. Positive symptoms reflect the appearance of some phenomena that were not present in the past, and include hallucinations and delusions. On the other hand, negative symptoms, such as anhedonia or apathy, reflect the loss of capacities or characteristics previously possessed. Finally,

the cognitive symptoms include alterations in attention, working memory, executive functions, and social cognition.

Numerous theories about the neurotransmission systems affected in schizophrenia have been postulated. Thus, almost all major neurotransmission systems (i.e., dopaminergic, glutamatergic, serotoninergic, GABAergic, and cholinergic) have been involved in schizophrenia, although none of these hypotheses fully explains all the pathological process(es) associated with the disease. Interestingly, one of the most sustained theories is based on a concomitant hyperdopaminergic–hypoglutamatergic phenomenon [65], even though at the beginning they were postulated as separate hypotheses (i.e., the "glutamatergic" and the "dopaminergic" hypothesis) [66, 67]. Indeed, current pharmacotherapy for schizophrenia is based on such a "dopamine" and "glutamate" hypothesis, which focus on a dopamine D_2 receptor (D_2R) hyperfunction in the striatum, a deficient stimulation of dopamine D_1 receptors (D_1Rs) in the prefrontal cortex (PFC), and a N-Methyl-D-aspartate (NMDA) receptor hypofunction in the PFC [68]. However, since negative symptoms, cognitive dysfunction, and decrements in psychosocial and vocational functioning are often still persistent upon available pharmacotherapy, the development of a next generation of pharmacologic agents tackling these resilient symptoms is needed [69]. Overall, more research based on non-dopaminergic and non-glutamatergic interventions will be necessary in order to improve the caveats in schizophrenia treatment.

As abovementioned, adenosine plays an important role in the CNS both as a homeostatic neuronal bioenergetic mediator and as a neuromodulator agent. Indeed, an adenosine-mediated modulation of dopaminergic and glutamatergic neurotransmission has been described [70–72]. Thus, adenosine agonists and antagonists produce behavioral effects similar to dopamine antagonists and dopamine agonists respectively [73]. In addition, adenosine tone can also modulate glutamatergic neurotransmission [74, 75]. Therefore, adenosine may play a unique role in integrating glutamatergic and dopaminergic neurotransmission systems, and thus the purinergic hypothesis of schizophrenia has been proposed [76]. Accordingly, early on Lara and co-workers postulated that a dysfunction in the purinergic system (i.e., reduced adenosinergic activity) would account for the imbalance observed between dopaminergic and glutamatergic neurotransmission, a phenomenon that would explain the schizophrenic phenotype [77]. Importantly, and in support of this hypothesis, cognitive impairments and anatomical changes related to psychotic symptoms were recently demonstrated in a mice lacking $A_{2A}R$ [78]. Overall, several elements of experimental evidence supported the adenosine hypothesis of schizophrenia, which will be reviewed here.

Preclinical Models of Schizophrenia: A Role for the Purinergic System?

There is still a considerable lack of knowledge about psychiatric illnesses in general, and schizophrenia in particular. Therefore, preclinical studies based in animal models mimicking some of the schizophrenia-associated symptoms may be useful, even in the case that they do not precisely mirror what exactly occurs in a schizophrenic human patient. Accordingly, preclinical models can be valuable experimental tools to shed light into the mechanisms behind the etiopathology of schizophrenia. As mentioned above, the adenosinergic hypothesis of schizophrenia was proposed to interconnect the schizophrenia-associated dopaminergic hyperfunction and glutamatergic hypofunction. Indeed, some evidence obtained from experimental animal models supported this adenosine contribution to schizophrenia, through the modulation of both dopaminergic and glutamatergic neurotransmission [79]. Hence, we will review here these animal models supporting the glutamatergic hypofunction theory (e.g., the phencyclidine model) and the hyperdopamergic hypothesis (e.g., the amphetamine model) and its relationship with adenosinergic neurotransmission.

The hypoglutamatergic-NMDA receptor hypothesis was formulated in the late 1950s,

when it was observed that phencyclidine (PCP) provoked a psychotic-like condition similar to that observed in schizophrenic patients [80]. However, nobody suspected that NMDA receptors were behind this phenomenon until the 1980s, when Lodge and colleagues [81] demonstrated that NMDA receptor blockade was in fact the primary mechanism of PCP-mediated psychotic actions. Indeed, blockade of NMDA receptors promoted both glutamate and dopamine release in the PFC [82], thus disrupting glutamatergic and dopaminergic neurotransmission in this brain region. It has since been postulated that this neurotransmitter unbalance may well be correlated with the cognitive and behavioral perturbations observed in schizophrenia [82–84]. Interestingly, the administration of NMDA receptor antagonists either in the late foetal or in the postnatal period of rats was shown to increase neuronal death by apoptosis [85], a phenomenon that would be linked to adult schizophrenia-like behaviour. Conversely, administration of the same kind of compounds in the adult animal increased the neuronal damage by necrosis with the subsequent gliosis [86], also associated with psychotic-like behaviour. Overall, these experimental observations supported a neurodevelopmental link between NMDA receptor antagonists and schizophrenia. Thus, the hypoglutamatergic-NMDA receptor theory postulates the existence of disturbances in the pre- and perinatal brain development that could provoke clinical manifestations in early adult life [87]. Nevertheless, despite the experimental evidence and some clinical observations, the precise mechanism involving NMDA receptors in schizophrenia is still unknown.

The hypofunction of NMDA receptors in adults, core of the glutamatergic hypothesis of schizophrenia [88], has been traditionally sustained by pharmacological animal models using NMDA receptor antagonists (i.e., PCP, ketamine, and dizocilpine). PCP is a dissociative drug firstly synthesized in 1926 as a surgical anesthetic. Despite its efficacy, the use of this drug was not extended because of its concomitant adverse effects (i.e., hallucinations, delusions, and agitation). Thus, since PCP mimics some schizophrenia symptoms in humans it has been extensively used in animals as a model of this illness. Indeed, in rodents, an acute administration of PCP produced hyperlocomotion [89], social withdrawal [90], and failures both in cognition [91] and in sensorimotor gating [92]. On the other hand, chronic PCP treatment also promoted hyperlocomotion (i.e., a positive symptom) and induced deficits in social behaviour and reduced mobility in the forced swimming test (i.e., negative symptoms). As for the cognitive symptoms, PCP-treated animals displayed sensorimotor gating deficits and cognitive dysfunctions when subjected to learning and memory tests [93]. Interestingly, in humans these PCP-mediated schizophrenic-like symptoms were maintained during several weeks after the chronic treatment [94, 95]. Therefore, the PCP-induced model of schizophrenia seems to partially mimic the pathology, although there also exist some criticisms to this PCP-based animal model. For instance, in animals, differently from the human disease, sensorimotor deficit in the prepulse inhibition test does not last after PCP withdrawal; and with regard to negative symptoms some discrepancies have been reported between clinical features and PCP-treated animals [93].

Importantly, adenosine receptors have been shown to modulate psychostimulant effects in PCP-treated animals. Hence, both A_1R and $A_{2A}R$ agonists (i.e., CPA and CGS21680 respectively) were able to counteract PCP-mediated hyperlocomotor activity [96, 97], while $A_{2A}R$ blockade, but not A_1R, prompted exacerbation of the motor-stimulant effects of the NMDA antagonist [98]. Indeed, PCP-induced psychomotor activities were enhanced in a KO mouse specifically lacking the striatal neuronal $A_{2A}R$ [99]. However, in a KO mouse lacking the forebrain $A_{2A}R$, thus with the $A_{2A}R$ deleted in the neurons of striatum as well as cerebral cortex and hippocampus, an opposite effect was observed. Thus, a critical role of $A_{2A}Rs$ in extrastriatal neurons was described in providing a major excitatory effect on psychomotor activity [99]. Overall, these results indicate that $A_{2A}Rs$ in striatal and extrastriatal neurons exert an opposing modulation of psychostimulant (i.e., PCP-mediated) effects.

Similarly, the dopaminergic hypothesis of schizophrenia had several important theoretical changes throughout its history. Thus, while at the beginning it was based on a generalized hyperdopaminergic brain function, it quickly evolved into a combined subcortical hyperdopaminergic-prefrontal hypodopaminergic dysfunction. However, Howes proposed an updated third version based on multiple changes of different neurotransmitters and neural systems, which together with other biological or environment influences would underlie the cognitive dysfunction and negative symptoms of schizophrenia. In Howes' words, rather than being a hypothesis of schizophrenia this new view is more accurately a "dopamine hypothesis of psychosis-in-schizophrenia". This hypothesis explains several environmental and genetics risks of schizophrenia, and proposes that these interact to funnel through one final common pathway of presynaptic striatal hyperdopaminergia [100].

The hyperdopaminergic status of schizophrenia has been largely studied by means of pharmacological animal models. Thus, the administration of drugs (i.e., amphetamine) increasing the brain dopamine content is a classical experimental approach to mainly study schizophrenia-associated positive symptoms. Amphetamine, first discovered in 1887 [101], is currently used as an attention deficit (i.e., ADHD) and narcolepsy treatment [102]. It is a drug that acts as a strong CNS stimulant by increasing dopamine concentration in the synaptic cleft and thus raising the response in the post-synaptic neuron. Apart from the well-known positive symptoms, its administration can also provoke long-term cognitive impairments [103, 104]. Overall, while several investigations have demonstrated that amphetamine treatment could induce some behavioral, molecular, cellular, and neurochemical changes, which were behind the striatal dopaminergic system [105–108], studies reporting amphetamine-mediated negative symptoms are rare.

Dopamine receptors on striatonigral and striatopallidal neurons (D_1R and D_2R respectively) play a pivotal role in the control of motor responses [109]; thus, the efficacy of many antipsychotic drugs correlates well with their ability to block D_2Rs [110]. Since $A_{2A}Rs$ antagonistically interact with D_2Rs [111, 112], adenosine is expected to exert a regulatory influence on psychomotor behaviour, and indeed a role for $A_{2A}R$ regulating amphetamine-induced psychomotor behaviour has been described [113]. Thus, $A_{2A}R$ activation restored responsiveness to amphetamine in adenosine-deficient mice [113]. Overall, the abovementioned preclinical data supported the involvement of adenosine in schizophrenia and the potential use of adenosine receptors as drug targets for this disease.

Clinical Evidence Supporting the Adenosine Hypothesis

Several lines of investigation support the notion that the adenosinergic system may be altered in schizophrenia. The first remarkable piece of information pointing to this consists of the discovery that $A_{2A}R$ expression was found to be altered in necropsies from schizophrenic individuals. Thus, $A_{2A}R$ binding was increased in the striatum of postmortem brains of chronic schizophrenics [114, 115]. Similarly, an increased expression of $A_{2A}R$ on perivascular astrocytes in the hippocampus of patients with schizophrenia has been described [116]. On the contrary, a reduced expression of $A_{2A}R$ in human postmortem putamens of patients suffering schizophrenia has been reported, thus proposing that there may be a subgroup of schizophrenic patients with reduced striatal $A_{2A}R$ levels accompanied by an altered motor phenotype [117]. Indeed, since the adenosinergic tone was shown to be altered in schizophrenia, $A_{2A}R$ up- and down-regulation in a brain region-dependent manner may correspond to adaptive physiological conditions that in turn would be associated to a concomitant hyperdopaminergic state [77]. Interestingly, the genetic linkage of adenosine receptors to schizophrenia has been evaluated. For instance, while an $A_{2A}R$ genetic variant (i.e., 1976 T > C) was not shown to confer susceptibility to schizophrenia [118] an A_1R gene polymorphism was associated with pathophysiological mechanisms underlying the

schizophrenia, thus becoming a potential useful biomarker of schizophrenia [119]. In addition, the most frequent functional polymorphism of adenosine deaminase (22G → A, ADA1*2), which is characterized by a reduced enzymatic activity and thus higher adenosine levels, is less frequent among schizophrenic patients [120]. Overall, these results support the hypothesis of lower adenosinergic activity in schizophrenia.

Based on the previous data, it seems feasible to think that the use of pro-adenosinergic drugs may be beneficial for the treatment of the pathology. However, this pharmacological proposal is still premature, although some data exist concerning this suggestion. Indeed, raising the endogenous pool of purines with allopurinol has been shown to produce some promising results as add-on therapy for schizophrenia [121, 122]. Allopurinol, a well-known hypouricemic drug that inhibits xanthine oxidase (Fig. 29.1) was used as an add-on drug in the treatment of poorly responsive schizophrenic patients [121]. Interestingly, in this short controlled trial (i.e., 23 patients treated with haloperidol 15 mg/day plus allopurinol 300 mg/day and 23 patients with haloperidol 15 mg/day plus placebo) it was observed that the combination of haloperidol and allopurinol showed a significant superiority over haloperidol alone in the treatment of positive symptoms and general psychopathology symptoms, as well as Positive and Negative Syndrome Scale (PANSS) total scores [121]. In a similar study, a double-blind, placebo-controlled, crossover clinical trial of add-on allopurinol (300 mg/day) for poorly responsive schizophrenia or schizoaffective disorder (DSM-IV criteria), was conducted in 22 patients [122]. In this case, allopurinol was an effective and well-tolerated adjuvant treatment, especially for refractory positive symptoms [122]. Also, allopurinol showed effectiveness as adjunctive medication in schizophrenia outpatients ($N = 59$) with persistent symptoms despite adequate pharmacotherapy [123]. And more recently in a case report, allopurinol prompted a rapid decrease in psychotic symptoms in a patient with schizophrenia [124]. Thus, within 2 weeks of allopurinol adjuvant therapy, the patient showed significant improvement with respect to his positive and negative symptoms of schizophrenia (PANSS scores went from 88 to 41 over a period of 2 weeks) [124]. Although some clinical controversy has been established around allopurinol [125], adenosine modulator adjuvant therapy was shown to be more beneficial in overall psychopathology (especially positive symptoms) in schizophrenia and in treating mania episodes of bipolar disorder when compared to placebo [126]. Overall, these clinical studies suggest that allopurinol might be an effective adjuvant drug in the management of patients with chronic schizophrenia who are poorly responsive to current treatments. However, larger, randomized clinical trials are needed before a broad clinical application of allopurinol is recommended as routinely used adjuvant therapy to antipsychotics [127].

Another piece of evidence supporting the link between the adenosinergic system and schizophrenia consists of the fact that the adenosine transport inhibitor dipyridamole was found to be beneficial in patients with schizophrenia [128]. Thus, raising extracellular adenosine levels with dipyridamole not only improved haloperidol-mediated amelioration of positive and general psychopathology symptoms, as well as PANSS total scores [128], but it also showed effectiveness when combined with lithium in the treatment of acute bipolar mania [129]. Overall, all the above-mentioned clinical data support the adenosine hypothesis of schizophrenia and highlight the potential pharmacological interest of combining antipsychotic drugs with purinergic-based compounds (i.e., allopurinol and dipyridamole) to tackle resilient schizophrenia symptoms.

Adenosine Receptors as Drug Targets in Schizophrenia

Adenosine receptor agonists have convincingly shown antipsychotic-like efficacy in hyperdopaminergic and hypoglutamatergic experimental animal models of schizophrenia (see above). Conversely, antagonists for the same receptors mostly promoted psychotic-like behaviour in

similar animal models. These results contrast with the well-documented negative impact of adenosine receptor agonists on learning and memory and the pro-cognitive properties of adenosine receptor antagonists. Thus, a pharmacological contradiction exists when adenosine receptor-based drugs are proposed to be used in schizophrenia treatment. Nevertheless, based on the adenosinergic hypothesis, $A_{2A}R$ agonists would be selected. However, the antipsychotics that are currently under clinical use have D_2R antagonistic activity. And due to the high level of expression of $A_{2A}Rs$ and the D_2Rs in the striatum [130] and the well-documented intramembrane $A_{2A}R$–D_2R mutual antagonistic interaction, an easy and simple association would lead to the proposal of $A_{2A}R$ agonists as potential antipsychotic agents [72]. Indeed, the idea that those $A_{2A}R$ agonists might be of interest for the treatment of schizophrenia initially derived from studies just showing the existence of the antagonistic intramembrane interaction between $A_{2A}R$ and D_2R. These results were obtained in some cases in animal models of schizophrenia, therefore a putative antipsychotic-like profile of $A_{2A}R$ agonists was postulated [72, 97]. In such a way, the systemic administration of CGS21680, an $A_{2A}R$ agonist, produced a dose-dependent blockage of spontaneous and amphetamine-mediated motor activity with similar potency [97]. Furthermore, this $A_{2A}R$ agonist was more potent than haloperidol or clozapine at antagonizing the motor activity induced by PCP than the amphetamine-mediated one [97]. Overall, these results demonstrated an apparent "atypical" antipsychotic profile (i.e., low probability of inducing extrapyramidal side-effects) of the $A_{2A}R$ agonist CGS21680.

Apart from the peripheral side-effects (i.e., severe cardiovascular and immunomodulatory adverse effects) that precluded their use in clinical trials [131], $A_{2A}R$ agonists also showed detrimental effects at the central level in animal models of learning and memory [132]. These associated problems of direct $A_{2A}R$ activation with specific agonists could be a consequence of the lack of spatial anatomical resolution of these compounds, a common generalized problem in pharmacology. Thus, in addition to target striatal $A_{2A}Rs$, which would counteract the schizophrenia-associated D_2R hyperfunction, these compounds would also block extrastriatal (e.g., cortical) and peripheral $A_{2A}Rs$, with the concomitant detrimental effects discussed above. This is the main reason why $A_{2A}R$ agonists are not yet available for human use. Interestingly, a therapeutic alternative might be the direct modulation of the ambient level of adenosine, and this can be achieved by targeting enzymes or nucleoside transporters that control the extracellular levels of adenosine [4]. However, again the anatomical resolution of the increase in adenosine might compromise its therapeutic efficacy, and an adenosine-based new drug has still not been developed for the treatment of the pathology.

Concluding Remarks

From the time when the first therapeutic consideration for adenosine (i.e., the 1930s) [133] until the present day, a remarkable wide range of diseases has been postulated to be alleviated with adenosine-based drugs [2, 133]. Indeed, agonists and antagonists of adenosine receptors have an enormous therapeutic potential for both peripheral and central diseases. Thus, selective agonists are well advanced in clinical trials for the treatment of atrial fibrillation, pain, neuropathy, and pulmonary and other inflammatory conditions, whereas antagonists are being explored for the treatment of Parkinson's disease and congestive heart failure, for which selective compounds are already in clinical trials. In addition, adenosine receptor-based drugs are under consideration for the management of more dreadful and challenging diseases, such as schizophrenia. However, the therapeutic proposal for schizophrenia is compromised by the anatomical distribution and functionality of these receptors. Thus, while $A_{2A}R$ agonists targeting striatal receptors might be particularly effective against schizophrenia symptoms linked to dopaminergic hyperfunction and/or NMDA receptor hypofunction, $A_{2A}R$ antagonists targeting extrastriatal receptors might be useful as adjuvant treatment to ameliorate cognitive deficits in schizophrenia that

are resistant to conventional antipsychotics. Overall, the success of adenosine receptor-based drugs in the pharmacotherapy of schizophrenia will depend on the ability to engineer specific drugs able to discriminate between subpopulations of $A_{2A}R$ in different brain regions.

Acknowledgements This work was supported by grants SAF2011-24779, Consolider-Ingenio CSD2008-00005 and PCIN-2013-019-C03-03 from Ministerio de Economía y Competitividad and ICREA Academia-2010 from the Catalan Institution for Research and Advanced Studies to F.C. Also, the authors belong to the "Neuropharmacology and Pain" accredited research group (Generalitat de Catalunya, 2009 SGR 232).

References

1. Drury AN, Szent-Gyorgyi A. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. J Physiol. 1929;68:213–37.
2. Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. Nat Rev Drug Discov. 2006; 5:247–64.
3. Abbracchio MP, Burnstock G, Verkhratsky A, Zimmermann H. Purinergic signalling in the nervous system: an overview. Trends Neurosci. 2009;32:19–29.
4. Boison D. Modulators of nucleoside metabolism in the therapy of brain diseases. Curr Top Med Chem. 2011;11:1068–86.
5. Burnstock G. Purinergic nerves. Pharmacol Rev. 1972;24:509–81.
6. Burnstock G. Cotransmission. Curr Opin Pharmacol. 2004;4:47–52.
7. Zimmermann H. Biochemistry, localization and functional roles of ecto-nucleotidases in the nervous system. Prog Neurobiol. 1996;49:589–618.
8. Newby AC. Adenosine and the concept of "retaliatory metabolites". Trends Biotechnol. 1984;9:42–4.
9. Brown RA, Spina D, Page CP. Adenosine receptors and asthma. Br J Pharmacol. 2008;153(Suppl):S446–56.
10. Burnstock G, Fredholm BB, Verkhratsky A. Adenosine and ATP receptors in the brain. Curr Top Med Chem. 2011;11:973–1011.
11. Johnston-Cox HA, Ravid K. Adenosine and blood platelets. Purinergic Signal. 2011;7:357–65.
12. Barletta KE, Ley K, Mehrad B. Regulation of neutrophil function by adenosine. Arterioscler Thromb Vasc Biol. 2012;32:856–64.
13. Ernst PB, Garrison JC, Thompson LF. Much ado about adenosine: adenosine synthesis and function in regulatory T cell biology. J Immunol. 2010;185:1993–8.
14. Burnstock G. Purinergic regulation of vascular tone and remodelling. Auton Autocoid Pharmacol. 2009;29:63–72.
15. Vallon V, Mühlbauer B, Osswald H. Adenosine and kidney function. Physiol Rev. 2006;86:901–40.
16. Fredholm BB, Sollevi A. Cardiovascular effects of adenosine. Clin Physiol. 1986;6:1–21.
17. Sebastião AM, Ribeiro JA. Tuning and fine-tuning of synapses with adenosine. Curr Neuropharmacol. 2009;7:180–94.
18. Fredholm BB. Adenosine and lipolysis. Int J Obes. 1981;5:643–9.
19. Sebastiao AM, Ribeiro JA. Fine-tuning neuromodulation by adenosine. Trends Pharmacol Sci. 2000;21:341–6.
20. Latini S, Pedata F. Adenosine in the central nervous system: release mechanisms and extracellular concentrations. J Neurochem. 2001;79:463–84.
21. Snyder SH. Adenosine as a neuromodulator. Annu Rev Neurosci. 1985;8:103–24.
22. Ferre S, Fuxe K. Adenosine as a volume transmission signal. A feedback detector of neuronal activation. Prog Brain Res. 2000;125:353–61.
23. Fredholm BB. Purinoceptors in the nervous system. Pharmacol Toxicol. 1995;76:228–39.
24. Pull I, McIlwain H. Adenine derivatives as neurohumoral agents in the brain. The quantities liberated on excitation of superfused cerebral tissues. Biochem J. 1972;130:975–81.
25. Sattin A, Rall TW. The effect of adenosine and adenine nucleotides on the cyclic adenosine 3′, 5'-phosphate content of guinea pig cerebral cortex slices. Mol Pharmacol. 1970;6:13–23.
26. Degubareff T, Sleator Jr W. Effects of caffeine on mammalian atrial muscle, and its interaction with adenosine and calcium. J Pharmacol Exp Ther. 1965;148:202–14.
27. Trost T, Stock K. Effects of adenosine derivatives on cAMP accumulation and lipolysis in rat adipocytes and on adenylate cyclase in adipocyte plasma membranes. Naunyn Schmiedebergs Arch Pharmacol. 1977;299:33–40.
28. Londos C, Cooper DM, Wolff J. Subclasses of external adenosine receptors. Proc Natl Acad Sci U S A. 1980;77:2551–4.
29. van Calker D, Muller M, Hamprecht B. Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. J Neurochem. 1979;33:999–1005.
30. Olah ME, Stiles GL. Adenosine receptor subtypes: characterization and therapeutic regulation. Annu Rev Pharmacol Toxicol. 1995;35:581–606.
31. Murray RD, Churchill PC. Effects of adenosine receptor agonists in the isolated, perfused rat kidney. Am J Phys. 1984;247:H343–8.
32. Anderson R, Sheehan MJ, Strong P. Characterization of the adenosine receptors mediating hypothermia in the conscious mouse. Br J Pharmacol. 1994;113:1386–90.
33. Yamamoto S, Nakanishi O, Matsui T, Shinohara N, Kinoshita H, Lambert C, et al. Intrathecal adenosine A1 receptor agonist attenuates hyperalgesia without inhibiting spinal glutamate release in the rat. Cell Mol Neurobiol. 2003;23:175–85.

34. De Lorenzo S, Veggetti M, Muchnik S, Losavio A. Presynaptic inhibition of spontaneous acetylcholine release induced by adenosine at the mouse neuromuscular junction. Br J Pharmacol. 2004;142:113–24.
35. Scholz KP, Miller RJ. Inhibition of quantal transmitter release in the absence of calcium influx by a G protein-linked adenosine receptor at hippocampal synapses. Neuron. 1992;8:1139–50.
36. Schnurr M, Toy T, Shin A, Hartmann G, Rothenfusser S, Soellner J, et al. Role of adenosine receptors in regulating chemotaxis and cytokine production of plasmacytoid dendritic cells. Blood. 2004;103:1391–7.
37. MacGregor DG, Miller WJ, Stone TW. Mediation of the neuroprotective action of R-phenylisopropyladenosine through a centrally located adenosine A1 receptor. Br J Pharmacol. 1993;110:470–6.
38. Varani K, Portaluppi F, Gessi S, Merighi S, Ongini E, Belardinelli L, et al. Dose and time effects of caffeine intake on human platelet adenosine A(2A) receptors: functional and biochemical aspects. Circulation. 2000;102:285–9.
39. Carroll MA, Doumad AB, Li J, Cheng MK, Falck JR, McGiff JC. Adenosine2A receptor vasodilation of rat preglomerular microvessels is mediated by EETs that activate the cAMP/PKA pathway. Am J Physiol Ren Physiol. 2006;291:F155–61.
40. Popoli P, Betto P, Reggio R, Ricciarello G. Adenosine A2A receptor stimulation enhances striatal extracellular glutamate levels in rats. Eur J Pharmacol. 1995;287:215–7.
41. Nagel J, Schladebach H, Koch M, Schwienbacher I, Müller CE, Hauber W. Effects of an adenosine A2A receptor blockade in the nucleus accumbens on locomotion, feeding, and prepulse inhibition in rats. Synapse. 2003;49:279–86.
42. Scammell TE, Gerashchenko DY, Mochizuki T, McCarthy MT, Estabrooke IV, Sears CA, et al. An adenosine A2a agonist increases sleep and induces Fos in ventrolateral preoptic neurons. Neuroscience. 2001;107:653–63.
43. Kemp BK, Cocks TM. Adenosine mediates relaxation of human small resistance-like coronary arteries via A2B receptors. Br J Pharmacol. 1999;126:1796–800.
44. Donoso MV, López R, Miranda R, Briones R, Huidobro-Toro JP. A2B adenosine receptor mediates human chorionic vasoconstriction and signals through arachidonic acid cascade. Am J Physiol Heart Circ Physiol. 2005;288:H2439–49.
45. Zhong H, Belardinelli L, Maa T, Feoktistov I, Biaggioni I, Zeng D. A(2B) adenosine receptors increase cytokine release by bronchial smooth muscle cells. Am J Respir Cell Mol Biol. 2004;30:118–25.
46. Dubey RK, Gillespie DG, Mi Z, Jackson EK. Adenosine inhibits PDGF-induced growth of human glomerular mesangial cells via A(2B) receptors. Hypertension. 2005;46:628–34.
47. Zhong H, Shlykov SG, Molina JG, Sanborn BM, Jacobson MA, Tilley SL, et al. Activation of murine lung mast cells by the adenosine A3 receptor. J Immunol. 2003;171:338–45.
48. Das S, Cordis GA, Maulik N, Das DK. Pharmacological preconditioning with resveratrol: role of CREB-dependent Bcl-2 signaling via adenosine A3 receptor activation. Am J Physiol Heart Circ Physiol. 2005;288:H328–35.
49. Hinschen AK, Rose'Meyer RB, Headrick JP. Adenosine receptor subtypes mediating coronary vasodilation in rat hearts. J Cardiovasc Pharmacol. 2003;41:73–80.
50. Avila MY, Stone RA, Civan MM. Knockout of A3 adenosine receptors reduces mouse intraocular pressure. Invest Ophthalmol Vis Sci. 2002;43:3021–6.
51. Stella L, de Novellis V, Marabese I, Berrino L, Maione S, Filippelli A, et al. The role of A3 adenosine receptors in central regulation of arterial blood pressure. Br J Pharmacol. 1998;125:437–40.
52. Kolakowski Jr LF. GCRDb: a G-protein-coupled receptor database. Recept Channels. 1994;2:1–7.
53. Fritze O, Filipek S, Kuksa V, Palczewski K, Hofmann KP, Ernst OP. Role of the conserved NPxxY(x)5,6F motif in the rhodopsin ground state and during activation. Proc Natl Acad Sci U S A. 2003;100:2290–5.
54. Rovati GE, Capra V, Neubig RR. The highly conserved DRY motif of class A G protein-coupled receptors: beyond the ground state. Mol Pharmacol. 2007;71:959–64.
55. Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci. 2001;24:31–55.
56. Palmer TM, Stiles GL. Adenosine receptors. Neuropharmacology. 1995;34:683–94.
57. Rosin DL, Hettinger BD, Lee A, Linden J. Anatomy of adenosine A2A receptors in brain: morphological substrates for integration of striatal function. Neurology. 2003;61:S12–8.
58. Marala RB, Mustafa SJ. Direct evidence for the coupling of A2-adenosine receptor to stimulatory guanine nucleotide-binding-protein in bovine brain striatum. J Pharmacol Exp Ther. 1993;266:294–300.
59. Ferre S, Karcz-Kubicha M, Hope BT, Popoli P, Burgueno J, Gutierrez MA, et al. Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. Proc Natl Acad Sci U S A. 2002;99:11940–5.
60. Feoktistov I, Biaggioni I. Adenosine A2B receptors. Pharmacol Rev. 1997;49:381–402.
61. Patel V. Universal health coverage for schizophrenia: a global mental health priority. Schizophr Bull. 2016;42:885–90.
62. Haahr U, Friis S, Larsen TK, Melle I, Johannessen JO, Opjordsmoen S, et al. First-episode psychosis: diagnostic stability over one and two years. Psychopathology. 2008;41:322–9.
63. Bromet EJ, Kotov R, Fochtmann LJ, Carlson GA, Tanenberg-Karant M, Ruggero C, et al. Diagnostic shifts during the decade following first admission for psychosis. Am J Psychiatry. 2011;168:1186–94.
64. Tandon R, Gaebel W, Barch DM, Bustillo J, Gur RE, Heckers S, et al. Definition and description of schizophrenia in the DSM-5. Schizophr Res. 2013;150:3–10.

65. Laruelle M, Kegeles LS, Abi-Dargham A. Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. Ann N Y Acad Sci. 2003;1003:138–58.
66. Javitt DC. Glutamatergic theories of schizophrenia. Isr J Psychiatry Relat Sci. 2010;47:4–16.
67. Carlsson A. The current status of the dopamine hypothesis of schizophrenia. Neuropsychopharmacology. 1988;1:179–86.
68. Leucht S, Heres S, Kissling W, Davis JM. Evidence-based pharmacotherapy of schizophrenia. Int J Neuropsychopharmacol. 2011;14:269–84.
69. Citrome L. Unmet needs in the treatment of schizophrenia: new targets to help different symptom domains. J Clin Psychiatry. 2014;74(Suppl 2):21–6.
70. Ferre S, Agnati LF, Ciruela F, Lluis C, Woods AS, Fuxe K, et al. Neurotransmitter receptor heteromers and their integrative role in "local modules": the striatal spine module. Brain Res Rev. 2007;55:55–67.
71. Fuxe K, Ferre S, Genedani S, Franco R, Agnati LF. Adenosine receptor-dopamine receptor interactions in the basal ganglia and their relevance for brain function. Physiol Behav. 2007;92:210–7.
72. Ferré S. Adenosine-dopamine interactions in the ventral striatum. Implications for the treatment of schizophrenia. Psychopharmacology. 1997;133:107–20.
73. Ferre S, Ciruela F, Quiroz C, Lujan R, Popoli P, Cunha RA, et al. Adenosine receptor heteromers and their integrative role in striatal function. ScientificWorldJournal. 2007;7:74–85.
74. Ferre S, Borycz J, Goldberg SR, Hope BT, Morales M, Lluis C, et al. Role of adenosine in the control of homosynaptic plasticity in striatal excitatory synapses. J Integr Neurosci. 2005;4:445–64.
75. Ciruela F, Casado V, Rodrigues RJ, Lujan R, Burgueno J, Canals M, et al. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J Neurosci. 2006;26:2080–7.
76. Lara DR, Souza DO. Schizophrenia: a purinergic hypothesis. Med Hypotheses. 2000;54:157–66.
77. Lara DR, Dall'Igna OP, Ghisolfi ES, Brunstein MG. Involvement of adenosine in the neurobiology of schizophrenia and its therapeutic implications. Prog Neuro-Psychopharmacol Biol Psychiatry. 2006;30:617–29.
78. Moscoso-Castro M, Gracia-Rubio I, Ciruela F, Valverde O. Genetic blockade of adenosine A2A receptors induces cognitive impairments and anatomical changes related to psychotic symptoms in mice. Eur Neuropsychopharmacol. 2016;26:1227–40.
79. Boison D, Singer P, Shen H-Y, Feldon J, Yee BK. Adenosine hypothesis of schizophrenia — opportunities for pharmacotherapy. Neuropharmacology. 2012;62:1527–43.
80. Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R. Study of a new schizophrenomimetic drug; sernyl. AMA Arch Neurol Psychiatry. 1959;81:363–9.
81. Lodge D, Anis NA. Effects of phencyclidine on excitatory amino acid activation of spinal interneurones in the cat. Eur J Pharmacol. 1982;77:203–4.
82. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci. 1997;17:2921–7.
83. Olney JW, Sharpe LG. Brain lesions in an infant rhesus monkey treated with monsodium glutamate. Science. 1969;166:386–8.
84. Adams B, Moghaddam B. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. J Neurosci. 1998;18:5545–54.
85. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vöckler J, Dikranian K, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science. 1999;283:70–4.
86. Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA. NMDA antagonist neurotoxicity: mechanism and prevention. Science. 1991;254:1515–8.
87. Weinberger DR. On the plausibility of "the neurodevelopmental hypothesis" of schizophrenia. Neuropsychopharmacology. 1996;14:1S–11S.
88. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry. 1991;148:1301–8.
89. Kalinichev M, Robbins MJ, Hartfield EM, Maycox PR, Moore SH, Savage KM, et al. Comparison between intraperitoneal and subcutaneous phencyclidine administration in Sprague-Dawley rats: a locomotor activity and gene induction study. Prog Neuro-Psychopharmacol Biol Psychiatry. 2008;32:414–22.
90. Sams-Dodd F. Distinct effects of d-amphetamine and phencyclidine on the social behaviour of rats. Behav Pharmacol. 1995;6:55–65.
91. Egerton A, Reid L, McKerchar CE, Morris BJ, Pratt JA. Impairment in perceptual attentional set-shifting following PCP administration: a rodent model of set-shifting deficits in schizophrenia. Psychopharmacology. 2005;179:77–84.
92. Mansbach RS, Geyer MA. Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. Neuropsychopharmacology. 1989;2:299–308.
93. Mouri A, Noda Y, Enomoto T, Nabeshima T. Phencyclidine animal models of schizophrenia: approaches from abnormality of glutamatergic neurotransmission and neurodevelopment. Neurochem Int. 2007;51:173–84.
94. Rainey JM, Crowder MK. Prolonged psychosis attributed to phencyclidine: report of three cases. Am J Psychiatry. 1975;132:1076–8.
95. Allen RM, Young SJ. Phencyclidine-induced psychosis. Am J Psychiatry. 1978;135:1081–4.
96. Gotoh L, Kawanami N, Nakahara T, Hondo H, Motomura K, Ohta E, et al. Effects of the adenosine A(1) receptor agonist N(6)-cyclopentyladenosine on phencyclidine-induced behavior and expression of the immediate-early genes in the discrete brain regions of rats. Brain Res Mol Brain Res. 2002;100:1–12.

97. Rimondini R, Ferre S, Ogren SO, Fuxe K. Adenosine A2A agonists: a potential new type of atypical antipsychotic. Neuropsychopharmacology. 1997;17:82–91.
98. Malec D, Poleszak E. Involvement of adenosine receptors in dizocilpine-induced motor activity in mice. Pharmacol Rep. 2006;58:101–6.
99. Shen H-Y, Coelho JE, Ohtsuka N, Canas PM, Day Y-J, Huang Q-Y, et al. A critical role of the adenosine A2A receptor in extrastriatal neurons in modulating psychomotor activity as revealed by opposite phenotypes of striatum and forebrain A2A receptor knock-outs. J Neurosci. 2008;28:2970–5.
100. Howes OD, Kapur S. The dopamine hypothesis of schizophrenia: version III — the final common pathway. Schizophr Bull. 2009;35:549–62.
101. Edeleanu L. Uber einige Derivate der Phenylmethacrylsäure und der Phenylisobuttersäure. Ber Dtsch Chem Ges. 1887;20:616.
102. Heal DJ, Smith SL, Gosden J, Nutt DJ. Amphetamine, past and present — a pharmacological and clinical perspective. J Psychopharmacol. 2013;27:479–96.
103. Deller T, Sarter M. Effects of repeated administration of amphetamine on behavioral vigilance: evidence for "sensitized" attentional impairments. Psychopharmacology. 1998;137:410–4.
104. Kondrad RL, Burk JA. Transient disruption of attentional performance following escalating amphetamine administration in rats. Psychopharmacology. 2004;175:436–42.
105. Castner SA, Vosler PS, Goldman-Rakic PS. Amphetamine sensitization impairs cognition and reduces dopamine turnover in primate prefrontal cortex. Biol Psychiatry. 2005;57:743–51.
106. Kolb B, Gorny G, Li Y, Samaha A-N, Robinson TE. Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens. Proc Natl Acad Sci U S A. 2003;100:10523–8.
107. Selemon LD, Begović A, Goldman-Rakic PS, Castner SA. Amphetamine sensitization alters dendritic morphology in prefrontal cortical pyramidal neurons in the non-human primate. Neuropsychopharmacology. 2007;32:919–31.
108. Wolf ME, Mangiavacchi S, Sun X. Mechanisms by which dopamine receptors may influence synaptic plasticity. Ann N Y Acad Sci. 2003;1003:241–9.
109. Durieux PF, Schiffmann SN, de Kerchove d'Exaerde A. Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. EMBO J. 2012;31:640–53.
110. Seeman P. Dopamine D2 receptors as treatment targets in schizophrenia. Clin Schizophr Relat Psychoses. 2010;4:56–73.
111. Ferre S, Fredholm BB, Morelli M, Popoli P, Fuxe K. Adenosine–dopamine receptor–receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci. 1997;20:482–7.
112. Ferre S, Ciruela F, Canals M, Marcellino D, Burgueno J, Casado V, et al. Adenosine A2A–dopamine D2 receptor–receptor heteromers. Targets for neuro-psychiatric disorders. Parkinsonism Relat Disord. 2004;10:265–71.
113. Shen H-Y, Singer P, Lytle N, Wei CJ, Lan J-Q, Williams-Karnesky RL, et al. Adenosine augmentation ameliorates psychotic and cognitive endophenotypes of schizophrenia. J Clin Invest. 2012;122:2567–77.
114. Kurumaji A, Toru M. An increase in [3H] CGS21680 binding in the striatum of postmortem brains of chronic schizophrenics. Brain Res. 1998;808:320–3.
115. Deckert J, Brenner M, Durany N, Zöchling R, Paulus W, Ransmayr G, et al. Up-regulation of striatal adenosine A(2A) receptors in schizophrenia. Neuroreport. 2003;14:313–6.
116. Hwang Y, Kim J, Shin JY, Kim JI, Seo JS, Webster MJ, et al. Gene expression profiling by mRNA sequencing reveals increased expression of immune/inflammation-related genes in the hippocampus of individuals with schizophrenia. Transl Psychiatry. 2013;3:e321.
117. Villar-Menéndez I, Díaz-Sánchez S, Blanch M, Albasanz JL, Pereira-Veiga T, Monje A, et al. Reduced striatal adenosine A2A receptor levels define a molecular subgroup in schizophrenia. J Psychiatr Res. 2014;51:49–59.
118. Hong C-J, Liu H-C, Liu T-Y, Liao D-L, Tsai S-J. Association studies of the adenosine A2a receptor (1976T>C) genetic polymorphism in Parkinson's disease and schizophrenia. J Neural Transm. 2005;112:1503–10.
119. Gotoh L, Mitsuyasu H, Kobayashi Y, Oribe N, Takata A, Ninomiya H, et al. Association analysis of adenosine A1 receptor gene (ADORA1) polymorphisms with schizophrenia in a Japanese population. Psychiatr Genet. 2009;19:328–35.
120. Dutra GP, Ottoni GL, Lara DR, Bogo MR. Lower frequency of the low activity adenosine deaminase allelic variant (ADA1*2) in schizophrenic patients. Rev Bras Psiquiatr. 2010;32:275–8.
121. Akhondzadeh S, Safarcherati A, Amini H. Beneficial antipsychotic effects of allopurinol as add-on therapy for schizophrenia: a double blind, randomized and placebo controlled trial. Prog Neuro-Psychopharmacol Biol Psychiatry. 2005;29:253–9.
122. Brunstein MG, Ghisolfi ES, Ramos FLP, Lara DR. A clinical trial of adjuvant allopurinol therapy for moderately refractory schizophrenia. J Clin Psychiatry. 2005;66:213–9.
123. Dickerson FB, Stallings CR, Origoni AE, Sullens A, Khushalani S, Sandson N, et al. A double-blind trial of adjunctive allopurinol for schizophrenia. Schizophr Res. 2009;109:66–9.
124. Linden N, Onwuanibe A, Sandson N. Rapid resolution of psychotic symptoms in a patient with schizophrenia using allopurinol as an adjuvant: a case report. Clin Schizophr Relat Psychoses. 2014;7:231–4.

125. Weiser M, Gershon AA, Rubinstein K, Petcu C, Ladea M, Sima D, et al. A randomized controlled trial of allopurinol vs. placebo added on to antipsychotics in patients with schizophrenia or schizoaffective disorder. Schizophr Res. 2012;138:35–8.
126. Hirota T, Kishi T. Adenosine hypothesis in schizophrenia and bipolar disorder: a systematic review and meta-analysis of randomized controlled trial of adjuvant purinergic modulators. Schizophr Res. 2013;149:88–95.
127. Buie LW, Oertel MD, Cala SO. Allopurinol as adjuvant therapy in poorly responsive or treatment refractory schizophrenia. Ann Pharmacother. 2006;40:2200–4.
128. Akhondzadeh S, Shasavand E, Jamilian H, Shabestari O, Kamalipour A. Dipyridamole in the treatment of schizophrenia: adenosine-dopamine receptor interactions. J Clin Pharm Ther. 2000;25:131–7.
129. Machado-Vieira R, Soares JC, Lara DR, Luckenbaugh DA, Busnello JV, Marca G, et al. A double-blind, randomized, placebo-controlled 4-week study on the efficacy and safety of the purinergic agents allopurinol and dipyridamole adjunctive to lithium in acute bipolar mania. J Clin Psychiatry. 2008;69:1237–45.
130. Svenningsson P, Le Moine C, Fisone G, Fredholm BB. Distribution, biochemistry and function of striatal adenosine A2A receptors. Prog Neurobiol. 1999;59:355–96.
131. Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. Adenosine and brain function. Int Rev Neurobiol. 2005;63:191–270.
132. Gomes CV, Kaster MP, Tomé AR, Agostinho PM, Cunha RA. Adenosine receptors and brain diseases: neuroprotection and neurodegeneration. Biochim Biophys Acta. 1808;2011:1380–99.
133. Kaiser S, Quinn R. Adenosine receptors as potential therapeutic targets. Drug Discov Today. 1999;4:542–51.

Neuroimaging in Chronic Pain, Fibromyalgia, and Somatization

30

Nicolás Fayed Miguel, Javier Garcia-Campayo, Eduardo González-Toledo, and Laura Viguera

Abstract

Neuroimaging research in psychiatry has been increasing exponentially in recent years, yet many psychiatrists are relatively unfamiliar with this field. The neuroimaging findings summarized here include alterations related to fibromyalgia, chronic pain, and coping in somatoform pain disorders. Magnetic resonance imaging is the imaging method of choice for standard clinical sequences. Improvements in imaging technology now allow advanced sequences, once used exclusively for research, to be used clinically. Magnetic resonance spectroscopy (showing metabolism) offers invaluable information on living tissues, with a special contribution to the diagnosis and prognosis of diseases of the central nervous system. Voxel-based morphometry (structural information) is a recent technique that can simultaneously visualize group differences or statistical effects on gray and white matter throughout the brain. Perfusion (marker of vascularity) offers higher spatial resolution than radionuclide techniques such as positron emission tomography and single-photon emission computed tomography. Diffusion-weighted imaging (a marker of cellularity) detects subtle degradation of white matter microstructure in fibromyalgia. Diffusion tensor imaging shows integrity of surrounding white matter tracts. Functional magnetic resonance imaging is used to identify eloquent cortex.

N.F. Miguel, MD, PhD (✉)
Department of Neuroradiology, Quirónsalud Hospital, Zaragoza 50006, Spain
e-mail: nicola33fr@yahoo.es

J. Garcia-Campayo, PhD
Department of Psychiatry, Miguel Servet Hospital, University of Zaragoza, Zaragoza, Spain
e-mail: jgarcamp@gmail.com

E. González-Toledo, MD, PhD
Department of Radiology, Neurology, and Anesthesiology, Louisiana State University Health Sciences Center, Shreveport, LA, USA
e-mail: ecgtoledo@gmail.com

L. Viguera, MD
Department of Anesthesiology, Miguel Servet Hospital, Zaragoza, Spain
e-mail: lauraviguera@gmail.com

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_30

These techniques, all of which have advanced our understanding of chronic pain and can be used to improve knowledge on the etiology of these illnesses, will be discussed.

Keywords
Chronic pain • Fibromyalgia • Somatization • Magnetic resonance imaging • Magnetic resonance spectroscopy • Structural brain imaging • Perfusion • Diffusion weighted imaging • Diffusion tensor imaging • Functional magnetic resonance imaging • Treatment effects

Introduction

Chronic pain is a major public health problem. The prevalence of chronic pain in Western, industrialized countries is estimated to be between 15% and 20% of the adult population [1–3]. Therefore, there is a need to better understand the mechanisms that lead to chronic pain. From a neurobiological perspective, the mechanisms contributing to the transition from acute to subacute and chronic pain are heterogeneous, and are thought to occur both within the peripheral nervous system and at various levels of the central nervous system (CNS). The role of the brain in chronic pain states remains to be fully elucidated.

The World Health Organization classifies fibromyalgia syndrome (FMS) under the heading of diseases of the musculoskeletal system and connective tissue, as does the International Association for the Study of Pain [4].

Fibromyalgia (FM) is a chronic rheumatic disease characterized by the presence of diffuse musculoskeletal pain, painful sensitivity to touch in at least 11 of 18 defined trigger points, and a constellation of symptoms including fatigue, disturbed sleep, cognitive problems, and distress [5]. Furthermore, it is accepted as a central sensitivity syndrome. The American College of Rheumatology in 2010 described new diagnosis criteria [6]. The prevalence of this syndrome in Europe is approximately 2.9% [7], and the prevalence in rheumatology consultations in Spain was found to be 12% [8].

Pain is the most common and disabling symptom of FM. This pain is suspected to be caused by the altered function of structures in the CNS, including the primary and secondary sensory and motor cortices, insula, anterior cingulate cortex, thalamus, dorsolateral prefrontal cortex, and basal ganglia. If we look for analogies or parallels between these and other insults, we encounter the clinical characteristics of a number of neuropathic pain syndromes. There are similarities from the point of view of symptoms, diagnosis, and therapeutic approach. However, FMS has not demonstrated any injury or association with any known disease that affects the nervous system, and therefore one that could be considered the origin of a somatosensory disorder. Neuropathic pain is defined as a "pain caused by direct injury or disease affecting the somatosensory system" [9]. As in the case of FM, diagnosis of various neuropathic pain syndromes are performed based on clinical criteria.

Somatoform disorders (SFDs), according to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [10], are defined by the presence of physical symptoms that suggest a general medical condition but that are not fully explained by a general medical condition, by the direct effects of a substance, or by another mental disorder. The most extreme form of this group is somatization disorder, a chronic and polysymptomatic disorder characterized by at least four unexplained gastrointestinal, sexual, and pseudo neurological symptoms [10].

The psychological profiles show the usual psychological characteristics of patients with an FM or SFD: high scores in anxiety and depression assessed with the Hospital Anxiety Depression Scale; high scores on the Pain

Catastrophizing Scale and in pain assessed with the Pain Visual Analogue Scale, and low quality of life as measured by the EuroQol 5D. The Mini-Mental State Exam scores suggest symptoms of cognitive dysfunction in FM and SFDs, but at levels less severe than those found in patients with dementia [11].

Over the past decade, brain-imaging studies have shed light on neural correlates of pain perception and pain modulation, and they have also begun to disentangle the neural mechanisms that underlie chronic pain. There is now general agreement that the CNS plays a prominent role in many chronic pain states because of the "centralization" of pain. Some of the findings that strongly support this paradigm shift are outlined later.

The clinical diagnosis of FM does not exist today, given that there is no analytical testing, validated image, or specific pathological condition that serves to confirm the diagnosis. However, numerous publications describe neuroimaging abnormalities in this syndrome that affects the somatosensory system [12–14].

The quantitative study of anatomical and biochemical variables is important in the diagnosis, prognosis, and monitoring of different diseases [15]. The morphometry study of certain brain structures, complemented by the identification of variations of some metabolites by nuclear magnetic resonance spectroscopy, is a promising tool for the elucidation of the morphobiological component of different pathological conditions.

The study of low frequency signals in brain activity through blood oxygen level–dependent (BOLD) contrast at rest revealed synchronized cortical activity patterns, allowing the intrinsic functional architecture of the human brain to be described. The international scientific community has shared resources that will help with this analysis by means of functional magnetic resonance imaging (fMRI) at rest to obtain more accurate and advanced diagnoses in the field of neuroscience treatments.

This chapter seeks to review published scientific evidence and studies of structural and functional imaging in FMS that consider the similarities and dissimilarities between the findings in FMS and related findings in a number of neuropathic pain syndromes. The ultimate goal is to find evidence of valid indicators with which to objectify the diagnosis of these syndromes.

Magnetic Resonance Spectroscopy (MRS)

This technique enables us to study the chemical composition of living tissues, and is based on the chemical shift of atoms. The concentration of a number of metabolites is determined from spectra that may be acquired in several ways. Proton magnetic resonance spectroscopy (1H–MRS) is one of the techniques used to assess potential disruptions in neuronal integrity and associated neurochemical dysregulations. The most commonly used spectroscopy is that originating from a hydrogen nucleus (proton 1H–MRS). The position of the metabolite signal is identified on the horizontal axis by its chemical shift, scaled in units referred to as parts per million (ppm).

The brain spectrum shows peaks corresponding to the different metabolites: myo-inositol (mI), 3.56 and 4.06 ppm; choline compounds (Cho), 3.23 ppm; creatine (Cr), 3.03 and 3.94 ppm; N-acetylaspartate (NAA), 2.02; glutamine (Gln) and glutamate (Glu), 2.1–2.55 ppm and 3.8 ppm. NAA is considered a neuronal–axonal marker with a neuronal bioenergetic role [16–18] found in the brain and spinal cord. Recent studies, however, have indicated that NAA reflects functional rather than structural neuronal characteristics [19], suggesting that NAA is most informative in the investigation of functional abnormality. Cr is involved in energy metabolism through the Cr kinase reaction generating phosphocreatine, and in turn, adenosine triphosphate [20]. Cho containing compounds of glycerol 3-phosphocholine and phosphocholine, which are present at high levels in glial cells [21], are intermediaries in the synthesis of acetylcholine [22]. Glutamine and glutamate (Glx) are strongly compartmentalized (in neurons and in astrocytes respectively), and are directly connected to energy metabolism and neurotransmission [23].

Previous studies (see Table 30.1) described lower NAA levels within the dorsolateral prefrontal cortex of patients with chronic back pain when compared with healthy controls [24]. Lower NAA levels have also been reported in the thalamus of patients with neuropathic pain included [25, 26].

These decreases in NAA might possibly reflect a neuronal loss, indicating a neurodegenerative process to be associated with chronic pain. Hippocampal dysfunction in patients with FM has also been explored using 1H–MRS [27]. In this case control study, 15 patients and ten controls were examined, and levels and interhippocampal ratios of metabolites such as NAA, Cho, and Cr were assessed. Patients with FM had lower NAA levels than those found in controls, representing a neuronal or axonal metabolic dysfunction in the hippocampus. As the hippocampus plays a crucial role in the maintenance of cognitive functions, sleep regulation, and pain perception, the authors suggest that hippocampal metabolic dysfunction may be implicated in the symptomatology of this puzzling syndrome. Consequently, a decrease was found in the NAA/Cr ratio in the right hippocampus [28], decreased Cho and N-acetylaspartate + N-acetylaspartate glutamate (NAA + NAAG) in the left hippocampus, and also a decrease in both hippocampi of myo-inositol (mI), glutamate (Glu), the Cho/Cr ratio, and the mI/Cr ratio [11, 29]. On the other hand, an increase in NAA was found in both

Table 30.1 Metabolic characteristics and anatomical areas in patients with fibromyalgia syndrome (FMS). Magnetic resonance spectroscopy (MRS)

Author	Caud N.	Amyg	Ínsula	ACC	PCC	VLPFC	Hp	DLPFC
Grachev	–	–	–	–	–	–	–	⇓ NAA
Petrou	⇑Cho/Cr R + L	–	–		–	⇑Cho/Cr R	–	–
Wood	–	–	–	–	–	–	⇓ NAA/Cr R	–
Harris	–	–	⇑ Glu post R	–	–	–	–	–
Fayed	–	–	–	–	⇑ Glx Glx/Cr	–	⇓ mI mI/Cr R + L ⇓ Cho L	–
Feraco	–	–	–	–	–	⇑ Glu/Cr Glx/Cr	–	–
Emad	–	–	–	–	–	–	⇓ NAA R + L ⇑ Cho R	–
Foerster	–	–	⇓ GABA ant R	⇑ GABA	–	–	–	–
Valdés	–	⇑ Glx	–	–	–	–	–	–
Fayed	–	–	⇓tNAA /Cr and NAA/Cr post	–	⇑Glx ⇓ Cho	–	⇓Glu ⇓ Cho/Cr ⇓mI and mI/Cr ⇓tNAA L	–

Caud N. caudate nucleus, *Amyg* amygdala, *Ins* ínsula, *ACC* anterior cingulate cortex, P*CC* posterior cingulate cortex, *VLPFC* ventrolateral prefrontal cortex, *Hp* Hippocampus, *DLPFC* dorsolateral prefrontal cortex, *Cho* choline, *Cr* creatine, *tNAA* N-acetylaspartate + N-acetylaspartate-glutamate, *NAA* N-acetylaspartate, *Glu* glutamate, *Glx* glutamate + glutamine, *mI* myo-inositol, *Ant* anterior, *Post* posterior, *R* right, *L* left

sides. Cho was increased on the right [27] and decreased on the left [29]. Our study confirms a significant reduction in Cho (both hippocampi / posterior cingulate cortex), mI (left hippocampus), NAA (left hippocampus / posterior insula), and Glu (left hippocampus) in both FMS and SFD groups compared with controls. Indeed, neurochemical changes that could be indicative of such damage have been reported previously [29–31].

These studies report a decrease in NAA in patients with chronic pain in the DLPFC and the thalamus respectively, two areas also involved in pain processing and perception. They attribute this loss to a neurodegenerative process present in chronic pain. Lower hippocampal and insular NAA levels suggest neuronal or axonal metabolic dysfunction, or a combination of these processes. We suggest that hippocampal dysfunction may be partly responsible for some of the phenomena associated with FM and somatization disorder. Blocking N-methyl-D-aspartate receptors (NMDAR) in the hippocampal formation reduces nociceptive behaviors; this reduction, in turn, supports the hypothesis that the hippocampal formation is involved in the pain-related neural processing and the expression of pain-related behaviors [32]. The default mode network (DMN) comprises a set of brain regions that are coactivated during passive task states, show an intrinsic functional relationship, and are connected via direct and indirect anatomic projections. In a previous study, we found elevated levels of Glu in the ventral posterior cingulate cortex, a key zone in the default mode network hypothesis [29]. We propose that high levels of Glu in certain regions of the brain [29] cause cellular damage and disruptions in circuits involved in the pain perception. This may be underlying the cognitive and behavioral impairments accompanying chronic pain. The chronic pain condition could cause a sustained lesion in the brain through Glu toxicity, and could explain the structural damage and significant atrophy seen in chronic pain patients.

Another remarkable fact is the correlation of all metabolites in the left hippocampus with pain and the correlation of choline in the posterior cingulate cortex with all psychological tests. In this sense, higher Cho levels and lower NAA/Cho ratios in both hippocampi have been reported in patients with FM [27]. The finding of metabolic brain differences between patients with FM and healthy controls in neural structures such as the hippocampus and amygdala (both of which pertain to the limbic system and are involved in fear, avoidance, and emotional responses experienced during pain) is compatible with a possible augmented emotional processing in patients with FM, in line with the augmented pain processing proposed by some authors [33].

Previous fMRI studies of FM have observed that augmented neural activity is due to an elevation in Glu levels, which leads to neuronal hyperexcitability. There is an increase in Glu in the amygdala [34], the posterior cingulate cortex [29], and the right posterior insula [35]. The degree of Glu elevation was associated with evoked pain sensitivity, suggesting that glutamatergic activity in this region of the brain might be partly responsible for the "gain" setting on central neural pain processing [35].

There is a study analyzing the neurotransmitter gamma aminobutyric acid (GABA) which describes a decrease in right anterior insula and an increase by the same amount in the anterior cingulate cortex [14]. Other studies report metabolic abnormalities in areas not studied by morphometry as an increased Cho/Cr ratio in the left and right caudate nuclei and right ventrolateral prefrontal cortex [36], and an increase in the Glu/Cr and Gln + Glu/Cr ratios [37].

A recent study has investigated the relationship between Glx- and GABA-derived spectroscopy values within the posterior cingulate, and the connectivity of this structure to the rest of the default mode network (DMN) [38]. The authors found that individuals with greater concentrations of Glu + Gln (Glx) and lower concentrations of GABA within the posterior cingulate have stronger connectivity values with other default mode network (DMN) regions.

One approach that might be particularly informative in FM would be to explore the association between insula connectivity and Glx/GABA levels in the same patient cohort. Alternatively, Glx

and GABA concentrations in the insula may also influence functional connectivity between other brain regions and networks, as the insula has widely distributed excitatory and inhibitory connections throughout the brain [39].

Lower GABA levels within the posterior insula were associated with greater sensitivity to experimental pain. These findings suggested that lower insular GABA may also play a role in pain, namely neuronal disinhibition. In neuropathic pain syndromes, studies on metabolism spectroscopy reflect a decrease in NAA in the thalamus [26], a decrease in GABA and GABA/Cr in right reticular nucleus, and NAA and NAA/Cr in the lateral ventral posterior nucleus [40] and in the posteromedial and posterolateral nucleus, and NAA/Cr as well as the Cho/Cr combination in the intralaminar nuclei [41]. In chronic pain syndromes, metabolic changes occur in various brain regions. For example, diabetic neuropathy shows decreased NAA in the thalamus, normal levels in the anterior cingulate, and a decrease in Cr in the dorsolateral prefrontal cortex (DLPFC); while temporomandibular joint dysfunction (TMJ) shows increased NAA and Cho in the posterior insula, increased Gln in the right insula, and decreased Gln in the left insula [42]. Our group observed a decrease in NAA and increased Glu/Cr in the posterior cingulate of chronic pain patients compared to patients without pain [11, 29].

The quantification of both brain metabolites and neurotransmitters is of great interest, as it can provide indirect evidence of local neural activity and/or excitability, and may even be a predictor for therapy response. Furthermore, whether altered neurotransmitter concentrations in patients with chronic pain reflect a global (whole brain) or region-specific phenomenon (e.g., confined to regions in the pain system) needs to be investigated in greater depth.

Recent studies show a significantly higher Glu/Gln ratio in the occipital cortex of migraine patients compared with healthy control subjects, and higher Glu levels and Glu/Cr + phosphocreatine ratios in the anterior paracingulate cortex. This situation could arise from a neuronal–glial coupling of glutamatergic metabolism differences or an increased neuron/astrocyte ratio [43]. Although these data may reflect a state of neuronal hyperexcitability, they may also be associated with a nonspecific pain process. Moreover, altered glutamatergic neurotransmission seems to mediate the relationship between abnormal cortical information processing and excitability in migraine patients [44, 45].

In a study of patients with tinnitus, the patients showed higher concentrations of Glu and NAA in the auditory cortical areas, most notably in Heschl's gyrus [46]. Another study that used MRS before, during, and after experimentally induced dental pain showed a significant absolute increase in Glu, Gln, and the Glu/Gln ratio in the insular cortex [47].

Our group has observed an increase in Glx in the posterior cingulate cortex in FM and, to a lesser extent, in somatization disorder compared with controls and levels of Glx correlates with pain-catastrophizing [11]. Our data suggest that Glx plays a role in this augmented pain processing in those individuals who have elevated Glx levels. Because higher Glx levels have been associated with an elevation in the pain catastrophizing syndrome (PCS), it is likely that Glx in the posterior cingulate is related to pain processing. We have hypothesized that increases in brain excitatory neurotransmitters could result in neuronal hyperexcitability. As part of its neurotransmitter role, Glx is an excitatory amino acid, and excessive Glx neurotransmission has been implicated in excitotoxic neuronal damage [48].

Structural Brain Imaging

Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [4]. Pain is therefore a multidimensional phenomenon that is heavily influenced by biopsychosocial factors.

In the field of structural imaging, new approaches such as T1 and T2* mapping, as well as magnetization transfer ratios, which are often acquired during the same scanning session (multiparametric approach), will be of clinical importance by unraveling new aspects of altered

microstructure in chronic pain conditions, and of methodological importance by providing a more detailed understanding of voxel-based morphometry findings [49].

Several brain structures are involved in the perception and experience of pain, such as the somatosensory cortex (primary and secondary), the cingulate gyrus (anterior), insula, thalamus, posterior parietal cortex, and prefrontal cortex. Studies show changes in the volume of brain areas involved in a "network of pain" in both FMS and neuropathic pain syndromes, and even in other forms of chronic pain. It has been suggested that prolonged nociceptive input to the brain might induce functional and morphologic maladaptive processes, which in turn further exacerbate the experience of chronic pain. Alternatively, morphologic changes might predispose toward vulnerability to develop a chronic pain state [50] (See Table 30.2). Interpretation of findings from morphometric studies must also take into account genetic and experiential factors that have recently been demonstrated to influence brain morphometry and the risk of developing chronic pain [51].

Reductions have been observed in the volume of gray matter in areas associated with pain: a level of emotional processing (anterior cingulate cortex and insula), cognitive (prefrontal cortex) and sensory (primary and secondary somatosensory cortex) processing, and the processing of stress (parahippocampal gyrus) [52].

Significant gray matter reduction has been found in the prefrontal cortex, anterior cingulate cortex, and insular cortex of FM patients [53]. These regions are known to be critically involved in the modulation of subjective pain experiences. The duration of pain or functional pain disability did not correlate with gray matter volumes. A trend of inverse correlation of gray matter volume reduction in the anterior cingulate cortex was detected with the duration of pain medication intake [54]. Furthermore, reductions in gray matter volume were seen in the postcentral gyri, amygdala, hippocampi, superior frontal gyri, and anterior cingulate gyri [55]. Other authors [56] found those patients with FM had significantly less total gray matter volume and an age-associated decrease in gray matter 3.3 times greater than that of healthy controls. Greater gray matter loss was seen in patients with a longer duration of the disorder, with each year of FM being equivalent to 9.5 times the loss in normal aging. In addition, patients with FM demonstrated significantly lower gray matter density than healthy controls in brain regions such as the cingulate, the insular and medial frontal cortices, and the parahippocampal gyri. In summary, FM appears to be associated with an acceleration of age-related changes in the brain in regions that are functionally linked to core features of the disorder, including affective disturbances and chronic widespread pain. However, these results require replication because the sample was somewhat small ($N = 10$), and matching between controls and patients with FM was not optimal.

Changes in volume have been found in the hippocampus and insula in posttraumatic stress disorder [57, 58], in the thalamus and the prefrontal cortex in back pain [59], and also bilaterally in the prefrontal cortex in chronic fatigue syndrome [60], with global changes in this volume [61]. One possible explanation for these changes may be atrophy secondary to chronic inflammation mediated by cytokines [59].

The thalamus plays a crucial role in the sensory–discriminative pain component. Both morphometry and diffusion tensor showed a volume decrease in different thalamic nuclei [55, 62]. In neuropathic pain syndromes (See Table 30.3), a decrease was also observed in the volume of the regions involved in the perception and processing of pain, and the thalamus [63, 64], the cortex of the region anterior cingulate gyrus [63, 65], and the insula [63].

The cingulate gyrus is part of the limbic system, and numerous neuroimaging studies demonstrate the involvement of the anterior cingulate region. Activation of this region is part of the emotional and cognitive component of pain [66, 67]. Both this region and the prefrontal cortex are involved in pain modulation (inhibition and facilitation).

Structural changes in these systems could contribute to the maintenance of pain and chronicity of symptoms, both in FMS and some

Table 30.2 Structural characteristics and anatomical areas in patients with fibromyalgia syndrome (FMS). Voxel-based morphometry (VBM) and diffusion tensor imaging (DTI)

Author	Image technique	Thal	Stri	Amyg	Cereb	Íns	TSG	ACC	PCC	Hp	PFC	OFG
Cagnie	VBM					⇓		⇓			⇓	
Valet						⇓		⇓			⇓	
Burgmer	VBM	–	–	⇓ L	–	–	–	⇓ R	–	–	–	–
Schmidt-Wilcke	VBM and DTI	⇓ L	⇑ R + L	–	⇑ L	–	⇓ R	–	–	–	–	⇑ L
Lutz	VBM and DTI	⇓	–	⇑	–	⇓	–	⇑	–	⇑	⇑	–
Wood	VBM	–	–	–	–	–	–	⇓ L	⇓ R	⇓ parahippocampal gyrus R + L	–	–
Kuchinad	VBM	–	–	–	–	⇓ L	–	–	⇓ girus R + L	⇓ parahippocampal gyrus L	–	–
Apkarian	VBM	⇓									⇓	
Robinson	VBM	–	–	–	–	⇓ medial	–	⇓	–	–	–	–

Thal thalamus, *Str* Striatum, *Amyg* amygdala, *Cereb* cerebellum, *Ins* ínsula, *TSG* temporal superior gyrus, *ACC* anterior cingulate cortex, *PCC* posterior cingulate cortex, *VLPFC* ventrolateral prefrontal cortex, *Hp* hippocampus, *PFC* prefrontal cortex, *OFC* orbito-frontal gyri, *VBM* voxel-based morphometry, *DTI* diffusion tensor imaging, *R* right, *L* left

Table 30.3 Neuroradiological characteristics and anatomical areas in patients with neuropathic pain syndromes (NPS). Voxel-based morphometry (VBM) and magnetic resonance spectroscopy (MRS)

Author	Image technique	Thal	Cereb	Íns	ACC	OFG
Schmidt-Wilcke	VBM	–	–	–	⇓ L	–
Obermann	VBM	⇓	⇓	⇓	⇓	⇓
Henderson	VBM	⇓ (reticular nucleus)	–	–	–	–
Mole	VBM	–	–	–	–	–
Fukui	MRS	⇓NAA	–	–	–	–
Gustin	MRS	⇓GABA, GABA/Cr R (reticular nucleus of the thalamus) ⇓NAA, NAA/Cr R (ventralis posterior)	–	–	–	–
Wang	MRS	⇓ NAA/Cr (ventralis intermedius posterior, lateralis dorsalis) ⇓ NAA/Cr ⇓ Cho/Cr (lamina medullaris)	–	–	–	–

Thal thalamus, *Cereb* cerebellum, *Ins* ínsula, *CCA* anterior cingulate cortex, *OFC* orbito-frontal gyri, *VBM* voxel-based morphometry, *MRS* magnetic resonance imaging, *NAA* N-acetylaspartate, *Cr* creatine, *Cho* choline, *R* right, *L* left

neuropathic pain syndromes. A number of authors [68] have proposed a model in which the transition from acute to chronic pain leads to a reorganization of cortical sensory and affective pain pathways, which would mean a contribution of independent CNS peripheral nociceptive input. The morphological variations relating to these chronic pain syndromes evolve over time and respond to concepts of neuroplasticity, not to irreversible structural loss mechanisms. NAA appears to decrease in hippocampus [11, 27] as a neuronal marker, providing the molecular correlate to the volume loss observed in studies of morphometry. It can therefore be deduced that there is chronic neuronal damage in this structure in FMS. This point only could be verified by postmortem neuropathological study.

The decrease in volume of the insula [55, 56, 69] correlates with increased Glu [35] and decreased GABA [14]. These alterations suggest a metabolic dysregulation due to CNS hyperexcitability, and this is probably responsible for the overall increase in sensitivity to noxious stimuli observed in these patients. The insula could encode or extract the magnitude of the painful stimulus regardless of sensory modality [70].

Perfusion Magnetic Resonance Imaging

Pioneering single-photon emission computed tomography (SPECT) studies on somatization disorder first described hypoperfusion, primarily in the nondominant hemisphere, in the frontal, prefrontal, temporoparietal, and cerebellar areas [71].

Other researchers [72] conducted an observational study using technetium- 99 m ethyl cysteinate dimer (Tc-99 m ECD) brain SPECT to detect abnormal regional cerebral blood flow (rCBF) in 92 patients with FM. They found rCBF heterogeneity in patients with both primary and concomitant FM compared with the homogeneous rCBF observed in control patients. This difference was observed primarily in the left temporoparietal area, but was also described in the thalamus, as well as in the right temporoparietal, frontal, and basal ganglia areas. Differences in rCBF

hypoperfusion in these areas between primary and concomitant FM groups were not significant. In conclusion, SPECT was not useful for differentiating primary and concomitant FM when the underlying disease activity was quiescent.

There are three main methods for studying brain perfusion by means of MRI:

Dynamic Susceptibility Contrast Imaging (DSCI)

This is the most widespread method of perfusion imaging, and is likely to be a standard sequence on most MR machines. It relies on the $T2^*$ signal drop caused by the passage of a gadolinium-containing contrast agent through the tissues. fMRI is based on measuring and analyzing the so-called BOLD effect. An increase in neural activity leads to a hemodynamic response, associated with an increase in regional blood flow and volume resulting in an increase of the oxyhemoglobin–deoxyhemoglobin ratio, which in turn leads to a reduction of local magnetic in homogeneity.

Dynamic Contrast Enhancement (DCE)

This method uses a rapid T1 sequence to measure changes in signal intensity as a bolus of gadolinium diffuses across the damaged blood-brain barrier into the extracellular, extravascular space.

Arterial Spin Labeling (ASL)

This is a newer MRI technique that uses water in arterial blood as a freely diffusible tracer to measure perfusion noninvasively, whereby the blood flowing into the brain is magnetically labeled (arterial spin labeling). This technique is still largely research-based, and provides truly quantitative values of cerebral blood flow. In future studies, the combination of arterial spin labeling and BOLD imaging might provide new insight into the interaction of neural activity with vascular responses, which is of particular importance, as neural activity cannot be measured directly using MRI techniques.

In a multimodal imaging study using H-MRS and ASL [40], it was possible to demonstrate that patients with neuropathic pain following spinal cord injury displayed lower levels of thalamic NAA, GABA, and regional blood flow (in the thalamus) compared to healthy controls and patients with spinal cord injury but no pain. This and other studies support the notion that neuropathic pain is associated with CNS reorganization, specifically within the thalamus, comprising functional as well as neurochemical mechanisms.

Diffusion Tensor Imaging (DTI)

Neuroimaging reveals changes in the white matter structure in the human brain. White matter comprises half of the human brain, and consists of bundles of myelinated axons connecting neurons in different brain regions [73]. Gray matter is composed of neuronal cell bodies and dendrites concentrated in the outer layers of the cortex.

Microstructural changes in white matter can be revealed by specialized MRI brain imaging techniques such as DTI. This method analyzes the diffusion of protons in tissue, which is more restricted in white matter than in gray matter.

Water molecules in the brain are in constant Brownian motion, and although the movement of these protons affects conventional structural imaging, diffusion-weighted imaging (DWI) and DTI allow quantification of this microscopic movement within each voxel. The main advantage of using DTI, rather than DWI, is that DTI reflects the underlying diffusion properties of the sample, independently of the orientation of the tissue with respect to the direction of measurements. DTI is thus a robust quantitative technique that is independent of how the subject has been oriented inside the scanner magnet and gradient coils.

The appropriate mathematical combination of the directional diffusion-weighted images provides quantitative measures of water diffusion for each voxel via the apparent diffusion coefficient (ADC), as well as the degree of diffusion directionality, or anisotropy. Myelin is a major

diffusion barrier for water, and gives white matter its high anisotropy [74]. Demyelinating diseases are characterized by partial or total loss of myelin, with consequent loss of neuronal function. Anisotropy increases with increased myelination, diameter, and axon compaction.

Previous studies have identified and confirmed the existence of an anatomic circuitry for the functionally characterized, top-down influences on pain processing via brainstem structures in humans [75]. Fractional anisotropy (FA) is a measure of the portion of the diffusion tensor from anisotropy. Previous studies with DTI in FM patients showed alterations in the right thalamus and significantly lower fractionated anisotropy in comparison with controls. A negative correlation was observed between the FA values in the right thalamus and clinical pain in the FM group [76]. Other authors have confirmed that DTI in the brain of patients with FM appeared to be more sensitive than volumetric imaging of voxel-based morphometry (VBM), and that increased pain intensity scores were correlated with changes in DTI measurements in the right superior frontal gyrus. Increased fatigue was correlated with changes in the left superior frontal and left anterior cingulate gyrus, and self-perceived physical impairment was correlated with changes in the left postcentral gyrus. Higher intensity scores for stress symptoms were correlated negatively with diffusivity in the thalamus and FA in the left insular cortex [55].

Functional Magnetic Resonance Imaging (FMRI)

Clinical studies with functional imaging show that pain is not a static condition with a pathophysiology that is only localized in the peripheral system of muscles or tendons, but that it is a highly plastic clinical pathology that affects multiple central neural systems and defines the so-called "neural matrix" of pain or network of cortico-subcortical areas involved in pain processing.

fMRI is a noninvasive technique that detects and locates focal brain activation taking place and involved in performing a task by means of a cognitive, emotional, or sensory-motor neuron circuit. Generally, fMRI studies are based on the acquisition of images during one sequence while the patient is at rest and another while performing a task. The subsequent statistical comparison between the two phases (rest and activation) represents, in an ideal case, the focal metabolic and vascular changes in the cerebral cortex that are in operation during performance of the task being studied, or by block-design paradigms that are well connected with the episode (event-related). However, fMRI can evaluate the metabolic and vascular condition that occurs at rest (resting-state) and the actual time duration of the pattern of brain activation when performing a functional certain task under study.

The DMN comprises a set of brain regions that are coactivated during passive task states. These show an intrinsic functional relationship, and are connected via direct and indirect anatomic projections. The medial temporal lobe subsystem provides information from previous experiences in the form of memories and associations, which are the building blocks of mental simulation. The medial prefrontal subsystem facilitates the flexible use of this information during the construction of self-relevant mental simulations. These two subsystems converge on important nodes of integration, including the ventral posterior cingulate cortex (vPCC) [77].

Multiple techniques have been devised to evaluate functional brain connectivity. For correlational analyses, the main techniques are seed correlation and independent component analysis (ICA). For seed correlation, the fMRI signal is extracted from a seed region of interest, and is then correlated with the fMRI time series taken from all other brain voxels [78]. Alternatively, ICA is a data-driven technique that considers all voxels in the brain and clusters them into spatio-temporally distinct networks, which are spatially independent of one another [79].

Recent functional neuroimaging studies have enabled the neuroanatomical differentiation of the classic dimensions of pain processing. Sensory and cognitive dimensions are at the top and brain dorsal portion (contralateral primary somatosensory cortex, bilateral secondary somatosensory cortex, insular cortex in its rear portion, opercular

area, thalamus, frontoparietal neocortex, and supplementary motor area). The emotional dimension involves the insular cortex, anterior cingulate cortex, basal ganglia, and prefrontal cortex. Consequently, the functional activation of the neural circuitry of pain can be modulated by either a sensory or emotional component, not to mention the involvement of the cognitive component.

The emotional component can mediate the intensity threshold at which a stimulus is perceived as painful. In fact, this threshold appears to be much lower in patients diagnosed with chronic functional pain (such as FM) than in healthy controls, and can generate an abnormal activation of the neuronal pain circuit. This has given rise to the collective denomination of these syndromes as central susceptibility syndromes. Patients with FM have been found to have greater connectivity between the DMN and the insular cortex, which is a brain region known to process evoked pain, and the executive attention network [80]. Resting-state functional magnetic resonance imaging (rfMRI) data from 18 patients with FM and 18 age-matched healthy control subjects were analyzed using dual-regression ICA, which is a data-driven approach for the identification of independent brain networks. Intrinsic, or resting-state, connectivity was evaluated in multiple brain networks: the DMN, the executive attention network (EAN), and the medial visual network (MVN), with the medial visual network serving as a negative control. Spontaneous pain levels were also analyzed for covariance with intrinsic connectivity. These findings indicate that resting brain activity within multiple networks is associated with spontaneous clinical pain in patients with FM. These findings may also have broader implications for how subjective experiences such as pain arise from a complex interplay among multiple brain networks (See Table 30.4).

While acute experimental pain induces default mode network deactivation in healthy subjects

Table 30.4 Neuroradiological characteristics and anatomical areas of functional magnetic resonance imaging (fMRI) activations in pain

Author	Image technique	Connectivity	Deactivation	Mitigated deactivation
Napadov	Resting state	⇑ Insula–DMN	–	–
Seminowicz	Resting state	–	DMN (acute experimental pain)	–
Baliki	Resting state	–	–	DMN chronic back pain
Pujol	Resting state	⇓ somatosensory system ⇑ DMN and somatosensory cortex	–	–
Cifre	Voxel	⇑ DMN and mPFC and PCC insula and ACC	Insula–DMN	–
Stoeter	fMRI	⇑ (thalamus, basal ganglia, and operculo-insular cortex)	–	–
Gundel	fMRI	⇑ amygdala, parahippocampal gyrus, and anterior insula	vmPFC/OFC	–
Raichle	fMRI	⇑ PFC and PCC–precuneus	Lateral parietal cortex	–

DMN default mode network, *mPFC* medial prefrontal cortex, *PCC* posterior cingulate cortex, *ACC* anterior cingulate cortex, *mPFC* medial prefrontal cortex, *vmFC* ventromedial prefrontal cortex, *OFC* orbito-frontal gyri, *PFC* prefrontal cortex

[81], chronic back pain is associated with mitigated DMN deactivation to visual attention tasks [82]. The DMN showed greater connectivity to the insula cortex and secondary somatosensory cortex (S2) (brain regions known to process evoked experimental pain and somatosensation), while the EAN showed greater intra-network connectivity in FM patients. Both the DMN and EAN were more connected to the insula in patients reporting greater spontaneous clinical pain at the time of the scan, which suggested a close link between DMN–insula connectivity and clinical pain.

Reduced resting connectivity within the somatosensory system and increased connectivity between the default mode network and somatosensory processing regions, such as the secondary somatosensory cortex, were recently reported [83]. Such independent, confirmatory data are important for any neuroimaging-based markers of disease in FM, and further research is needed. Interestingly, this study also found altered connectivity with brain regions supporting visual and auditory processing, which may relate to the multisensory dysfunction sometimes reported in these patients.

A study used a seed voxel region of interest approach, and showed a pattern of both increased and decreased brain connectivity in FM patients [84]. Increased connectivity was found between DMN areas such as medial prefrontal cortex (mPFC) and posterior cingulate cortex (PCC), and also between anterior cingulate cortex (ACC) and the insula. These results support the fact that DMN and insula resting connectivity is disrupted in FM.

A recent study explored structural and fMRI changes in FM patients, and found an interesting association with age [85]. Younger, but not older, FM patients showed decoupling between the insula and anterior mid-cingulate cortex, two brain regions that are normally strongly connected in healthy adults, as part of a salience network. Another study reported increased frequency power (for a broad 0.01–0.25 Hz band) in somatosensory (primary somatosensory cortex, S1), cognitive (DLPFC), and affective (amygdala) brain regions in FM patients [86].

During mild pain events, a relationship has been demonstrated between catastrophizing and activity in cortical regions associated with affective, attention, and motor aspects of pain. This includes the dorsolateral prefrontal, insular, rostral anterior cingulate, premotor, and parietal cortices. During more intense pain, prefrontal cortical regions involved in top-down pain modulation are negatively correlated with catastrophizing. An explanation for this may be that a cortical vigilance network is engaged during mild pain. However, diminished prefrontal cortical modulation impedes disengagement and suppression during more intense pain [87]. These findings may also involve catastrophizing in the progression toward or persistence of chronic pain.

Patients with somatoform pain disorders are thought to have an early-acquired defect in stress regulation. The fMRI [88] was used to search for common alterations in the pain-responsive and stress-responsive cortical areas. They studied a group of 17 patients and an age-matched control group by inducing pin-prick pain, cognitive stress, and emotional stress. The patients demonstrated increased activation of pain-processing areas (thalamus, basal ganglia, and operculo-insular cortex) during first pain exposure, and increased activation of some prefrontal, temporal, and parietal regions was also observed. Temporal and parietal areas were also activated during cognitive stress, and activation was reduced during emotional stress. However, hippocampal volume was not significantly reduced in the patient group. This study supports the current concept that central processing of pain and cognitive stress is increased in these patients, possibly owing to exaggerated memory or anticipation of pain exposure, or both, and to a disturbance in stress regulating systems. Though surprising, the finding of a reduced responsiveness to emotional stress is not contradictory to this hypothesis. Some sort of neglect or coping mechanisms may have developed over time as a response to earlier adverse events.

Another fMRI study [89] researched the cerebral processing of noxious heat stimuli as objective markers for pain sensation in 12 right-handed women diagnosed with somatoform pain disorder and 13 age-matched, healthy volunteers. Compared with controls, patients with pain

disorder responded to induced pain with hypoactivation of the ventromedial prefrontal/orbitofrontal cortex, and hyperactivation of the amygdala, parahippocampal gyrus, and anterior insula. The finding of altered cerebral processing of experimentally induced pain in patients with somatoform pain disorder supports the hypothesis of dysfunctional pain processing, particularly in affect-regulating regions.

An fMRI analysis [13] revealed no differences in activity in brain regions related with attention and affection, or regions with sensory projections from the stimulated body area. However, when there was a primary lesion in the descending pain regulating system (the rostral anterior cingulate cortex), the patients failed to respond to pain provocation. The attenuated response to pain in these cases is the first demonstration of a specific brain region where the impairment of pain inhibition in FMS patients is expressed. These results validate previous reports of dysfunctional endogenous pain inhibition in FM, and advance the understanding of the central pathophysiologic mechanisms, providing a new direction for the development of successful treatments in FM.

Although the etiology of this disorder remains largely unknown, emerging data suggest that FM arises through augmentation of central pain processing pathways. This hypothesis is largely based upon findings of previous functional neuroimaging studies, showing that FM patients display augmented neuronal responses to both innocuous and painful stimuli [12, 33], confirming the allodynia and hyperalgesia seen in this condition [90].

Studies with functional neuroimaging support the hypothesis of central pain augmentation in FM. Differences of activation in the frontocingulate cortex, the supplemental motor areas, and the thalamus were found between both groups with distinct differences in BOLD signal changes over the duration of pain stimulation, and even during anticipation of pain. These results support the hypothesis that central mechanisms of pain processing in the medial pain system and favorable cognitive/affective factors even during the anticipation of pain may play an important role for pain processing in patients with FMS [54].

The default network is disrupted during painful stimuli [91, 92] in FM [93] and depression [94], thereby further encouraging researchers to consider how the functions of the DMN might be important in understanding diseases of the mind. The functional connectivity pattern within the DMN is altered during pain, selectively in the prefrontal cortex and posterior cingulate cortex–precuneus (increased connectivity), and in the lateral parietal cortex (decreased connectivity). A limited number of functional neuroimaging pain studies have shown that the activity in a network, including the posterior cingulate cortex–precuneus, the inferior parietal lobule, and the medial prefrontal cortex, was consistently reduced in response to a range of painful stimuli [91, 92].

New acquisition techniques and new analysis strategies have emerged that enable new conceptual approaches to the acquisition of data, such as network and multivariate pattern analyses, and in particular, support vector machines (SVM) [95]. The emergence of connectivity analyses, both functional connectivity (resting state) and structural connectivity, as enabled by DTI and tractography, have allowed not only for the analysis of the connectedness of two remote brain areas, but also for the construction and analysis of large networks consisting of multiple brain sites. One such approach is the graph theory, where graphs are mathematical structures to model relations between objects.

The use of the graph theory in the analysis of chronic pain states has been limited. When investigating patients with migraine (without aura) and healthy controls, and applying the graph theory based on resting-state functional connectivity analyses [96], a disruption was found in whole-brain networks with an increase in disease duration, in which areas implicated in sensory discrimination constituted an abnormal network configuration.

Treatment Effects

Mindfulness meditation has beneficial effects on a number of psychiatric, functional somatic, and stress-related symptoms, and therefore has been

increasingly incorporated into psychotherapeutic programs [97, 98], with subjects reporting better pain-related quality of life and greater life satisfaction [99]. Altogether, 25 systematic reviews were found; they investigated the evidence of complementary and alternative medicine (CAM) for the FMS [100]. In general, they were exercised-based CAM therapies, manipulative therapies, mind–body therapies, acupuncture, hydrotherapy, phytotherapy, and homeopathy. Consistently positive results were found for tai chi, yoga, meditation and mindfulness-based interventions, hypnosis or guided imagery, electromyogram (EMG) biofeedback, and balneotherapy/hydrotherapy. Inconsistent results were found for qigong, acupuncture, chiropractic interventions, electroencephalogram (EEG) biofeedback, and nutritional supplements. Inconclusive results were found for homeopathy and phytotherapy. Despite a growing body of scientific evidence of CAM therapies for the management of fibromyalgia syndrome (FMS), systematic reviews still show methodological flaws that limit definite conclusions about their efficacy and safety.

Neuroimaging studies were conducted to analyze the brains of people with and without meditation experience. During the first 20 min inside the MRI scanner, they had spontaneous thoughts, and for the next 20 min they developed a simple exercise task, which consisted of focusing only on their breathing. As they began to practice this exercise, meditation with the usual respiratory concentration, medial prefrontal cortex activity decreased in all patients. This part of the DMN is considered relevant to self-centered mental processes. Moreover, although the blood flow in the medial prefrontal region of the inexperienced meditators decreased a few minutes later than that of the experienced meditators, the blood supply of the area was reduced for the duration of the exercise, suggesting the calming effects of meditation [101].

Anatomical likelihood estimation (ALE) meta-analysis found eight brain regions of GM that were consistently enhanced in meditators [102]. Three studies [103–105] showed an apparent pattern of structural increase in WM in meditators versus controls. Our research found that meditators showed a lower apparent diffusion coefficient (ADC) in the left posterior parietal white matter than did controls, and that the ADC was negatively correlated with years of meditation.

Similar research [106] addressed the functional connectivity of the DMN in subjects who commonly practiced mindfulness versus subjects who did not. Their results indicated both reduced activation of two main nodes of the DMN (posterior cingulated cortex and medial prefrontal cortex), and that experienced meditators showed activation of the medial prefrontal cortex, insula, and temporal lobes during meditation, a differential pattern of functional connectivity both during resting and during mindfulness exercises. Other authors [107] show that the activity in a subregion of the DMN, the ventromedial prefrontal cortex, is inversely correlated with years of meditation experience, suggesting that the experience of meditation can enable more efficient cognitive processes subserved by this region. Another study [108], also reported a higher functional connectivity in the DMN in meditator subjects (medial prefrontal cortex), suggesting that meditation practice is associated with functional changes in areas of the DMN even when not practicing. In summary, existing studies suggest differential patterns in meditators' functional connectivity, consistent with reduced mind-wandering, a greater awareness of the present moment, and self-referential processing than those found in non-meditators [106, 109].

Meditation may be able to reinforce positive feelings, especially compassion and benevolence. To test this hypothesis [110], subjects performed compassion exercises while lying down in a brain scanner. Half of the 30 volunteers had several years of experience in Buddhist meditation techniques. The control group comprised age-matched participants with no experience in this type of group meditation. Emotional reactions were provoked with either the laughter of a baby or a deeply distressed groan. Such acoustic signals primarily stimulated those areas that had been shown in other studies to process emotional stimuli (the insula, the anterior cingulate cortex, and secondary somatosensory area). The major

differences between experienced meditators and novices were observed in the insula. Many of these phenomena are explained through mechanisms of neuronal plasticity: An intense effort results in alterations in the structure and mode of operation of certain areas of the brain.

One review [111] described up to 17 research studies in which the therapeutic potential of mindfulness in pain was analyzed. Another study [112] showed that Zen meditators have pain sensitivity thresholds higher than non-meditator subjects. This is where regulation comes into play as a basic feature of meditation [113]. It appears that one effect of reduced activation of certain areas is a reduction in the connectivity between them. Connectivity has been associated with complex functions that are performed by multiple brain structures in combination. The study showed increased activation of areas typically associated with pain, such as the insula, thalamus, anterior cingulate cortex, and prefrontal cortex [33, 114]. If only this increased activity is observed, it might seem that meditators are feeling more pain than nonmeditators, which contrasts with the poor results obtained when they were asked to rate their pain. Connectivity studies, however, show that meditation reduces the connectivity between these areas related to pain regulation.

Similarly, several authors have studied the role of the prefrontal cortex using emotion-regulation tasks. For example, in the first study to address this [115], meditators were asked to perform a task of emotion recognition, with results showing lower connectivity between the prefrontal cortex and the right amygdala than in participants who did not practice meditation. The authors hypothesized that meditators tend to treat emotional states as "objects" of care. By treating these conditions as transient mental products, this allows the meditator to maintain greater distance from emotional experiences. This contrasts with the usual way of thinking and feeling emotions and thoughts, in which they are considered "facts" or "reality". Results in this field have been obtained by other authors [116, 117], although some of these [118] pointed to the amygdala as a major participant in the regulation of emotions. These authors studied the regulation of anxiety through meditation techniques, which showed reduced amygdala activity after performing a series of exercises.

However, other researchers [119] showed that DMN–insula connectivity, which was increased in FM patients, was reduced following 4 weeks of nonpharmacological acupuncture and sham acupuncture therapy, resulting in reduced pain in these patients. The authors suggested that connectivity between the DMN and insula may serve as a possible surrogate biomarker for pain reduction in FM.

Such studies could indeed play a role in clinical practice, as they could, in addition to behavioral measures such as anxiety and catastrophizing scores, help to identify patients at a high risk of developing chronic pain, implying the necessity of early therapeutic intervention. Likewise, it is desirable to determine whether or not patients are likely to respond to a certain therapy. In two recently performed studies, functional connectivity was demonstrated to predict clinical improvement in response to pregabalin or milnacipran intake in FM patients [120, 121].

Interestingly, functional brain imaging has also been used to predict response to placebo treatment [121–123]. In the context of personalized medicine, placebo treatment might indeed be a therapeutic option in several patients with chronic pain, and brain imaging could help to identify patients who are likely to benefit from a placebo treatment.

Other authors investigating analgesic mechanisms of pregabalin in an FM group have seen that reductions in clinical pain were associated with reductions in functional connectivity between the DMN and the posterior insular cortex, corroborating the theory that the interaction of these two regions might play a specific role in chronic pain [120].

A recent study by our group, with 63 patients, showed preliminary evidence of the utility of memantine for the treatment of FM. Compared with a placebo group, memantine significantly decreased ratings on a pain visual analog scale (Cohen's d = 1.43 at 6 months) and pain measured with a sphygmomanometer (d = 1.05). All other secondary outcomes except anxiety also improved, with moderate-to-large effect sizes at

6 months. Compared with placebo, the absolute risk reduction obtained with memantine was 16.13% (95% confidence interval = 2.0–32.6%), and the number needed to treat was 6.2 (95% confidence interval = 3–47) [124].

Interestingly, in another study conducted by our group using MRS at baseline and 6 months, in 13 patients with FM treated with memantine and 12 with placebo, the patients treated with memantine exhibited a significant increase in Glu, the Glu/Cr ratio, Glx, and total NAA + NAAG in the posterior cingulate cortex compared with those on placebo. Furthermore, the memantine group exhibited increases in Cr and Cho in the right posterior insula, while a correlation between Cho and the Fibromyalgia Impact Questionnaire (FIQ) in the posterior insula was also observed, demonstrating that memantine treatment resulted in an increase in cerebral metabolism in FM patients, and suggesting its utility for the treatment of the illness [125].

The combination of ASL and BOLD imaging might provide new insight into the interaction of neural activity with vascular responses, which is of particular importance given that neural activity cannot be measured directly using MRI techniques. Some authors have applied ASL and a Gaussian process binary classifier to distinguish intraindividually between a nonpain condition (prior to molar extraction) and postsurgical pain (after third molar extraction from the lower jaw), reaching a classification accuracy of 95 [126]. Others have reported that the functional connectivity between the nucleus accumbens (NAc) and the prefrontal cortex in patients with subacute back pain was predictive of whether the pain persisted [127]. A similar association was described for the structural connectivity of the prefrontal cortex as assessed by DTI [128].

Conclusions

This chapter discussbes several techniques used for the diagnosis of FM. At present, there are no other noninvasive techniques that can provide equivalent information and, as a consequence, MRS, DTI tractography, and fMRI are expected to be a powerful combined technique for researching brain anatomy and disease in situ in human beings.

The main findings among patients with chronic pain are an increased functional connectivity between the pain system and the DMS, decrease in gray matter volume in the insular cortex and anterior cingulate cortex, and also decreased GABA concentrations in the insular cortex or thalamus.

New acquisition techniques and new analysis strategies have emerged that enable new conceptual approaches to the acquisition of data, such as network and multivariate pattern analyses, and in particular, support vector machines (SVM).

Continued improvements in the design of imaging equipment and analysis algorithms are progressively improving the specificity of the biological parameters that can be calculated, allowing detailed quantitative characterization of microvascular structure in a wide range of pathological tissues, including FM.

References

1. Sjøgren P, Ekholm O, Peuckmann V, Grønbaek M. Epidemiology of chronic pain in Denmark: an update. Eur J Pain. 2009;13:287–92.
2. Blyth FM, March LM, Brnabic AJ, Jorm LR, Williamson M, Cousins MJ. Chronic pain in Australia: a prevalence study. Pain. 2001;89:127–34.
3. Manchikanti L, Boswell MV, Singh V, Derby R, Fellows B, Falco FJ, Datta S, Smith HS, Hirsch JA. Comprehensive review of neurophysiologic basis and diagnostic interventions in managing chronic spinal pain. Pain Physician. 2009;12:E71–120.
4. International Association for the Study of Pain (IASP). Classification of chronic pain. 2nd ed. IASP Task Force on Taxonomy, Merskey H, Bogduk N, editors. Seattle: IASP Press; 1994.
5. Wolfe F, Smythe HA, Yunus MB, Bennet RM, Bombardier C, Goldenberg ADL. American College of Rheumatology 1990. Criteria for the classification of fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum. 1990;33:160–72.
6. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthritis Care Res. 2010;62:600–10.
7. Branco JC, Bannwarth B, Failde I, Abello Carbonell J, Blotman F, Spaeth M, Saraiva F, Nacci F, Thomas E, Caubère JP, Le Lay K, Taieb C, Matucci-Cerinic M. Prevalence of fibromyalgia: a survey in five

European countries. Semin Arthritis Rheum. 2010;39:448–53.
8. Gamero Ruiz F, Gabriel Sánchez R, Carbonell Abelló J, Tornero Molina J, Sanchez-Magro I. Pain in Spanish rheumatology outpatient offices: EPIDOR epidemiological study. Rev Clin Esp. 2005;205:157–63.
9. Treede RD, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, Hansson P, Hughes R, Nurmikko T, Serra J. Neuropathic pain: redefinition and a grading system for clinical and research purposes. Neurology. 2008;70:1630–5.
10. American Psychiatric Association. Diagnostic and statistical manual of mental disorders DSM-IV-TR. 4th (text revision) ed. Washington, DC: American Psychiatric Association; 2000.
11. Fayed N, Andres E, Rojas G, Moreno S, Serrano-Blanco A, Roca M, Garcia-Campayo J. Brain dysfunction in fibromyalgia and somatization disorder using proton magnetic resonance spectroscopy: a controlled study. Acta Psychiatr Scand. 2012;126:115–25.
12. Cook DB, Lange G, Ciccone DS, Liu WC, Steffener J, Natelson BH. Functional imaging of pain in patients with primary Fibromyalgia. J Rheumatol. 2004;31:364–78.
13. Jensen KB, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, Williams SC, Choy E, Giesecke T, Mainguy Y, Gracely R, Ingvar M. Evidence of dysfunctional pain inhibition in Fibromyalgia reflected in rACC during provoked pain. Pain. 2009;144:95–100.
14. Foerster BR, Petrou M, Edden RAE, Clauw DJ, Sundgren PC, Schmidt-Wilcke T, Lowe SE, Harte SE, Clauw DJ, Harris RE. Reduced insular gamma-aminobutyric acid in fibromyalgia. Arthritis Rheum. 2012;64:579–83.
15. Ashburner J. Computational Neuroanatomy. PhD thesis, London: University College; 2000.
16. Barker PB. N-acetyl aspartate – a neuronal marker? Ann Neurol. 2001;49:423–4.
17. Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AM. N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. Prog Neurobiol. 2007;81:89–131.
18. Urenjak J, Williams SR, Gadian DG. Specific expression of N-acetylaspartate in neurons, oligodendrocyte-type-2 astrocyte progenitors, and immature oligodendrocytes in vitro. J Neurochem. 1992;59:55–61.
19. Baslow MH. The vertebrate brain, evidence of its modular organization and operating system: insights in to the brain's basic units of structure, function, and operation and how they influence neuronal signaling and behavior. Front Behav Neurosci. 2011;5:5.
20. Sartorius A, Lugenbiel P, Mahlstedt MM. Proton magnetic resonance spectroscopic creatine correlates with creatine transporter protein density in rat brain. J Neurosci Methods. 2008;172:215–9.
21. Gill SS, Small RK, Thomas DG, Patel P, Porteous R, Van Bruggen N, Gadian DG, Kauppinen RA, Williams SR. Brain metabolites as 1H NMR markers of neuronal and glial disorders. NMR Biomed. 1989;2:196–200.
22. Katz-Brull R, Koudinov AR, Degani H. Choline in the aging brain. Brain Res. 2002;951:158–65.
23. Escartin C, Valette J, Lebon V, Bonvento G. Neuron–astrocyte interactions in the regulation of brain energy metabolism: a focus on NMR spectroscopy. J Neurochem. 2006;99:393–401.
24. Grachev ID, Fredrickson BE, Apkarian AV. Abnormal brain chemistry in chronic back pain: an in vivo proton magnetic resonance spectroscopy study. Pain. 2000;89:7e18.
25. Gustin SM, Peck CC, Wilcox SL, Nash PG, Murray GM, Henderson LA. Different pain, different brain: thalamic anatomy in neuropathic and non-neuropathic chronic pain syndromes. J Neurosci. 2011;31:5956–64.
26. Fukui S, Matsuno M, Inubushi T, Nosaka S. N-Acetylaspartate concentrations in the thalami of neuropathic pain patients and healthy comparison subjects measured with (1) H-MRS. Magn Reson Imaging. 2006;24:75–9.
27. Emad Y, Ragab Y, Zeinhom F, El-Khouly G, Abou-Zeid A, Rasker JJ. Hippocampus dysfunction may explain symptoms of fibromyalgia syndrome. A study with single-voxel magnetic resonance spectroscopy. J Rheumatol. 2008;35:1371–7.
28. Wood PB, Glabus MF, Simpson R, Patterson JC. Changes in gray matter density in fibromyalgia: correlation with dopamine metabolism. J Pain. 2009;10:609–18.
29. Fayed N, Garcia-Campayo J, Magallón R, Andrés-Bergareche H, Luciano JV, Andres E, Beltrán J. Localized 1H-NMR spectroscopy in patients with fibromyalgia: a controlled study of changes in cerebral glutamate/glutamine, inositol, choline, and N-acetylaspartate. Arthritis Res Ther. 2010;12:R134.
30. Grachev ID, Thomas PS, Ramachandran TS. Decreased levels of N-acetylaspartate in dorsolateral prefrontal cortex in a case of intractable severe sympathetically mediated chronic pain (complex regional pain syndrome, type I). Brain Cogn. 2002;49:102–13.
31. Pattany PM, Yezierski RP, Widerström-Noga EG, Bowen BC, Martinez-Arizala A, Garcia BR, Quencer RM. Proton magnetic resonance spectroscopy of the thalamus in patients with chronic neuropathic pain after spinal cord injury. AJNR Am J Neuroradiol. 2002;23:901–5.
32. Mckenna JE, Melzack R. Blocking NMDA receptors in the hippocampal dentate gyrus with AP5 produces analgesia in the formalin pain test. Exp Neurol. 2001;172:92–9.
33. Gracely RH, Petzke F, Wolf JM, Clauw DJ. Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. Arthritis Rheum. 2002;46:1333–43.

34. Valdés M, Collado A, Bargalló N, Vázquez M, Rami L, Gómez E, Salamero M. Increased glutamate/glutamine compounds in the brains of patients with fibromyalgia: a magnetic resonance spectroscopy study. Arthritis Rheum. 2010;62:1829–36.
35. Harris RE, Sundgren PC, Craig AD, Kirshenbaum E, Sen A, Napadow V, Clauw DJ. Elevated insular glutamate in fibromyalgia is associated with experimental pain. Arthritis Rheum. 2009;60:3146–52.
36. Petrou M, Harris RE, Foerster BR, McLean SA, Sen A, Clauw DJ, Sundgren PC. Proton MR spectroscopy in the evaluation of cerebral metabolism in patients with fibromyalgia: comparison with healthy controls and correlation with symptom severity. Am J Neuroradiol. 2008;29:913–8.
37. Feraco P, Bacci A, Pedrabissi F, Passamonti L, Zampogna G, Malavolta N, Leonardi M. Metabolic abnormalities in pain-processing regions of patients with fibromyalgia: a 3 T MR spectroscopy study. Am J Neuroradiol. 2011;32:1585–90.
38. Kapogiannis D, Reiter DA, Willette AA, Mattson MP. Posteromedial cortex glutamate and GABA predict intrinsic functional connectivity of the default mode network. Neuroimage. 2013;64:112–9.
39. Napadow V, Harris RE. What has functional connectivity and chemical neuroimaging in fibromyalgia taught us about the mechanisms and management of 'centralized' pain? Arthritis Res Ther. 2014;16:425.
40. Gustin SM, Wrigley PJ, Youssef AM, McIndoe L, Wilcox SL, Rae CD, Edden RA, Siddall PJ, Henderson LA. Thalamic activity and biochemical changes in individuals with neuropathic pain after spinal cord injury. Pain. 2014;155:1027–36.
41. Wang Y, Li D, Bao F, Ma S, Guo C, Jin C, Zhang M. Thalamic metabolic alterations with cognitive dysfunction in idiopathic trigeminal neuralgia: a multivoxel spectroscopy study. Neuroradiology. 2014;56:685–93.
42. Chang L, Munsaka SM, Kraft-Terry S, Ernst T. Magnetic resonance spectroscopy to assess neuroinflammation and neuropathic pain. J Neuroimmune Pharmacol. 2013;8:576–93.
43. González de la Aleja J, Ramos A, Mato-Abad V, Martínez-Salio A, Hernández-Tamames JA, Molina JA, Hernández-Gallego J, Alvarez-Linera J. Higher glutamate to glutamine ratios in occipital regions in women with migraine during the interictal state. Headache. 2013;53:365–75.
44. Siniatchkin M, Sendacki M, Moeller F, Wolff S, Jansen O, Siebner H, Stephani U. Abnormal changes of synaptic excitability in migraine with aura. Cereb Cortex. 2012;22:2207–16.
45. Reyngoudt H, Achten E, Paemeleire K. Magnetic resonance spectroscopy in migraine: what have we learned so far? Cephalalgia. 2012;32:845–59.
46. Cacace AT, Silver SM. Applications of magnetic resonance spectroscopy to tinnitus research: initial data, current issues, and future perspectives. Prog Brain Res. 2007;166:71–81.
47. Gutzeit A, Meier D, Froehlich JM, Hergan K, Kos S, V Weymarn C, Lutz K, Ettlin D, Binkert CA, Mutschler J, Sartoretti-Schefer S, Brügger M. Differential NMR spectroscopy reactions of anterior/posterior and right/left insular subdivisions due to acute dental pain. Eur Radiol. 2013;23:450–60.
48. Bleich S, Römer K, Wiltfang J, Kornhuber J. Glutamate and the glutamate receptor system: a target for drug action. Int J Geriatr Psychiatry. 2003;18:S33–40.
49. Lorio S, Lutti A, Kherif F, Ruef A, Dukart J, Chowdhury R, Frackowiak RS, Ashburner J, Helms G, Weiskopf N, Draganski B. Disentangling in vivo the effects of iron content and atrophy on the ageing human brain. Neuroimage. 2014;103:280–9.
50. Schmidt-Wilcke T. Variations in brain volume and regional morphology associated with chronic pain. Curr Rheumatol Rep. 2008;10:467–74.
51. Wood PB. Variations in brain gray matter associated with chronic pain. Curr Rheumatol Rep. 2010;12:462–9.
52. Cagnie B, Coppieters I, Denecker S, Six J, Danneels L, Meeus M. Central sensitization in fibromyalgia? A systematic review on structural and functional brain MRI. Semin Arthritis Rheum. 2014;44:68–75.
53. Valet M, Gündel H, Sprenger T, Sorg C, Mühlau M, Zimmer C, Henningsen P, Tölle TR. Patients with pain disorder show gray-matter loss in pain-processing structures: a voxel-based morphometric study. Psychosom Med. 2009;71:49–56.
54. Burgmer M, Pogatzki-Zahn E, Gaubitz M, Wessoleck E, Heuft G, Pfleiderer B. Altered brain activity during pain processing in fibromyalgia. Neuroimage. 2009;44:502–8.
55. Lutz J, Jäger L, de Quervain D, Krauseneck T, Padberg F, Wichnalek M, Beyer A, Stahl R, Zirngibl B, Morhard D, Reiser M, Schelling G. White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. Arthritis Rheum. 2008;58:3960–9.
56. Kuchinad A, Schweinhardt P, Seminowicz DA, Wood PB, Chizh BA, Bushnell MC. Accelerated brain gray matter loss in fibromyalgia patients: premature aging of the brain? J Neurosci. 2007;27:4004–7.
57. Villarreal G, Hamilton DA, Petropoulos H, Driscoll I, Rowland LM, Griego JA, Kodituwakku PW, Hart BL, Escalona R, Brooks WM. Reduced hippocampal volume and total white matter volume in posttraumatic stress disorder. Biol Psychiatry. 2002;52:119–25.
58. Chen S, Xia W, Li L, Liu J, He Z, Zhang Z, Yan L, Zhang J, Hu D. Gray matter density reduction in the insula in fire survivors with posttraumatic stress disorder: a voxel-based morphometric study. Psychiatry Res. 2006;146:65–72.
59. Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR. Chronic back pain is

associated with decreased prefrontal and thalamic gray matter density. J Neurosci. 2004;24:10410–5.
60. Okada T, Tanaka M, Kuratsune H, Watanabe Y, Sadato N. Mechanisms underlying fatigue: a voxel-based morphometric study of chronic fatigue syndrome. BMC Neurol. 2004;4:14.
61. de Lange FP, Kalkman JS, Bleijenberg G, Hagoort P, van der Meer JW, Toni I. Gray matter volume reduction in the chronic fatigue syndrome. Neuroimage. 2005;26:777–81.
62. Schmidt-Wilcke T, Luerding R, Weigand T, Jürgens T, Schuierer G, Leinisch E, Bogdahn U. Striatal grey matter increase in patients suffering from fibromyalgia. A voxel-based morphometry study. J Pain. 2007;132:S109–16.
63. Obermann M, Rodriguez-Raecke R, Naegel S, Holle D, Mueller D, Yoon MS, Theysohn N, Blex S, Diener HC, Katsarava Z. Gray matter volume reduction reflects chronic pain in trigeminal neuralgia. Neuroimage. 2013;74:352–8.
64. Henderson LA, Peck CC, Petersen ET, Rae CD, Youssef AM, Reeves JM, Wilcox SL, Akhter R, Murray GM, Gustin SM. Chronic pain: lost inhibition? J Neurosci. 2013;33:7574–82.
65. Schmidt-Wilcke T, Hierlmeier S, Leinisch E. Altered regional brain morphology in patients with chronic facial pain. Headache. 2010;50:1278–85.
66. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain. 2005;9:463–84.
67. Ridderinkhof KR, Ullsperger M, Crone EA, Nieuwenhuis S. The role of the medial frontal cortex in cognitive control. Science. 2004;306:443–7.
68. Apkarian AV, Baliki MN, Geha PY. Towards a theory of chronic pain. Prog Neurobiol. 2009;87:81–97.
69. Robinson ME, Craggs JG, Price DD, Perlstein WM, Staud R. Gray matter volumes of pain related brain areas are decreased in fibromyalgia syndrome. Gray matter volumes of pain related brain areas are decreased in fibromyalgia syndrome. J Pain. 2011;12:436–43.
70. Baliki MN, Geha PY, Apkarian AV. Parsing pain perception between nociceptive representation and magnitude estimation. J Neurophysiol. 2009;101:875–87.
71. Garcia-Campayo J, Sanz C, Baringo T, Ceballos C. SPECT scan in somatisation disorder patients: an exploratory study of eleven cases. Aust N Z J Psychiatry. 2001;35:359–63.
72. Chen JJ, Wang JY, Chang YM. Regional cerebral blood flow between primary and concomitant fibromyalgia patients: a possible way to differentiate concomitant fibromyalgia from the primary disease. Scand J Rheumatol. 2007;36:22.
73. Fields RD. White matter in learning, cognition and psychiatric disorders. Trends Neurosci. 2008;31:317–76.
74. Sundgren PC, Dong Q, Gómez-Hassan D, Mukherji SK, Maly P, Welsh R. Diffusion tensor imaging of the brain: review of clinical applications. Neuroradiology. 2004;46:339–50.
75. Hadjipavlou G, Dunckley P, Behrens TE, Tracey I. Determining anatomical connectivities between cortical and brainstem pain processing regions in humans: a diffusion tensor imaging study in healthy controls. Pain. 2006;123:169–78.
76. Sundgren PC, Petrou M, Harris RE. Diffusion-weighted and diffusion tensor imaging in fibromyalgia patients: a prospective study of whole brain diffusivity, apparent diffusion coefficient, and fraction anisotropy in different regions of the brain and correlation with symptom severity. Acad Radiol. 2007;14:839–46.
77. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci. 2008;1124:1–38.
78. Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn Reson Med. 1995;34:537–41.
79. Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. Philos Trans R Soc Lond B Biol Sci. 2005;360:1001–13.
80. Napadow V, LaCount L, Park K, As-Sanie S, Clauw DJ, Harris RE. Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. Arthritis Rheum. 2010;62:2545–55.
81. Seminowicz DA, Davis KD. Interactions of pain intensity and cognitive load: the brain stays on task. Cereb Cortex. 2007;17:1412–22.
82. Baliki M, Geha P, Apkarian A, Chialvo D. Impaired brain de-activation in chronic pain. In: Society for Neuroscience Annual Meeting. San Diego: Society for Neuroscience. 2007;825-822/II819.
83. Pujol J, Macia D, Garcia-Fontanals A, Blanco-Hinojo L, Lopez-Sola M, Garcia-Blanco S, Poca-Dias V, Harrison BJ, Contreras-Rodriguez O, Monfort J, Garcia-Fructuoso F, Deus J. The contribution of sensory system functional connectivity reduction to clinical pain in fibromyalgia. Pain. 2014;155:1492–503.
84. Cifre I, Sitges C, Fraiman D, Munoz MA, Balenzuela P, Gonzalez-Roldan A, Martinez-Jauand M, Birbaumer N, Chialvo DR, Montoya P. Disrupted functional connectivity of the pain network in fibromyalgia. Psychosom Med. 2012;74:55–62.
85. Ceko M, Bushnell MC, Fitzcharles MA, Schweinhardt P. Fibromyalgia interacts with age to change the brain. Neuroimage Clin. 2013;3:249–60.
86. Kim JY, Kim SH, Seo J, Kim SH, Han SW, Nam EJ, Kim SK, Lee HJ, Lee SJ, Kim YT, Chang Y. Increased power spectral density in resting-state pain-related brain networks in fibromyalgia. Pain. 2013;154:1792–7.
87. Seminowicz DA, Davis KD. Cortical responses to pain in healthy individuals depends on pain catastrophizing. Pain. 2006;120:297–306.

88. Stoeter P, Bauermann T, Nickel R, Corluka L, Gawehn J, Vucurevic G, Vossel G, Egle UT. Cerebral activation in patients with somatoform pain disorder exposed to pain and stress: an fMRI study. Neuroimage. 2007;36:418–30.
89. Gundel H, Valet M, Sorg C, Huber D, Zimmer C, Sprenger T, Tölle TR. Altered cerebral response to noxious heat stimulation in patients with somatoform pain disorder. Pain. 2008;137:413–21.
90. Petzke F, Clauw DJ, Ambrose K, Khine A, Gracely RH. Increased pain sensitivity in fibromyalgia: effects of stimulus type and mode of presentation. Pain. 2003;105:403–13.
91. Lui F, Duzzi D, Corradini M, Serafini M, Baraldi P, Porro CA. Touch or pain? Spatiotemporal patterns of cortical fMRI activity following brief mechanical stimuli. Pain. 2008;138:362–74.
92. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc Natl Acad Sci U S A. 2001;98:676–82.
93. Jorge LL, Amaro Jr E. Brain imaging in fibromyalgia. Curr Pain Headache Rep. 2012;16:388–98.
94. Sheline YI, Barch DM, Price JL, Rundle MM, Vaishnavi SN, Snyder AZ, Mintun MA, Wang S, Coalson RS, Raichle ME. The default mode network and self-referential processes in depression. Proc Natl Acad Sci U S A. 2009;106:1942–7.
95. O'Doneel LJ, Schultz T. Statistical and machine learning methods for neuroimaging: examples, challenges, and extensions to diffusion imaging data. In: Hotz I, Schultz T, editors. Visualization and processing of higher order descriptors for multi-valued data, mathematics and visualization. Cham: Springer; 2015.
96. Liu J, Zhao L, Lei F, Zhang Y, Yuan K, Gong Q, Liang F, Tian J. Disrupted resting-state functional connectivity and its changing trend in migraine suffers. Hum Brain Mapp. 2015;36:1892–907.
97. Baer RA. Mindfulness training as a clinical intervention: a conceptual and empirical review. Clin Psychol Sci Pract. 2003;10:125–43.
98. Grossman P, Niemann L, Schmidt S, Walach H. Mindfulness-based stress reduction and health benefits. A meta-analysis. J Psychosom Res. 2004;57:35–43.
99. Lauche R, Langhorst J, Paul A, Dobos G, Cramer H. Self-reported health and satisfaction of patients with chronic diseases who meditate: a case–control study. Qual Life Res. 2014;23:2639–44.
100. Lauche R, Cramer H, Häuser W. A systematic overview of reviews for complementary and alternative therapies in the treatment of the fibromyalgia syndrome. Evid Based Complement Alternat Med. 2015;1–13.
101. Hölzel BK, Ott U, Hempel H, Hackl A, Wolf K, Stark R, Vaitl D. Differential engagement of anterior cingulate and adjacent medial frontal cortex in adept meditators and non-meditators. Neurosci Lett. 2007;421:16–21.
102. Fox KC, Nijeboer S, Dixon ML, Floman JL, Ellamil M, Rumak SP, Sedlmeier P, Christoff K. Is meditation associated with altered brain structure? A systematic review and meta-analysis of morphometric neuroimaging in meditation practitioners. Neurosci Biobehav Rev. 2014;43C:48–73.
103. Fayed N, Lopez del Hoyo Y, Andres E, Serrano-Blanco A, Bellón J, Aguilar K, Cebolla A, Garcia-Campayo J. Brain changes in long-term Zen meditators using proton magnetic resonance spectroscopy and diffusion tensor imaging: a controlled study. PLoS One. 2013;8:e58476.
104. Kang D, Jo HJ, Jung WH, Kim SH, Jung YH, Choi CH, Lee US, An SC, Jang JH, Kwon JS. The effect of meditation on brain structure: cortical thickness mapping and diffusion tensor imaging. Soc Cogn Affect Neurosci. 2013;8:27–33.
105. Tang YY, Lu Q, Fan M, Yang Y, Posner MI. Mechanisms of white matter changes induced by meditation. Proc Natl Acad Sci. 2012;109:10570–4.
106. Brewer JA, Worhunsky PD, Gray JR, Tang YY, Weber J, Kober H. Meditation experience is associated with differences in default mode network activity and connectivity. Proc Natl Acad Sci. 2011;108:20254–9.
107. Hasenkamp W, Barsalou LW. Effects of meditation experience on functional connectivity of distributed brain networks. Front Hum Neurosci. 2012;6:38.
108. Jang JH, Jung WH, Kang DH, Byun MS, Kwon SJ, Choi CH, Kwon JS. Increased default mode network connectivity associated with meditation. Neurosci Lett. 2011;487:358–62.
109. Taylor VA, Daneault V, Grant J, Scavone G, Breton E, Roffe-Vidal S, Lavarenne AS, Marrelec G, Benali H, Beauregard M. Impact of meditation training on the default mode network during a restful state. Soc Cogn Affect Neurosci. 2013;8:4–14.
110. Lutz A, Brefczynski-Lewis J, Johnstone T, Davidson RJ. Regulation of the neural circuitry of emotion by compassion meditation: effects of meditative expertise. PLoS One. 2008;26:e1897.
111. Khoury B, Lecomte T, Fortin G, Masse M, Therien P, Bouchard V, Hofmann SG. Mindfulness-based therapy: a comprehensive meta-analysis. Clin Psychol Rev. 2013;33:763–71.
112. Grant JA, Courtemanche J, Rainville P. A non-elaborative mental stance and decoupling of executive and pain-related cortices predicts low pain sensitivity in Zen meditators. Pain. 2011;152:150–6.
113. Hölzel BK, Lazar SW, Gard T, Schuman-Olivier Z, Vago DR, Ott U. How does mindfulness meditation work? Proposing mechanisms of action from a conceptual and neural perspective. Perpsect Psychol Sci. 2011;6:537–59.
114. Price DD. Psychological and neural mechanisms of the affective dimension of pain. Science (New York, NY). 2000;288:1769–72.
115. Creswell JD, Way BM, Eisenberger NI, Lieberman MD. Neural correlates of dispositional mindfulness during affect labeling. Psychosom Med. 2007;69:560–5.

116. Harris RE, Clauw DJ, Scott DJ, McLean SA, Gracely RH, Zubieta JK. Decreased central mu-opioid receptor availability in fibromyalgia. J Neurosci. 2007;27:10000e6.
117. Arienzo D, Babiloni C, Ferretti A, Caulo M, Del Gratta C, Tartaro A, Rossini PM, Romani GL. Somatotopy of anterior cingulate cortex (ACC) and supplementary motor area (SMA) for electric stimulation of the median and tibial nerves: an fMRI study. Neuroimage. 2006;33:700–5.
118. Goldin PR, Gross JJ. Effects of mindfulness-based stress reduction (MBSR) on emotion regulation in social anxiety disorder. Emotion. 2010;10:83–91.
119. Napadow V, Kim J, Clauw DJ, Harris RE. Decreased intrinsic brain connectivity is associated with reduced clinical pain in fibromyalgia. Arthritis Rheum. 2012;64:2398–403.
120. Harris RE, Napadow V, Huggins JP, Pauer L, Kim J, Hampson J, Sundgren PC, Foerster B, Petrou M, Schmidt-Wilcke T, Clauw DJ. Pregabalin rectifies aberrant brain chemistry, connectivity, and functional response in chronic pain patients. Anesthesiology. 2013;119:1453–64.
121. Schmidt-Wilcke T, Ichesco E, Hampson JP, Kairys A, Peltier S, Harte S, Clauw DJ, Harris RE. Resting state connectivity correlates with drug and placebo response in fibromyalgia patients. Neuroimage Clin. 2014;6:252–61.
122. Hashmi JA, Baria AT, Baliki MN, Huang L, Schnitzer TJ, Apkarian AV. Brain networks predicting placebo analgesia in a clinical trial for chronic back pain. Pain. 2012;153:2393–402.
123. Sevel LS, O'Shea AM, Letzen JE, Craggs JG, Price DD, Robinson ME. Effective connectivity predicts future placebo analgesic response: a dynamic causal modeling study of pain processing in healthy controls. Neuroimage. 2015;110:87–94.
124. Olivan-Blázquez B, Herrera-Mercadal P, Puebla-Guedea M, Pérez-Yus MC, Andrés E, Fayed N, López-Del-Hoyo Y, Magallon R, Roca M, Garcia-Campayo J. Efficacy of memantine in the treatment of fibromyalgia: a double-blind, randomised, controlled trial with 6-month follow-up. Pain. 2014;155:2517–25.
125. Fayed N, Olivan-Blázquez B, Herrera-Mercadal P, Puebla-Guedea M, Pérez-Yus MC, Andrés E, López del Hoyo Y, Magallon R, Viguera L, Garcia-Campayo J. Changes in metabolites after treatment with memantine in fibromyalgia. A double-blind randomized controlled trial with magnetic resonance spectroscopy with a 6-month follow-up. CNS Neurosci Ther. 2014;20:999–1007.
126. O'Muircheartaigh J, Marquand A, Hodkinson DJ, Krause K, Khawaja N, Renton TF, Huggins JP, Vennart W, Williams SC, Howard MA. Multivariate decoding of cerebral blood flow measures in a clinical model of on-going postsurgical pain. Hum Brain Mapp. 2015;36:633–42.
127. Baliki MN, Petre B, Torbey S, Herrmann KM, Huang L, Schnitzer TJ, Fields HL, Apkarian AV. Corticostriatal functional connectivity predicts transition to chronic back pain. Nat Neurosci. 2012;15:1117–9.
128. Mansour AR, Baliki MN, Huang L, Torbey S, Herrmann KM, Schnitzer TJ, Apkarian AV. Brain white matter structural properties predict transition to chronic pain. Pain. 2013;154:2160–8.

31 Utility of Magnetic Resonance Findings in Elucidating Structural and Functional Brain Impairment in Traumatic Brain Injury

Eduardo González-Toledo, Nicolás Fayed Miguel, Laura Viguera, Kanika Sharma, Piyush Kalakoti, Navdeep Samra, Anil Nanda, and Hai Sun

Abstract

Traumatic brain injury (TBI) is a major cause of death and disability in the United States, contributing to about 30% of all injury-related deaths. TBI survivors often develop clinical impairments and long-term disabilities. These include impaired thinking or memory, effects on movement and sensations such as vision, hearing, or emotional functioning including personality changes, depression, burst of anger, abnormal social behavior, and insomnia. These issues not only affect individuals but can have a deleterious impact on families and communities. The advances in computer software applied to a non-invasive acquisition of images containing digital data, provides us with objective examination of brain structure and function. Magnetic resonance (MR) imaging of the brain makes it possible to investigate morphological and functional connectivity without exposing the patient to ionizing radiations. In patients with TBI, computed tomography and conventional MR scans seldom show limited or no abnormalities to explain clinical symptomatology. For these reasons, we propose an "ad hoc" protocol that exploits advances in MR sequences to predict long-term outcomes including evaluation of cortical thickness, detecting hemosiderin

E. González-Toledo, MD, PhD (✉)
Department of Radiology, Neurology, and Anesthesiology, Louisiana State University Health Sciences Center, Shreveport, LA, USA
e-mail: egonz1@lsuhsc.edu; ecgtoledo@gmail.com

N.F. Miguel, MD, PhD
Radiology, Hospital Quiron, Madrid, Spain

L. Viguera, MD
Miguel Servet University Hospital, University of Zaragoza, Zaragoza, Spain

K. Sharma, MD • P. Kalakoti, MD • A. Nanda, MD, MPH • H. Sun, MD, PhD
Neurosurgery, Louisiana State University Health Sciences Center, Shreveport, LA, USA

N. Samra, MD
Trauma and Surgical Critical Care, Louisiana State University Health Sciences Center, Shreveport, LA, USA

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_31

deposits via magnetic susceptibility weighted images, to explore indemnity of fiber tracts using diffusion tensor with fractional anisotropy measurement, to assess metabolic changes in the frontal lobe and cingulate cortex by utilizing the properties of magnetic resonance spectroscopy, and lastly to detect abnormal connectivity in the brain networks via resting-state functional magnetic resonance imaging. Meticulous application of our protocol can potentially detect subtle abnormalities in patients with mild TBI such as detection of iron or mineral deposits, abnormal cortical thickness, abnormal metabolites, disruption of white matter tracts, and decreased or loss connectivity in brain networks. Application of special MR sequences as described in our protocol can optimize clinical outcomes, offer predictive capabilities of short and long-term prognosis, and aid in risk-stratification tailored upon individual comorbidities.

Keywords

Traumatic brain injury • Diffuse axonal injury • DTI • Rs-fMRI • Cortical thickness • Susceptibility imaging • DWI • MRS

Introduction

In technical parlance, traumatic brain injury (TBI) is described as "an alteration in brain function, or other evidence of brain pathology, caused by an external force." Synonymous with its nomenclature, TBI simply refers to structural and functional changes in the brain attributable to external trauma [1, 2]. The desideratum for an external force distinguishes TBI from various acquired brain injuries, including vascular insults, and neoplastic and degenerative pathologies [3]. Typical mechanisms leading to TBI, albeit heterogeneous in nature, include blunt trauma, penetrating injury, blast waves, and sudden acceleration or deceleration. Both the magnitude and transfer of impact to the scalp vault and its contents determine the severity of damage, presenting variably in the form of cerebral edema, focal contusions, hematomas, and shearing of white matter tracts leading to diffuse axonal injury (DAI).

Affecting population across all age groups, TBI has become one of the leading causes of mortality and disability in children and adolescents worldwide [4–8]. In the United States (US), TBI accounts for approximately 30% of all deaths resulting from injury, averaging 138 deaths per day [9]. The dramatic increment in the cumulative rates for TBI-related emergency department (ED) visits, hospitalizations, and mortality across the globe as witnessed during the previous decades have been concerning from a public health perspective. In the US, this upsurge translated from approximately 1.5 million cases in 2003 to 2.5 million in 2010, an increase in incidence to over 50%, from 538 per 100,000 at baseline to 823 per 100,000 in 2010 [4–10]. During the same period, the average mortality from TBI declined from 18.2 deaths per 100,000 to 17.1 per 100,000, a meager rate of 6% [4] Considering under-reporting of events and limited accountability for uninsured patients with non-fatal TBI without access to healthcare facilities and those seen at private clinics, the projected estimates far undervalue the actual magnitude of burden posed by TBI. The economic impact from these low estimates is equally colossal. In 2010 alone, healthcare spending including direct and indirect costs for management of patients with TBI stood at a staggering US$ 76.5 billion, [11] contributing to approximately 3% of the national health expenditures for that year [12]. A major proportion of this economic burden is attributed to long-term residual disability seen in patients with TBI, in the form of motor and sensory deficits, cognitive impairments, and emotional disturbances. Insomnia,

cognitive decline, post-traumatic headache, and depression are common factors limiting a patient's reintegration into the community and return to employment [13–18]. Post-traumatic depression following TBI contributes independently to cognitive decline [16–18], which affects quality of life over the long term. TBI has also been implicated in delayed-onset neurodegenerative syndromes such as Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE). Brain pathology following a single incident of severe TBI mimics closely that seen in AD during early amyloid pathology, whereas repetitive TBI can produce tauopathy with or without amyloidosis, which resembles the pathology of boxers' dementia [19–21]. Although clinical assessment of TBI severity based upon the Glasgow coma scale (GCS) [22] provides some insight into the extent of severity at the time of presentation, it is often under-predictive of the actual extent of structural and impending functional damage to the brain, and is often deemed unreliable for predicting acute prognosis or long-term sequelae. To this effect, neuroimaging, particularly the MR imaging, plays a crucial role in determining the extent of injury, providing guidance for surgical management, and predicting prognosis. In this article, we provide a comprehensive overview on the utility of magnetic resonance (MR) imaging in explaining anatomical and functional brain impairment in patients with TBI.

Neuroimaging in TBI

Integration of technological advancements in the digital world has led to development and enhancement of non-invasive neuroimaging modalities employed for objective assessment of anatomical, functional, and metabolic milieu of the brain. This has led to improved diagnosis and subsequent management for patients with TBI. Ability to assess these changes confers risk-stratification via gauging severity, predicting prognosis, and streamlining management for these patients. While head roentgenogram may have become obsolete in today's era, other neuroimaging modalities such as computed tomography, MR imaging, positron emission tomography (PET), and single-photon emission computed tomography (SPECT) provide valuable insights on brain abnormalities.

Conventional computed tomography (CT) is a routinely employed diagnostic procedure to assess acute head injury requiring observation or admission [23]. By using the degree of X-ray attenuation, CT scans can differentiate between normal brain with the presence of bleed, contusions, discontinuity in scalp or facial bones, edema, and ischemia. With the advent of high-resolution, multi-detector scanners, scanning duration has dramatically reduced, and offers selective re-scanning of slices affected by motion artifacts [24]. Three-dimensional (3D) reconstruction depicts bony injury and intracranial pathologies, if any [25]. Despite obvious advantages of CT in the initial detection of head injury with surgical guidance in management of acute cases and its cost-effectiveness, conventional CT scans have limitations in detecting the subtle neuronal damage and diffuse axonal injury seen in over 50% patients with TBI. These subtle changes form the basis of residual disability and cognitive impairment from TBI [26–29]. Most of these limitations in detecting these neuronal changes can be mitigated through the use of specialized magnetic resonance (MR) sequences. Structural MR sequences in conjunction with functional MR imaging can potentially provide accurate assessment of extent and severity of brain injury in these patients.

Magnetic Resonance Imaging: An Overview

Of the various available neuroimaging modalities, developments in MR technology have been remarkable. It is based upon the principle of nuclear magnetic resonance. In the presence of a static magnetic field, nuclei of atoms (mainly protons) resonate when varying electromagnetic fields are applied at a fixed frequency. The MR machine computes an image based on the "resonance" signals to compute spatial orientation based on processing the frequency and phase in these signals. Diverse MR sequences exploit the physical properties of the target tissue (protons) to provide information on morphological and functional integrity. MR signals are obtained from several parameters such as T1, T2, proton

density and flow, chemical shift, and molecular diffusion [30]. Unlike CT scans, MR imaging neutralizes the risk of being exposed to ionizing radiation, thus eliminating the risk of radiation-induced DNA damage that has been implicated as a potential risk factor for carcinogenesis [31, 32]. With increasing availability in emergency settings, MR imaging constitutes a valuable tool for baseline assessment in practically all patients with TBI, albeit with some contraindication. An absolute contraindication for brain MR is for patients with cardiac pacemakers, penile implants, cochlear implants, and ferromagnetic materials, and relative contraindications are metallic implants including, but not limited to, vascular clips, coronary and peripheral arterial stents, prosthetic heart valves, cardiac devices, aortic stent grafts, vena cava filters, hemodynamic monitoring, and pacing devices [23]. Claustrophobic patients and those with tattoos are some relative contraindications for MR scans. Some MR scans utilize contrast agents, therefore those with renal insufficiency or hypersensitivity, or are pregnant or breastfeeding may not be eligible candidates [33].

Structural MR Imaging

In contrast to CT scans, conventional MR scans are more sensitive in depicting minute areas of petechial hemorrhages, contusions, or extra-axial hematomas, axonal injury [34–37], and white matter abnormalities [38]. In patients with mild TBI, conventional MR scans depict abnormal findings in approximately one third of patients with normal CT scans [35–39]. T1-weighted MR scans provides descriptive overview of anatomic affection of the brain, if any, such as midline shift, ventricular distortion, or mass effect. Although gadolinium-based contrast may offer few advantages over non-contrast scans in regards to structural anatomical changes in mild TBI, special MR sequences such as fluid attenuation inversion recovery (FLAIR) and gradient echo have shown particularly high sensitivity for appreciating axonal injury, and in predicting outcomes [40, 41].

- *FLAIR*: The FLAIR technique permits detection of periventricular and superficial cortical lesions [42]. By diminishing the signal from CSF while concurrently amplifying the intensity of lesions that are non-fluid-containing, FLAIR is of utility in identifying lesions in close proximity to the CSF-filled subarachnoid and ventricular spaces. Areas of T2 prolongation appear as bright, while normal CSF signals are depicted dark [42]. FLAIR is helpful in detecting non-hemorrhagic DAI and sub-arachnoid hemorrhage.
- *Gradient echo sequence* (*GRE*): T2-weighted gradient echo MR is sensitive to signal intensity loss that results from changes in magnetic susceptibility. GRE is sensitive in detecting the presence of blood breakdown products such as deoxyhemoglobin, intracellular methemoglobin, ferritin and hemosiderin. This is useful in detecting hemorrhagic DAI and contusions.
- *Susceptibility-weighted imaging* (*SWI*): This is a relatively newer contrast type of MR that differs from T1- or T2-weighted imaging that exploits magnetic susceptibility differences across various tissues such as calcium and iron, and uses phase image signals to detect these differences. It is sensitive in detecting microbleeds in the form of paramagnetic hemoglobin or intracellular hemorrhages [43]. It is also used to image venous blood via the blood-oxygen-level-dependent (BOLD) technique.
- *Short tau inversion recovery* (*STIR*): STIR signals attenuate fat signals, and provide distinction of water-containing lesions in areas with relative fat abundance such as the orbit, head and neck, or spine. STIR improves T1 or T2 lesion conspicuity, and is useful in avoiding chemical shift artifacts. While its utility as a diagnostic tool is limited in TBI, STIR is often used to differentiate between lipomas and hemorrhage, evaluation of optic nerve injury, and vertebral body compression fractures in patients with head trauma.
- *Diffusion-weighted imaging* (*DWI*): DWI processes information based upon differences in water molecule diffusion rate by employing echo-planar or line-scan spin echo MR technique. The measure of mobility of water molecules is reflected via the apparent diffusion coefficient (ADC). Regions with relatively higher degree of diffusion such as

that of the CSF appear hypo-intense with a high ADC value, while areas with restricted diffusion, such as protons within grey or white matter, appear hyper-intense with low ADC value. A distinction between cytotoxic and vasogenic edema can be made using DWI. While the former depicts characteristics of restricted diffusion, vasogenic edema demonstrates signs of increased diffusion. In patients with mild TBI, focal areas of restricted diffusion associated with cerebral edema or DAI are often seen. In contrast to FLAIR and T2-weighted imaging, DWI demonstrates a greater degree and extent of abnormalities in patients with TBI. Regions with acute DAI brighten up and appear dark in ADC due to restricted pattern of diffusion from plausible cellular death.

- *Diffusion tensor imaging* (*DTI*): DTI is an extension of DWI that senses diffusion of water molecules across several directions, along the course of nerve fibers, with a tensor applied to describe diffusion in an anisotropic system. This forms the basis for the 3D reconstruction of the fiber tracts (white matter), thus enabling the possibility of exploring broken connections [44]. Key approaches to assess microstructural damage include whole-brain voxel-based analysis, region-of-interest (ROI) analysis and in-vivo tractography. A quantified estimate of DTI data is derived from the functional anisotropy (FA) value, which ranges from 0 to 1. An FA value of zero depicts an isotropic diffusion occurring in all directions, while FA value of 1 indicates a unidirectional diffusion. A standardized color coding is applied in 2D representation to depict direction of fibers; red representing lateral commissural pathways, green for anterior–posterior pathways, and blue indicating cranial–caudal pathways. In patients with mild TBI with normal CT scans and GCS 15, DTI is regarded as a potential biomarker as it detects micro-structural changes in white matter, even in patients with mild TBI, as opposed to other MR sequences [45–49].

A decreased FA value corresponds to axonal degradation and fiber discontinuity owing to intertract or perivascular accumulation of water, and can be detected as early as 24 h after TBI [50–53].

DTI studies have confirmed decreased FA value in the corpus callosum, which sustains a high degree of deformation [53, 54]. Structural abnormalities in the corpus callosum as shown by DTI indices correlated clinically with cognitive, somatic, and affective disorders as seen post injury in these patients. An association between quantitative measures of gait function and DTI findings demonstrate white matter integrity in the genu of corpus callosum to be an important marker of gait [55]. Other common brain regions affected in mild TBI detected on DTI include anterior and posterior cingulum, middle cerebellar peduncles, and inferior longitudinal (ILF) and uncinate fasciculi (UF). As cingulum is the fiber tract related to the limbic system, any structural abnormality is associated clinically with depression, memory loss, lack of social restraint, aggressiveness, heightened sexuality, and bulimia [56]. The anterior cingulum is linked to emotion, especially apathy and depression, while the posterior cingulum is more related to cognitive functions [57, 58]. Structural abnormalities as detected in the ILF bundle using DTI can explain functional impairments such as thought disorders, visual emotion, and cognitive impairment [59]. Studies have demonstrated abnormalities in DTI to correlate with symptom severity, and with predicting long-term cognitive impairments [48, 52, 60, 61]. Disruption of the UF may cause problems with expression of memory, decision making, and acquisition of certain types of learning and memory. Additionally, uncinate involvement in TBI often extends beyond memory to include social–emotional problems and low motivation [62].

- *Magnetic resonance angiography* (*MRA*): This is a specialized form of MR imaging that visualizes blood vessels as opposed to brain tissues. It can detect bleed or patency of blood vessels, and is often used to screen for evidence of vascular injury in the head and neck region in patients with TBI [63].
- *Cortical thickness*: Using high-resolution T1 anatomical MR images, evaluation of cortical changes using an automated, vertex-based reconstruction for measurement of thickness

of the brain cortex can be performed [62–77]. This provides baseline assessment of cortical integrity. Cortical thinning occurs in TBI, and correlates with measures of PTSD, depression, executive functioning, declarative memory loss, and post-concussive symptoms [68, 78–80]. Precuneus thickness is correlated to acute traumatic stress symptoms in TBI survivors. Recent evidence suggests structural changes in frontal cortex over 3 months following mild TBI [81].

Functional Imaging

- *Magnetic resonance spectroscopy* (*MRS*): MRS is similar to conventional MR that uses properties of magnetism. As opposed to MR that utilizes time domain to obtain T1 and T2 relaxation times that are processed as images, MRS data uses frequency-domain information to display a spectrum of signal intensity from different brain metabolites [82]. The main metabolites are N-acetyl aspartate (NAA) related to neurons, creatine (Cr) related to energetic metabolism, choline (Cho) representing membrane metabolism, and myoinositol (mI) representing glial cells. Data is quantified as a ratio of all metabolites with respect to creatinine. In children with TBI, a disturbance in brain metabolites is predictive of overall outcomes relating to behavioral and cognitive functions both in acute and long-term phase [83–86].
- *Resting-state fMRI*: Several studies have demonstrated that damage to white matter alters structural integrity, which leads to impairment in functional connectivity across regions of the brain. Structural and functional disruptions are implicated in cognitive impairment in TBI [87–90]. Resting-state fMRI assesses functional connectivity in the brain following severe TBI and even in patients with mild TBI during the initial phase [90–96]. As it processes brain connectivity in the absence of any task or activity, this modality of MR permits functional evaluation irrespective of severity and cognitive functions. Using advanced neuroimaging processing tools, functional connectivity during resting state can be studied effectively. The most commonly studied functional connectivity network during resting state is the default mode network (DMN) [97]. Although commonly related to the cognitive process, DMN can be affected in a broad range of disorders affecting the brain [98]. Resting-state fMRI assesses changes in oxygen delivery to various centers that are synchronously connected within a time duration of 8 min while the patient is at rest (not performing any task). The data processed and reconstructed to depict any synchrony across various regions is compared to a pool of normal controls. This generates a brain map with areas of abnormally decreased connectivity or increased connectivity within target centers.

Findings in Traumatic Brain Injury

In our protocol to study morphological, metabolic, and functional characteristics of the patients with TBI, we routinely employ T1-weighted images, T2 and proton density, diffusion-weighted sequence, tensor sequence, SWI, FLAIR sequence, magnetic resonance spectroscopy, and resting-state fMRI.

With no likely abnormality being seen on CT scans and conventional MRI in most patients with TBI, an "ad-hoc" protocol is recommended for unanimous implementation across centers for complete MR evaluation for patients with TBI. This should mandate cortical thickness reconstruction, magnetic susceptibility weighted sequences for detecting any hemosiderin deposits, DTI for measurement of FA values for structural integrity of white matter tracts, and lastly resting-state fMRI for functional regional connectivity across the brain. The protocol is viable even for patients with mild TBI, as they present a pattern with one or more of the following:

(a) Hemosiderin deposits in temporal and frontal poles that could be picked up with magnetic susceptibility weighted sequences.
(b) Cortical thickness or abnormal integrity in frontal dorsomedial and central decreased

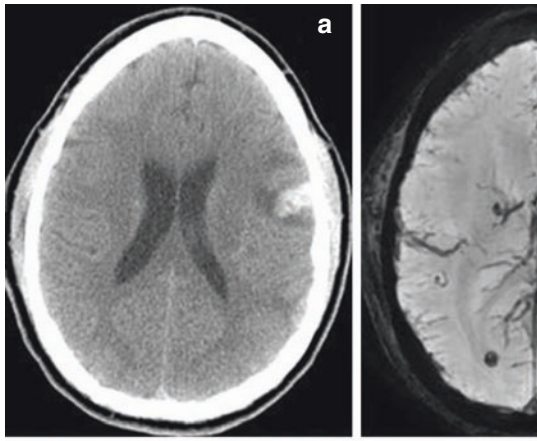
cortical thickness which may extend to the parietal, depending on the power of the impact, as well as in the ventral surfaces of the brain such as the orbitofrontal cortex, temporal poles and temporo-occipital areas [99–105].

(c) Abnormal fractional anisotropy values in the genu of the corpus callosum and cingulum fibers [106–120].

(d) Decreased NAA in magnetic resonance spectroscopy indicating neuronal loss, mostly in frontal lobes [121, 122].

(e) A decrease in or loss of connectivity to the frontal cortex from anterior and posterior cingulum on rsfMRI [92, 123–125].

Implementation of the aforementioned protocol at presentation, short- and long-term follow up can help unveil microstructural changes to explain and predict long-term outcomes. These findings may intuitively form the basis of rehabilitation, and an octagonal approach for long-term care, and plausibly attenuate residual disability (Figs. 31.1, 31.2, 31.3 and 31.4).



e

WM tract	Patient FA	Normal	SD
Genu cc	0.539396	0.74	0.04
Splenium cc	0.525055	0.78	0.03
cing R	0.465562	0.45	0.02
cing L	0.482117	0.45	0.02
ILF R	0.35729	0.41	0.03
ILF L	0.322366	0.41	0.03
UF R	0.277559	0.33	0.02
UF L	0.305373	0.33	0.02
SLF R	0.549114	0.42	0.02
SLF L	0.51181	0.42	0.02

Fig. 31.1 Patient is a 20-year-old male admitted to the hospital for 30 ft. fall from oil rig. Patient was helicoptered in from the field and intubated due to low GCS 4–5. Presented with subarachnoid hemorrhage, brain laceration in the left frontal lobe, multiple skull fractures. *After* 3 *years the patient showed cognitive decline, depression, bursts of anger, decreased capacity for planning, bad social interaction*. Never returned to work. (**a**) Computed tomography in transverse view showing laceration and hematoma in left frontal lobe. (**b**) Magnetic resonance with susceptibility sequence depicts the frontal hemorrhage and blood deposits in the ventricles. (**c**) Hemosiderin deposits in microglia appear 3 years after first magnetic resonance in the susceptibility sequence. (**d**) Decreased cortical thickness (*blue*) in the frontal lobe in the same patient pinpointing Brodmann's areas involved. (**e**) Diffusion tensor imaging performed in the same patient with decreased fractional anisotropy values in corpus callosum and inferior longitudinal fasciculus

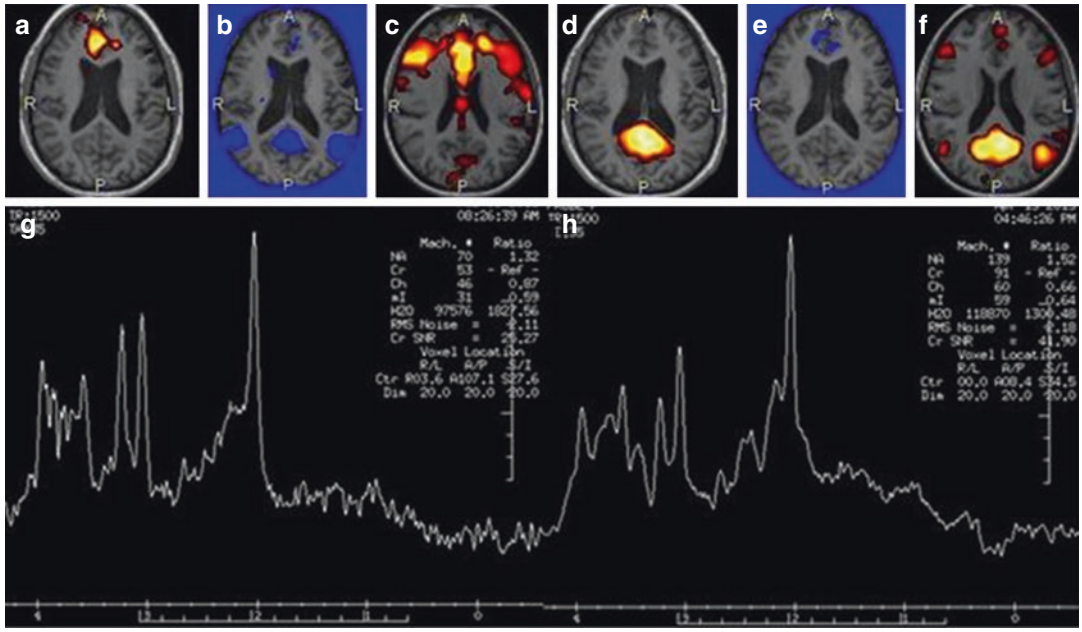


Fig. 31.2 Resting-state functional magnetic resonance. (**a**) Functional connectivity. A seed was placed in the anterior cingulum. No connectivity with posterior cingulum and dorsal frontal cortex compared with normal in (**c**). (**b**) Z-test, patient compared to 20 normal individuals depicting decreased connectivity in the posterior cingulum. (**d**) Normal frsfMRI with seed in anterior cingulum. (**e**) Resting-state functional magnetic resonance. Compared to normal in (**f**), there is no connectivity with posterior cingulum, frontal cortex, angular cortex. (**f**) Z-test showing decreased connectivity in the patient's anterior cingulum. (**f**, **g**) Magnetic resonance spectroscopy. Decreased n-acetyl aspartate in the frontal lobe. NAA is a marker for neurons, indicating decreased neuronal content in the frontal lobe. There is also an increase in myoinositol, a marker for glia. This correlates with increase in scarring and fibrillary content

ROI	FA	Normal	SD
genucc	0.375139	0.74	0.04
spleniumcc	0.419388	0.78	0.03

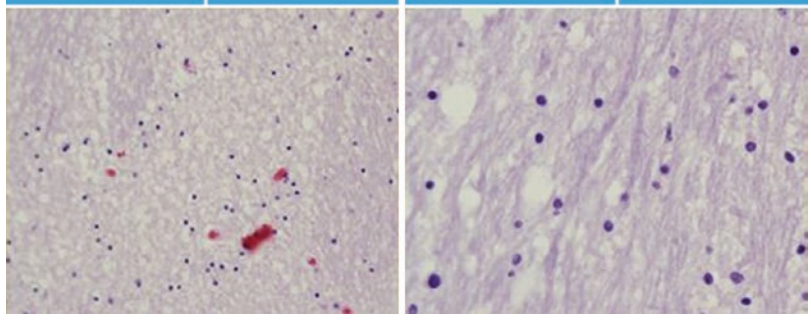


Fig. 31.3 Autopsy in a patient who died from TBI. MR was obtained before death. Correlation of abnormal fractional anisotropy with pathology. Swollen and disrupted fibers in genu and splenium of corpus callosum correlate with fractional anisotropy (FA) abnormal values

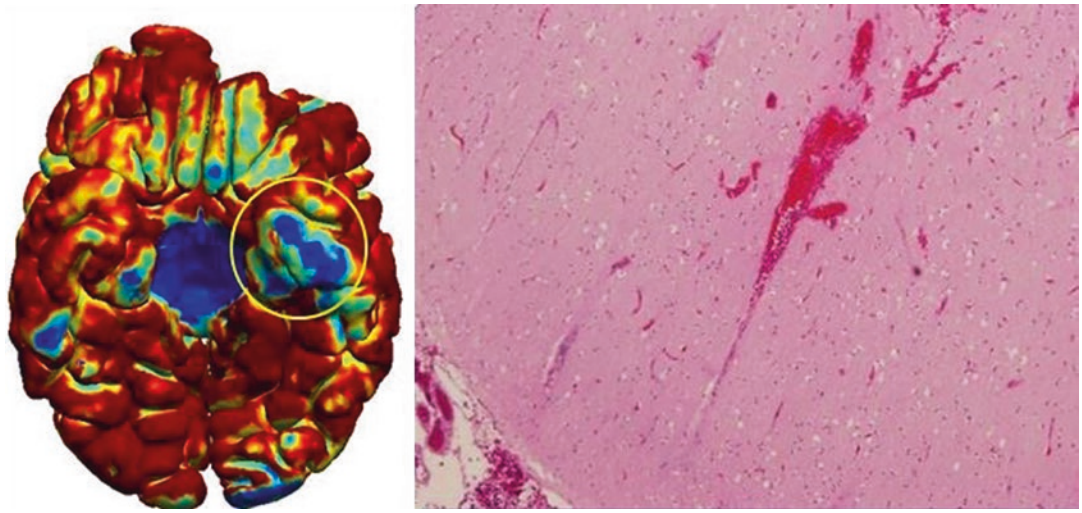


Fig. 31.4 Autopsy case. Patient with encephalomalacia in frontal pole after surgery for resection of a meningioma. He sustained a seizure while driving. MR obtained before death. Correlation between abnormal cortical thickness (*blue*) and contusion demonstrated by pathology

Conclusions

Neuroimaging has increasingly become a vital tool for management of patients with head injury. While conventional CT and MR modalities offer rapid structural assessment ensuring prompt institution of surgical management for selective cases, functional modalities allow accurate prediction of overall functional and clinical outcomes in patients with TBI. With easy accessibility to MR technology, complex MR sequences entailing deeper insights into structural and functional impairments should routinely be employed in assessment of patients with TBI. MR imaging techniques additionally enhance our knowledge base relating to anatomic abnormalities and functional outcomes. Higher resolution scans, integration of digital software for data processing, and technical advancements offer a viable solution for automation in image processing and interpretation.

References

1. Kalakoti P, Notarianni C. Revisiting traumatic brain injury in the pediatric population. World Neurosurg. 2016;91:635–7.
2. Menon DK, Schwab K, Wright DW, et al. Position statement: definition of traumatic brain injury. Arch Phys Med Rehabil. 2010;91(11):1637–40.
3. McAllister TW. Neurobiological consequences of traumatic brain injury. Dialogues Clin Neurosci. 2011;13(3):287–300.
4. Centers for Disease Control and Prevention. Injury prevention & control: traumatic brain injury & concussion: rates of TBI-related emergency department visits, Hospitalizations, and deaths — United States, 2001–2010. Last accessed: 28 June 2016 [URL: http://www.cdc.gov/traumaticbraininjury/data/rates.html].
5. Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G, Kobusingye OC. The impact of traumatic brain injuries: a global perspective. NeuroRehabilitation. 2007;22(5):341–53.
6. Langlois JA, Rutland-Brown W, Thomas KE. The incidence of traumatic brain injury among children in the United States: differences by race. J Head Trauma Rehabil. 2005;20(3):229–38.
7. Reid SR, Roesler JS, Gaichas AM, Tsai AK. The epidemiology of pediatric traumatic brain injury in Minnesota. Arch Pediatr Adolesc Med. 2001;155(7):784–9.
8. Winthrop AL, Brasel KJ, Stahovic L, Paulson J, Schneeberger B, Kuhn EM. Quality of life and functional outcome after pediatric trauma. *J Trauma.* 2005;58(3):468–73; discussion 473–64.
9. Centers for Disease Control and Prevention. Injury prevention & control: traumatic brain injury & concussion: basic information. Last accessed: 28 June 2016 [URL:http://www.cdc.gov/traumaticbraininjury/get_the_facts.html].

10. Rutland-Brown W, Langlois JA, Thomas KE, Xi YL. Incidence of traumatic brain injury in the United States, 2003. J Head Trauma Rehabil. 2006;21(6):544–8.
11. Centers for Disease Control and Prevention. Injury prevention & control: traumatic brain injury & concussion: basic information. Last accessed: 28 June 2016 [URL:http://www.cdc.gov/traumaticbraininjury/severe.html].
12. Centers for Medicare & Medicaid Services, Office of the Actuary, National Health Statistics Group; U.S. Department of Commerce, Bureau of Economic Analysis; and U.S. Bureau of the Census. National health expenditure data. Last acessed: 28 June 2016 [URL: https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/NationalHealthExpendData/NationalHealthAccountsHistorical.html].
13. Pangilinan PH, Kelly BM, Hornyak JEI, et al. Classification and complications of traumatic brain injury. Medscape. June 10, 2016 Last accessed: 16 June 2016 [URL: http://emedicine.medscape.com/article/326643-overview].
14. Adams JH, Graham DI, Jennett B. The structural basis of moderate disability after traumatic brain damage. J Neurol Neurosurg Psychiatry. 2001;71(4):521–4.
15. Evans SA, Airey MC, Chell SM, Connelly JB, Rigby AS, Tennant A. Disability in young adults following major trauma: 5 year follow up of survivors. BMC Public Health. 2003;3:8.
16. Chamelian L, Feinstein A. The effect of major depression on subjective and objective cognitive deficits in mild to moderate traumatic brain injury. J Neuropsychiatry Clin Neurosci. 2006;18(1):33–8.
17. Jorge RE, Robinson RG, Moser D, Tateno A, Crespo-Facorro B, Arndt S. Major depression following traumatic brain injury. Arch Gen Psychiatry. 2004;61(1):42–50.
18. Fann JR, Burington B, Leonetti A, Jaffe K, Katon WJ, Thompson RS. Psychiatric illness following traumatic brain injury in an adult health maintenance organization population. Arch Gen Psychiatry. 2004;61(1):53–61.
19. DeKosky ST, Blennow K, Ikonomovic MD, Gandy S. Acute and chronic traumatic encephalopathies: pathogenesis and biomarkers. Nat Rev Neurol. 2013;9(4):192–200.
20. Deb S, Burns J. Neuropsychiatric consequences of traumatic brain injury: a comparison between two age groups. Brain Inj. 2007;21(3):301–7.
21. Till C, Colella B, Verwegen J, Green RE. Postrecovery cognitive decline in adults with traumatic brain injury. Arch Phys Med Rehabil. 2008;89(12 Suppl):S25–34.
22. Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. Lancet. 1974;2(7872):81–4.
23. National Institute for Clinical Excellence. Head injury: Triage, Assessment, Investigation and Early Management of Head Injury in Infants, Children and Adults. National Collaborating Centre for Acute Care (UK). Source London: National Collaborating Centre for Acute Care (UK); 2007.
24. Jones TR, Kaplan RT, Lane B, Atlas SW, Rubin GD. Single- versus multi-detector row CT of the brain: quality assessment. Radiology. 2001;219(3):750–5.
25. Newberg AB, Alavi A. Neuroimaging in patients with head injury. Semin Nucl Med. 2003;33(2):136–47.
26. Ogawa T, Sekino H, Uzura M, et al. Comparative study of magnetic resonance and CT scan imaging in cases of severe head injury. Acta Neurochir Suppl (Wien). 1992;55:8–10.
27. Levin HS, Amparo E, Eisenberg HM, et al. Magnetic resonance imaging and computerized tomography in relation to the neurobehavioral sequelae of mild and moderate head injuries. J Neurosurg. 1987;66(5):706–13.
28. Lee H, Wintermark M, Gean AD, Ghajar J, Manley GT, Mukherjee P. Focal lesions in acute mild traumatic brain injury and neurocognitive outcome: CT versus 3 T MRI. J Neurotrauma. 2008;25(9):1049–56.
29. Hammoud DA, Wasserman BA. Diffuse axonal injuries: pathophysiology and imaging. Neuroimaging Clin N Am. 2002;12(2):205–16.
30. Hunter JV, Wilde EA, Tong KA, Holshouser BA. J Neurotrauma. 2012;29(4):654–71.
31. Abdelhalim AN, Alberico RA. Pediatric neuroimaging. Neurol Clin. 2009;27(1):285–301, x.
32. Ketonen LM, Valanne L. Neuroimaging of pediatric diseases. Semin Neurol. 2008;28(4):558–69.
33. Dill T. Contraindications to magnetic resonance imaging: non-invasive imaging. Heart. 2008;94(7):943–8.
34. Hesselink JR, Dowd CF, Healy ME, Hajek P, Baker LL, Luerssen TG. MR imaging of brain contusions: a comparative study with CT. AJR Am J Roentgenol. 1988;150(5):1133–42.
35. Hughes DG, Jackson A, Mason DL, Berry E, Hollis S, Yates DW. Abnormalities on magnetic resonance imaging seen acutely following mild traumatic brain injury: correlation with neuropsychological tests and delayed recovery. Neuroradiology. 2004;46(7):550–8.
36. Wilberger Jr JE, Rothfus WE, Tabas J, Goldberg AL, Deeb ZL. Acute tissue tear hemorrhages of the brain: computed tomography and clinicopathological correlations. Neurosurgery. 1990;27(2):208–13.
37. Yuh EL, Mukherjee P, Lingsma HF, et al. Magnetic resonance imaging improves 3-month outcome prediction in mild traumatic brain injury. Ann Neurol. 2013;73(2):224–35.
38. Garnett MR, Cadoux-Hudson TA, Styles P. How useful is magnetic resonance imaging in predicting severity and outcome in traumatic brain injury? Curr Opin Neurol. 2001;14(6):753–7.
39. Mittl RL, Grossman RI, Hiehle JF, et al. Prevalence of MR evidence of diffuse axonal injury in patients

with mild head injury and normal head CT findings. *AJNR*. Am J Neuroradiol. 1994;15(8):1583–9.
40. Kampfl A, Franz G, Aichner F, et al. The persistent vegetative state after closed head injury: clinical and magnetic resonance imaging findings in 42 patients. J Neurosurg. 1998;88(5):809–16.
41. Kampfl A, Schmutzhard E, Franz G, et al. Prediction of recovery from post-traumatic vegetative state with cerebral magnetic-resonance imaging. Lancet. 1998;351(9118):1763–7.
42. Coles JP. Imaging after brain injury. Br J Anaesth. 2007;99(1):49–60.
43. Haacke EM, Xu Y, Cheng YC, Reichenbach JR. Susceptibility weighted imaging (SWI). Magn Reson Med. 2004;52(3):612–8.
44. Hofer S, Frahm J. Topography of the human corpus callosum revisited – comprehensive fiber tractography using diffusion tensor magnetic resonance imaging. Neuroimage. 2006;32(3):989–94.
45. Bigler ED, Bazarian JJ. Diffusion tensor imaging: a biomarker for mild traumatic brain injury? Neurology. 2010;74(8):626–7.
46. Mac Donald CL, Johnson AM, Cooper D, et al. Detection of blast-related traumatic brain injury in U.S. military personnel. N Engl J Med. 2011;364(22):2091–100.
47. Mayer AR, Ling J, Mannell MV, et al. A prospective diffusion tensor imaging study in mild traumatic brain injury. Neurology. 2010;74(8):643–50.
48. Wilde EA, McCauley SR, Hunter JV, et al. Diffusion tensor imaging of acute mild traumatic brain injury in adolescents. Neurology. 2008; 70(12):948–55.
49. Brandstack N, Kurki T, Tenovuo O. Quantitative diffusion-tensor tractography of long association tracts in patients with traumatic brain injury without associated findings at routine MR imaging. Radiology. 2013;267(1):231–9.
50. Lipton ML, Gulko E, Zimmerman ME, et al. Diffusion-tensor imaging implicates prefrontal axonal injury in executive function impairment following very mild traumatic brain injury. Radiology. 2009;252(3):816–24.
51. Arfanakis K, Haughton VM, Carew JD, Rogers BP, Dempsey RJ, Meyerand ME. Diffusion tensor MR imaging in diffuse axonal injury. AJNR Am J Neuroradiol. 2002;23(5):794–802.
52. Huisman TA, Schwamm LH, Schaefer PW, et al. Diffusion tensor imaging as potential biomarker of white matter injury in diffuse axonal injury. AJNR Am J Neuroradiol. 2004;25(3):370–6.
53. Perlbarg V, Puybasset L, Tollard E, Lehericy S, Benali H, Galanaud D. Relation between brain lesion location and clinical outcome in patients with severe traumatic brain injury: a diffusion tensor imaging study using voxel-based approaches. Hum Brain Mapp. 2009;30(12):3924–33.
54. Greenberg G, Mikulis DJ, Ng K, DeSouza D, Green RE. Use of diffusion tensor imaging to examine subacute white matter injury progression in moderate to severe traumatic brain injury. Arch Phys Med Rehabil. 2008;89(12 Suppl):S45–50.
55. Bhadelia RA, Price LL, Tedesco KL, et al. Diffusion tensor imaging, white matter lesions, the corpus callosum, and gait in the elderly. Stroke J Cereb Circ. 2009;40(12):3816–20.
56. Angelini L, Mazzucchi A, Picciotto F, Nardocci N, Broggi G. Focal lesion of the right cingulum: a case report in a child. J Neurol Neurosurg Psychiatry. 1981;44(4):355–7.
57. Metzler-Baddeley C, Jones DK, Steventon J, Westacott L, Aggleton JP, O'Sullivan MJ. Cingulum microstructure predicts cognitive control in older age and mild cognitive impairment. J Neurosci. 2012;32(49):17612–9.
58. Devinsky O, Morrell MJ, Vogt BA. Contributions of anterior cingulate cortex to behaviour. Brain J Neurol. 1995;118(Pt 1):279–306.
59. Chanraud S, Zahr N, Sullivan EV, Pfefferbaum A. MR diffusion tensor imaging: a window into white matter integrity of the working brain. Neuropsychol Rev. 2010;20(2):209–25.
60. Virji-Babul N, Borich MR, Makan N, et al. Diffusion tensor imaging of sports-related concussion in adolescents. Pediatr Neurol. 2013;48(1):24–9.
61. Gu L, Li J, Feng DF, et al. Detection of white matter lesions in the acute stage of diffuse axonal injury predicts long-term cognitive impairments: a clinical diffusion tensor imaging study. J Trauma Acute Care Surg. 2013;74(1):242–7.
62. Von Der Heide RJ, Skipper LM, Klobusicky E, Olson IR. Dissecting the uncinate fasciculus: disorders, controversies and a hypothesis. Brain J Neurol. 2013;136(Pt 6):1692–707.
63. Barkley JM, Morales D, Hayman LA, Diaz-Marchan PJ. Static neuroimaging in the evaluation of TBI. In: Zasler ND, Katz DI, Zafonte RD, editors. Brain injury medicine: principles and practice. New York: Demos; 2007.
64. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage. 1999;9(2):179–94.
65. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc Natl Acad Sci U S A. 2000;97(20):11050–5.
66. Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis. II: inflation, flattening, and a surface-based coordinate system. Neuroimage. 1999;9(2):195–207.
67. Kim JS, Singh V, Lee JK, et al. Automated 3-D extraction and evaluation of the inner and outer cortical surfaces using a Laplacian map and partial volume effect classification. Neuroimage. 2005;27(1):210–21.
68. Palacios EM, Sala-Llonch R, Junque C, et al. Long-term declarative memory deficits in diffuse TBI: correlations with cortical thickness, white matter integrity and hippocampal volume. Cortex. 2013;49(3):646–57.
69. Tomaiuolo F, Carlesimo GA, Di Paola M, et al. Gross morphology and morphometric sequelae in

the hippocampus, fornix, and corpus callosum of patients with severe non-missile traumatic brain injury without macroscopically detectable lesions: a T1 weighted MRI study. J Neurol Neurosurg Psychiatry. 2004;75(9):1314–22.
70. Turken AU, Herron TJ, Kang X, et al. Multimodal surface-based morphometry reveals diffuse cortical atrophy in traumatic brain injury. BMC Med Imaging. 2009;9:20.
71. Yang F, Kruggel F. Automatic segmentation of human brain sulci. Med Image Anal. 2008;12(4):442–51.
72. Han X, Xu C, Tosun D, Prince JL. Cortical surface reconstruction using a topology preserving geometric deformable model. In: Proceedings of the IEEE Workshop on Mathematical Methods in Biomedical Image Analysis (MMBIA '01); December 2001; p. 213–20.
73. Han X, Xu C, Prince JL. A topology preserving level set method for geometric deformable models. IEEE Trans Pattern Anal Mach Intell. 2003;25(6):755–68.
74. Yezzi A, Prince JL. A PDE approach for measuring tissue thickness. In: Proceedings of the IEEE Computer Society Conference on Computer Vision and Pattern Recognition (CVPR '01), vol. 1; December 2001; p. I87–I92.
75. Rocha KR, Yezzi Jr AJ, Prince JL. A hybrid Eulerian–Lagrangian approach for thickness, correspondence, and gridding of annular tissues. IEEE Trans Image Process. 2007;16(3):636–48.
76. Kruggel F, von Cramon DY. Measuring the cortical thickness. In: Proceedings of the IEEE Workshop on Mathematical Methods in Biomedical Image Analysis (MMBIA '00); June 2000; p. 154–61.
77. Osechinskiy S, Kruggel F. PDE-based reconstruction of the cerebral cortex from MR images. Conf Proc IEEE Eng Med Biol Soc. 2010;2010:4278–83.
78. Michael AP, Stout J, Roskos PT, et al. Evaluation of cortical thickness after traumatic brain injury in military veterans. J Neurotrauma. 2015;32(22):1751–8.
79. Wilde EA, Merkley TL, Bigler ED, et al. Longitudinal changes in cortical thickness in children after traumatic brain injury and their relation to behavioral regulation and emotional control. Int J Dev Neurosci Off J Int Soc Dev Neurosci. 2012;30(3):267–76.
80. Wilde EA, Newsome MR, Bigler ED, et al. Brain imaging correlates of verbal working memory in children following traumatic brain injury. Int J Psychophysiol Off J Int Organ Psychophysiol. 2011;82(1):86–96.
81. Wang X, Xie H, Cotton AS, et al. Early cortical thickness change after mild traumatic brain injury following motor vehicle collision. J Neurotrauma. 2015;32(7):455–63.
82. Wintermark M, Sanelli PC, Anzai Y, Tsiouris AJ, Whitlow CT, American College of Radiology Head Injury Institute. Imaging evidence and recommendations for traumatic brain injury: advanced neuro- and neurovascular imaging techniques. AJNR Am J Neuroradiol. 2015;36(2):E1–E11.
83. Walz NC, Cecil KM, Wade SL, Michaud LJ. Late proton magnetic resonance spectroscopy following traumatic brain injury during early childhood: relationship with neurobehavioral outcomes. J Neurotrauma. 2008;25(2):94–103.
84. Babikian T, Freier MC, Ashwal S, Riggs ML, Burley T, Holshouser BA. MR spectroscopy: predicting long-term neuropsychological outcome following pediatric TBI. J Magn Reson Imaging JMRI. 2006;24(4):801–11.
85. Parry L, Shores A, Rae C, et al. An investigation of neuronal integrity in severe paediatric traumatic brain injury. Child Neuropsychol J Norm Abnorm Dev Child Adolesc. 2004;10(4):248–61.
86. Ashwal S, Holshouser BA, Shu SK, et al. Predictive value of proton magnetic resonance spectroscopy in pediatric closed head injury. Pediatr Neurol. 2000;23(2):114–25.
87. Kasahara M, Menon DK, Salmond CH, et al. Traumatic brain injury alters the functional brain network mediating working memory. Brain Inj. 2011;25(12):1170–87.
88. Marquez de la Plata CD, Garces J, Shokri Kojori E, et al. Deficits in functional connectivity of hippocampal and frontal lobe circuits after traumatic axonal injury. Arch Neurol. 2011;68(1):74–84.
89. Palacios EM, Sala-Llonch R, Junque C, et al. White matter integrity related to functional working memory networks in traumatic brain injury. Neurology. 2012;78(12):852–60.
90. Sharp DJ, Beckmann CF, Greenwood R, et al. Default mode network functional and structural connectivity after traumatic brain injury. Brain J Neurol. 2011;134(Pt 8):2233–47.
91. Arenivas A, Diaz-Arrastia R, Spence J, et al. Three approaches to investigating functional compromise to the default mode network after traumatic axonal injury. Brain Imaging Behav. 2014;8(3):407–19.
92. Bonnelle V, Ham TE, Leech R, et al. Salience network integrity predicts default mode network function after traumatic brain injury. Proc Natl Acad Sci U S A. 2012;109(12):4690–5.
93. Hillary FG, Slocomb J, Hills EC, et al. Changes in resting connectivity during recovery from severe traumatic brain injury. Int J Psychophysiol Off J Int Organ Psychophysiol. 2011;82(1):115–23.
94. Mayer AR, Mannell MV, Ling J, Gasparovic C, Yeo RA. Functional connectivity in mild traumatic brain injury. Hum Brain Mapp. 2011;32(11):1825–35.
95. Shumskaya E, Andriessen TM, Norris DG, Vos PE. Abnormal whole-brain functional networks in homogeneous acute mild traumatic brain injury. Neurology. 2012;79(2):175–82.
96. Slobounov SM, Gay M, Zhang K, et al. Alteration of brain functional network at rest and in response to YMCA physical stress test in concussed athletes: RsFMRI study. Neuroimage. 2011;55(4):1716–27.

97. Smith SM, Fox PT, Miller KL, et al. Correspondence of the brain's functional architecture during activation and rest. Proc Natl Acad Sci U S A. 2009;106(31):13040–5.
98. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci. 2008;1124:1–38.
99. Ding K, Marquez de la Plata C, Wang JY, et al. Cerebral atrophy after traumatic white matter injury: correlation with acute neuroimaging and outcome. J Neurotrauma. 2008;25(12):1433–40.
100. Gale SD, Baxter L, Roundy N, Johnson SC. Traumatic brain injury and grey matter concentration: a preliminary voxel-based morphometry study. J Neurol Neurosurg Psychiatry. 2005;76(7):984–8.
101. Lindemer ER, Salat DH, Leritz EC, McGlinchey RE, Milberg WP. Reduced cortical thickness with increased lifetime burden of PTSD in OEF/OIF Veterans and the impact of comorbid TBI. Neuroimage Clin. 2013;2:601–11.
102. Maxwell WL, MacKinnon MA, Stewart JE, Graham DI. Stereology of cerebral cortex after traumatic brain injury matched to the Glasgow outcome score. Brain J Neurol. 2010;133(Pt 1):139–60.
103. Merkley TL, Bigler ED, Wilde EA, McCauley SR, Hunter JV, Levin HS. Diffuse changes in cortical thickness in pediatric moderate-to-severe traumatic brain injury. J Neurotrauma. 2008;25(11):1343–5.
104. Warner MA, Youn TS, Davis T, et al. Regionally selective atrophy after traumatic axonal injury. Arch Neurol. 2010;67(11):1336–44.
105. Wilde EA, Hunter JV, Newsome MR, et al. Frontal and temporal morphometric findings on MRI in children after moderate to severe traumatic brain injury. J Neurotrauma. 2005;22(3):333–44.
106. Bendlin BB, Ries ML, Lazar M, et al. Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging. Neuroimage. 2008;42(2):503–14.
107. Kim EY, Park HJ, Kim DH, Lee SK, Kim J. Measuring fractional anisotropy of the corpus callosum using diffusion tensor imaging: mid-sagittal versus axial imaging planes. Korean J Radiol. 2008;9(5):391–5.
108. Gale SD, Johnson SC, Bigler ED, Blatter DD. Nonspecific white matter degeneration following traumatic brain injury. J Int Neuropsychol Soc. 1995;1(1):17–28.
109. Kennedy MR, Wozniak JR, Muetzel RL, et al. White matter and neurocognitive changes in adults with chronic traumatic brain injury. J Int Neuropsychol Soc. 2009;15(1):130–6.
110. Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, Little DM. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. Brain J Neurol. 2007;130(Pt 10):2508–19.
111. Kumar R, Husain M, Gupta RK, et al. Serial changes in the white matter diffusion tensor imaging metrics in moderate traumatic brain injury and correlation with neuro-cognitive function. J Neurotrauma. 2009;26(4):481–95.
112. Lipton ML, Gellella E, Lo C, et al. Multifocal white matter ultrastructural abnormalities in mild traumatic brain injury with cognitive disability: a voxel-wise analysis of diffusion tensor imaging. J Neurotrauma. 2008;25(11):1335–42.
113. Mac Donald CL, Dikranian K, Bayly P, Holtzman D, Brody D. Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. J Neurosci. 2007;27(44):11869–76.
114. Maller JJ, Thomson RH, Lewis PM, Rose SE, Pannek K, Fitzgerald PB. Traumatic brain injury, major depression, and diffusion tensor imaging: making connections. Brain Res Rev. 2010;64(1):213–40.
115. Marsh PD, Keevil CW, McDermid AS, Williamson MI, Ellwood DC. Inhibition by the antimicrobial agent chlorhexidine of acid production and sugar transport in oral streptococcal bacteria. Arch Oral Biol. 1983;28(3):233–40.
116. Nakayama N, Okumura A, Shinoda J, et al. Evidence for white matter disruption in traumatic brain injury without macroscopic lesions. J Neurol Neurosurg Psychiatry. 2006;77(7):850–5.
117. Niogi SN, Mukherjee P, Ghajar J, et al. Extent of microstructural white matter injury in postconcussive syndrome correlates with impaired cognitive reaction time: a 3 T diffusion tensor imaging study of mild traumatic brain injury. AJNR Am J Neuroradiol. 2008;29(5):967–73.
118. Wada T, Asano Y, Shinoda J. Decreased fractional anisotropy evaluated using tract-based spatial statistics and correlated with cognitive dysfunction in patients with mild traumatic brain injury in the chronic stage. AJNR Am J Neuroradiol. 2012;33(11):2117–22.
119. Westlye LT, Walhovd KB, Bjornerud A, Due-Tonnessen P, Fjell AM. Error-related negativity is mediated by fractional anisotropy in the posterior cingulate gyrus — a study combining diffusion tensor imaging and electrophysiology in healthy adults. Cereb Cortex. 2009;19(2):293–304.
120. Zappala G, Thiebaut de Schotten M, Eslinger PJ. Traumatic brain injury and the frontal lobes: what can we gain with diffusion tensor imaging? Cortex. 2012;48(2):156–65.
121. Cohen BA, Inglese M, Rusinek H, Babb JS, Grossman RI, Gonen O. Proton MR spectroscopy and MRI-volumetry in mild traumatic brain injury. AJNR Am J Neuroradiol. 2007;28(5):907–13.
122. Garnett MR, Blamire AM, Corkill RG, Cadoux-Hudson TA, Rajagopalan B, Styles P. Early proton magnetic resonance spectroscopy in normal-appearing brain correlates with outcome in patients

following traumatic brain injury. Brain J Neurol. 2000;123(Pt 10):2046–54.
123. Pandit AS, Expert P, Lambiotte R, et al. Traumatic brain injury impairs small-world topology. Neurology. 2013;80(20):1826–33.
124. van den Heuvel M, Mandl R, Luigjes J, Hulshoff PH. Microstructural organization of the cingulum tract and the level of default mode functional connectivity. J Neurosci. 2008;28(43):10844–51.
125. Fujiwara E, Schwartz ML, Gao F, Black SE, Levine B. Ventral frontal cortex functions and quantified MRI in traumatic brain injury. Neuropsychologia. 2008;46(2):461–74.

Liaison Psychiatry: Playing "Hide and Seek" with Delirium

32

María Soledad Barboza, Julia Cittadini,
Milagros de Hertelendy,
Mauricio Sebastián Farías, and Natacha Loiacono

Abstract

In the general medical setting, as many as 30% of patients have a psychiatric disorder. Delirium is detected in 10% of all medical inpatients, and is detected in over 30% in some high-risk groups. Two-thirds of patients who are high users of medical care have a psychiatric disturbance: Around 23% have depression, 22% anxiety, and 20% somatization. Only a small subset of the population at risk is currently being adequately identified. Education of non-psychiatric physicians and allied health professionals about medical and psychiatric issues related to a patient's illness is a core component of the liaison model. Possibly because of the psychiatric nature of its manifestations, delirium is poorly recognized by non-psychiatric house staff. The aim of this study is to evidence incidence rates of under-diagnosis and provide an overview including prevention, diagnosis, and early management of delirium in general hospitals. A retrospective study was conducted at the Centenario Provincial Hospital of Rosario, Argentina, a tertiary care academic hospital. It was carried out during the period January 2010–June 2011, following the referrals of the house staff for 345 adult inpatients. The incidence of delirium, as well as the staff's diagnostic ability was analyzed. Of the total sample, the 19% developed an acute confusional state (ACS). Diagnosis had been correct in 51% of the cases, whereas misdiagnosis reached 49%. The under-diagnosis of ACS due to semiological misrecognition has been modified after an educational effort in the acquisition of screening skills carried out by the psychiatric and non-psychiatric staff together.

M.S. Barboza • J. Cittadini • M.S. Farías
N. Loiacono
Department of Psychiatry, Provincial Centenary Hospital, Rosario National University, Rosario, Argentina

M. de Hertelendy, MD (✉)
Department of Psychiatry, Provincial Centenary Hospital, Rosario National University, Rosario, Argentina

Department of Psychiatry, National University of Rosario (Universidad Nacional de Rosario, UNR), Maipu 1065 (Institutional) Postal code 2000, Rosario 5500, Argentina
e-mail: mdehertelendy@gmail.com

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_32

Keywords
Delirium • General Hospital • Consultation–liaison psychiatry • Referrals • Under-diagnose

Introduction

Consultation–liaison (C–L) psychiatry is the subspecialty of psychiatry concerned with medically and surgically ill patients [1]. The C–L consultant must have an extensive clinical understanding of physical/neurological disorders and their relation to abnormal illness behavior. The C–L consultant must be a skilled diagnostician, and be able to tease apart and formulate a patient's multiaxial disorders and to develop an effective treatment plan. The C–L consultant must also have knowledge of psychotherapeutic and psychopharmacological interventions as well as knowledge of the wide array of medicolegal aspects of psychiatric and medical illness and hospitalization.

In the general medical setting, as many as 30% of patients have a psychiatric disorder [2–4]. Delirium is detected in 10% of all medical inpatients [5] and in over 30% in some high-risk groups. Two-thirds of patients who are high users of medical care have a psychiatric disturbance: 23% have depression, 22% have anxiety, and 20% have somatization [6, 7]. Clearly, psychiatric comorbidity has an impact on health care economics [8–12]. The presence of a psychiatric disturbance has repeatedly been shown to be a robust predictor of increased hospital length of stay [13–16]. Nearly 90% of 26 studies have demonstrated either an increased length of stay or an increased medical readmission rate among patients with psychiatric comorbidity [17]. Only a small subset of the population at risk is currently being adequately identified. The percentage of admitted patients receiving psychiatric consultation varies from institution to institution [18], ranging from 1% to 10% [18–21].

Each institution is responsible for the continuing medical education of medical/surgical staff about the psychological consequences of illness and the indications for psychiatric consultation. Areas of focus should include the recognition of substance abuse, delirium, dementia, affective disorders, anxiety disorders, and suicidal ideation.

Education of non-psychiatric physicians and allied health professionals about medical and psychiatric issues related to a patient's illness is a core component of the liaison model. Liaison services lead to heightened sensitivity by medical staff, which results in earlier detection and more cost-effective management of patients with psychiatric problems.

Delirium or acute confusional state (ACS), also called either acute mental syndrome or organic mental syndrome, is a disorder located in the interface between psychiatry and the rest of the medical specialties. Delirium is defined as an acute change in cognition and a disturbance of consciousness, with impaired attention that fluctuates during the day [22].

It is defined as a syndrome of acute onset and fluctuating course, characterized by impairment of superior cognoscitive functions, with an deleterious effect on consciousness, alertness, and temporospatial orientation. With a complex and multifactorial etiology, its incidence is high in hospitalized patients, affecting the aged in particular. The generalized effect on superior functions expresses itself in the decline of the capacity to think clearly and correctly evaluate the environment, to which the patient might answer with alterations in his conduct.

At the same time, these superficial manifestations may confuse the professionals and lead to misdiagnosis and wrong treatments.

Multiple factors contribute to the development of delirium, including cognitive dysfunction, alcohol and/or drug withdrawal, sedative use, altered or inadequate sleep, painful procedures,

lack of a focal point, infection, and disordered physiology [23, 24].

When the ACS appears, it usually implies a prolonged hospital stay, and an increased morbidity and mortality. It is often under-diagnosed, and in many cases it receives an inappropriate or late therapeutic approach.

Taking into account these observations, a retrospective observational analysis was carried out in a polyvalent hospital, directed towards the evaluation on confidence in diagnosing ACS by the house staff of the departments of Internal Medicine and General Surgery.

Aim

The aim of this study—from the liaison psychiatry perspective—is to show incidence rates of under-diagnosis and provide a summary including prevention, diagnosis, and early management of delirium in general hospitals.

Materials and Methods

A retrospective analysis was carried out by the resident staff of the Psychiatry Department of Rosario's Provincial Centenary Hospital from January 2010 to June 2011. The study included 345 hospitalized patients whose referrals had been requested by medical and surgical house staff (typically interns and junior residents).

The referrals were assessed through templates which registered patient's demographic data, the service on which the patient was treated, including the Emergency Department, the calendar month and year of admission, days of hospitalization, and discharge date. In addition, the chief complaint, consultation request (date of request and motive given by non-psychiatric house staff), underlying pathologies, psychiatrists' intervention, and finally, the diagnosis according to DSM-IV TR, were recorded.

The requests of the different departments were clustered and classified as "correct" or "incorrect" referrals. A "correct" referral for delirium by the house staff consultee included: (1) "delirium" or acute confusional state, (2) related synonyms such as psychomotor agitation, sensory perception disorder, behavioral disorder, sleep disturbances, hypoprosexia, central nervous system depression, and disorientation (Table 32.1).

Absence of delirium or "incorrect" referrals were put together in related groups as noted in Table 32.2. The patients frequently received more than one diagnosis. Nevertheless, if one of the diagnoses given was "delirium" or a synonym, the consultee was given "credit" for an accurate diagnosis of the patient.

Table 32.1 Correct referrals

Correct consultation request	33
Confusional state	6
Psychomotor agitation	15
Sensory-perceptive alterations	5
Behavioral disorder	2
Sleep disturbances	1
Hypoprosexia	2
Central nervous system depression	1
Disorientation	1

Table 32.2 Incorrect referrals

Incorrect consultation request	32
Lack of adherence to treatment	4
Distress, anguish	5
Assessment	6
Negativism	2
Apathy/abulia	1
Fear	1
Psychiatric medication check	1
Depression	4
Withdrawal síndrome	1
Intoxication	1
Dementia	1
Anxiety	1
Suicidal ideation	1
Emocional lability	1
Cholecystitis	1
Amputation	1

Results

From the total sample (*N = 345*), 65 patients (19%) developed ACS; 20 (31%) were female patients and 45 (69%) male patients. Forty-nine percent (49%) of the patients diagnosed as delirious by the Psychiatry staff had been misdiagnosed by the non-psychiatric consultees, whereas in fifty-one percent (51%) of the cases the diagnosis was correctly made by medical and surgical staff. It remains interesting to highlight though, that throughout the year 2010 the rate of under-diagnosis was higher than in the period January – June 2011. Psychiatry's resident staff had been working throughout the year making use of each request to give medical house staff the indications for recognizing, managing, and treating ACS. After those interventions, the percentage of misdiagnosis diminished to 37% while correct diagnoses reached 63% [see Fig. 32.1a, b].

Patient study sample demographics included a mean age of 56.5 (range: 18–95), revealing a significant divergence between the periods January–December 2010 and January–June 2011. During the first period, the group which was mainly affected involved patients between 60–69 years old, with a lower peak that extended to the 50–59-year-old group. During the second period, the age range varied between 20 and 29, in direct relation with the increase of traumatic brain injury and substance abuse.

Among the "correct" referrals, 46% were diagnosed as psychomotor agitation, 18% as acute mental state, and 15% as sensoperceptive alterations. With regard to the "incorrect" requests, 19% were requests for assessment, approximately 40% were grouped as mood symptoms, and 13% demonstrated lack of adherence to medical treatment.

Taking into account that this disorder obeys organic etiology which, at the same time, determines the evolution and prognosis of the patient, it is of interest to recall that the most prevalent related diseases during 2010 were infectious processes (34%), malignant formations (15%), and kidney impairment and polytrauma (12%).

In 2011, the percentage of infectious processes remained in the first place, though to a decreased extent (26%), and polytrauma and substance abuse became the highlights (14%) (See Fig. 32.1a, b).

Discussion

This study identified over an 18-month period, out of 345 patients, that 65 suffered from delirium. This was consistent with the bibliography [25], and what is expected for an institution with the characteristics of a polyvalent hospital whose population is around 55 years old. The patients come from a low cultural and income level, and

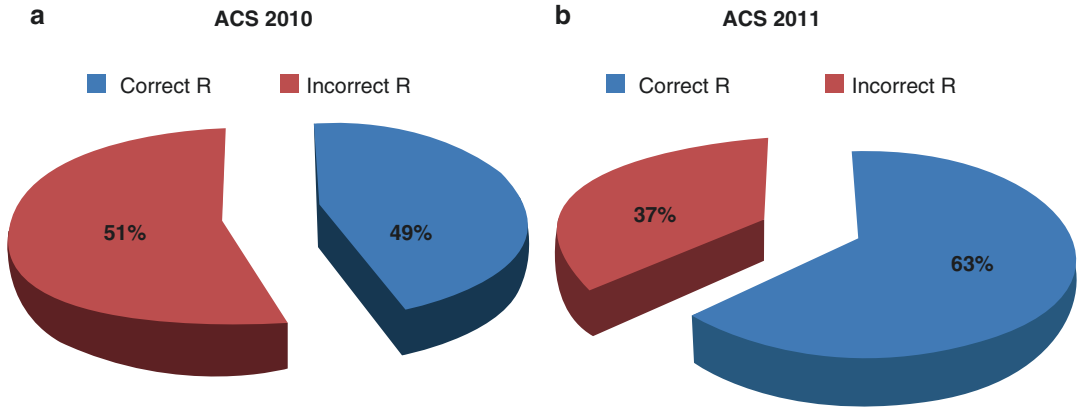


Fig. 32.1 (**a**, **b**) Correct and incorrect ACS diagnosis before and after Psychiatry Staff's intervention in educating house staff

carry multiple comorbidities which make them liable to polypharmacy.

The house staff that referred the patients to the Psychiatry Department included medical specialties that related to inpatient wards, for example, surgical specialties (General Surgery, Orthopedics and Traumatology, and Urology), the Intensive Care Unit, the Coronary Unit and the Emergency Department. Most request forms came from the medical staff, followed by the surgical staff, Orthopedics being the service to solicit the fewest request forms.

Taking the 18 months as a whole, the percentage of under-diagnosis reached 49%, in accordance with current bibliography [22]. Nevertheless, when split into two periods (January–December 2012/January–June 2011), a difference could be observed (Fig. 32.1a, b) between the two with regard to recognition of ACS.

This change may result from two elements. On one hand, the educational activity that the Psychiatry Department undertook towards the training of non-psychiatric house staff concerning screening and recognition of ACS, and on the other hand, to the fact that by January 2011 the resident doctors were going through the last third of their academic year, which means that they had probably gained many new skills and could provide a better semiological performance concerning this condition.

According to the correct referrals, confusional state (17%), sensory-perceptive alterations (15%), and psychomotor agitation (48%) had the highest incidence. On the other hand, among incorrect referrals, the highest were lack of adherence to treatment (15%), distress or anguish (19%), and request for assessment (23%). Circa 45% of referrals identified depressive symptoms when, in fact, it was a question of hypoactive delirium.

In many countries, the symptoms of delirium are attributed to dementia or depression, and a delay in correct diagnosis entails a longer stay and risks major complications [26].

A propos the psychomotor subtype, various similarities were found between the Provincial Centenary Hospital and other polyvalent hospitals with regard to the major incidence of mixed delirium [27]. This explains why this condition turns out so difficult to recognize, leading to mismanagement, a late referral to the specialized service, under-diagnosis, and under-estimation of a medical condition with high morbidity/mortality.

As far as gender is concerned, throughout the study, male patients outnumbered women patients [28, 29].

A remarkable variance in the case of this hospital is that it does not go totally along with the rest in regard to the age of onset of delirium. Although during the year 2010, the prevalent age had a mean of 60–69 years old, during 2011 there was an increase in onset of delirium in patients between 20 and 29 years old. There is a similar reference in a descriptive study from San Jorge Hospital, where 28 patients suffering from acute traumatic brain injury were admitted and were followed up with Folstein's Mini-Mental State Exam. When statistics were examined, it was found that 46% developed delirium sometime during admission. In that study, the authors establish a direct association among acute traumatic brain injury, substance abuse, and incidence of delirium, the highest incidence relating to moderate brain traumatism and with a male/female correlation of about 3:1, mostly between 15 and 29 years of age [30].

The analysis of underlying pathologies suggests that during 2010 the greatest percentage concerned infections (34%), followed by growth or tumor (15%), polytrauma, and renal failure (12%). Not far behind come vascular diseases, diabetes, and finally strokes, liver disease, and surgical pathologies. Curiously, during that period, there was no indication related to substance abuse as a positive risk factor (Fig. 32.2).

This state of affairs changes in 2011 and, even though the infections remain first in prevalence, polytrauma, strokes, diabetes, and malignant growth take second place, closely followed by the occurrence of substance abuse as a predisposing factor of delirium (Fig. 32.2b).

In some research studies conducted in Intensive Care Units, infections also hold the first place as reason for admittance [31], while in other investigations, infections take third place, overtaken by fluid imbalance and trauma [22].

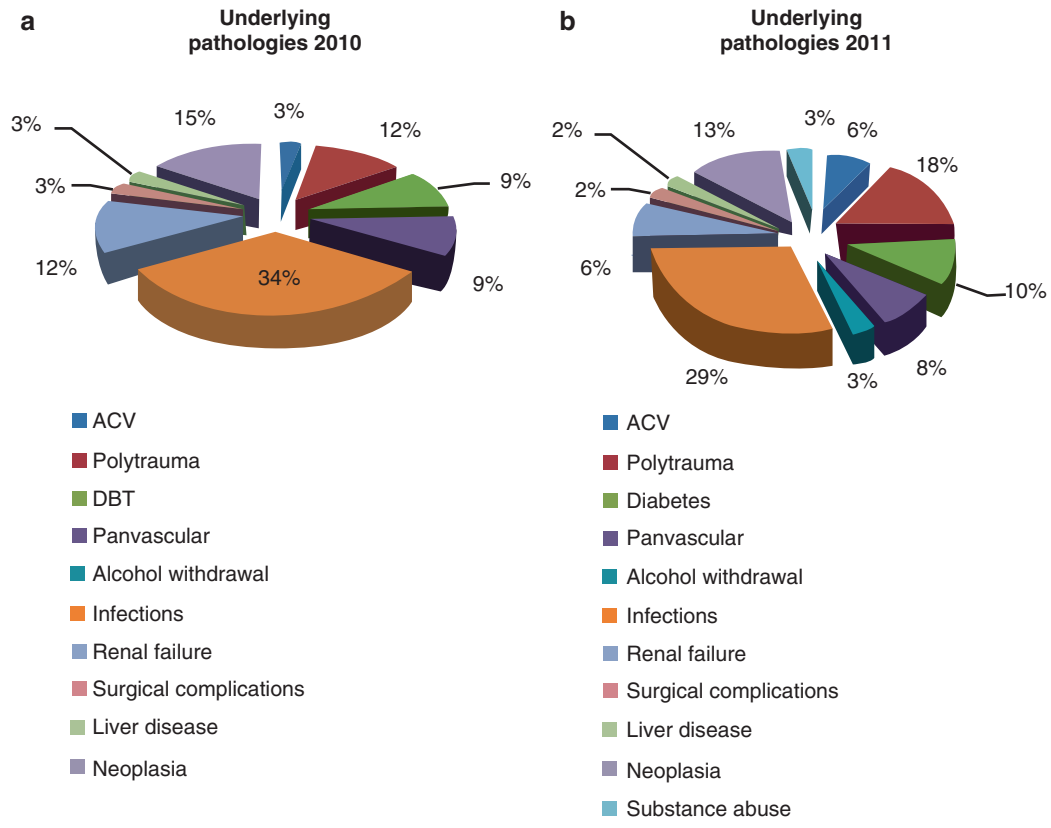


Fig. 32.2 (**a**, **b**) Most frequent underlying etiologic agents and comorbid factors seen in hospitalized patients

Whether misdiagnosis of delirium influenced the duration of hospitalization remains open because this study did not carry out a follow-up with regard to that possibility.

Conclusion

Evaluation of the mental health of patients with serious medical illness, formulation of their problems and diagnosis, and organization and implementation of an effective treatment plan involve complex clinical skills that require specialized training. Commonly, the overt reason for initiating a consultation may not be as serious as a comorbid, but unrecognized, problem.

The aim of the study was to assess the underdiagnosis of delirium as a starting point to enable the Liaison Psychiatry team to provide to non-psychiatric house staff an framework with regard to prevention, diagnosis, and early management of this condition. Secondarily, to make a follow-up of the medical team whose learning experience could be shown over the months, in regard to the outcome for the patient.

References

1. Academy of Psychosomatic Medicine: proposal for the designation of consultation-liaison psychiatry as a subspecialty: internal report. Chicago: Academy of Psychosomatic Medicine ;1992.
2. Strain JJ. Needs for psychiatry in the general hospital. Hosp Community Psychiatry. 1982;33:996–1002.
3. VonAmmon R, Cavanaugh S, Wettstein RM. Emotional and cognitive dysfunction associated with medical disorders. J Psychosom Res. 1989;33:505–14.
4. Spitzer RL, Kroenke K, Linzer M, et al. Health-related quality of life in primary care patients with mental disorders: results as from the PRIME-MD 1000 study. JAMA. 1994;274:1511–7.
5. Lipowski ZJ. Delirium (acute confusional state). JAMA. 1987;258:1789–92.

6. Katon W, Von Korf M, Lin E, et al. A randomized trial of psychiatric consultation with distressed high utilizers. Gen Hosp Psychiatry. 1992;14:86–98.
7. Von Korf M, Ormel J, Katon W, et al. Disability and depression among high utilizers of health care. Arch Gen Psychiatry. 1992;49:91–9.
8. Simon GE, Von Korf M. Somatization and psychiatric disorders in the NIMH Epidemiologic Catchment Area Study. Am J Psychiatry. 1991;148:1494–500.
9. Simon GE, Von Korf M, Barlow W. Health care costs of primary care patients with recognized depression. Arch Gen Psychiatry. 1995;52:850–6.
10. Shaw J, Creed F. The cost of somatization. J Psychosom Res. 1991;35:307–12.
11. Escobar JL, Golding JM, Hough RL, et al. Somatization in the community: relationship to disability and use of services. Am J Public Health. 1987;77:837–40.
12. Linn LD, Yager J. Screening of depression in relationship to subsequent patient and physician behavior. Med Care. 1982;20:1233.
13. Fink P. The use of hospitalization by persistent somatizing patients. Psychol Med. 1992;22:173–80.
14. Fulop G, Strain JJ, Vita J, et al. Impact of psychiatric comorbidity on length of stay for medical/surgical patients. Am J Psychiatry. 1987;144:878–82.
15. Huyse FJ, Strain JJ, Hammer JS. Psychiatric comorbidity and length of hospital stay. Am J Psychiatry. 1988;145:1319.
16. Thomas RI, Cameron DJ, Fahs MC. A prospective study of delirium and hospital stay. Arch Gen Psychiatry. 1988;45:937–40.
17. Saravay SM. Psychiatric interventions in the medically ill: outcome and effectiveness research. Psychiatr Clin North Am. 1996;19:1–14.
18. Lipowski ZJ. Review of consultation psychiatry and psychosomatic medicine, II. Psychosom Med. 1967;29:201–24.
19. Pablo RY, Lamarre CJ. Psychiatric consultation on a general hospital. Can J Psychiatry. 1988;33:224–30.
20. Swigar ME, Sanguineti VR, Piscatelli RL. A retrospective study on the perceived need for and actual use of psychiatric consultation in older medical patients. Int J Psychiatry Med. 1992;22:239–49.
21. Wallen J, Pincus HA, Goldman HH, et al. Psychiatric consultations in short-term general hospitals. Arch Gen Psychiatry. 1987;44:163–16.
22. Goulia P, Mantas C, Hyphantis T. Delirium, a 'confusing' condition in general hospitals: the experience of a consultation liaison psychiatry unit in Greece. Int J Gen Med. 2009;29:201–7.
23. Griffiths RD, Jones C. Delirium cognitive dysfunction and posttraumatic stress disorder. Curr Opin Anesthesiol. 2007;20:124–9.
24. King CS, Moores LK, Epstein SK. Should patients be able to follow commands prior to extubation? Respir Care. 2010;55:56–62.
25. Armstrong SC, Kelly LC, Watanabe KS. The misdiagnosis of delirium. Psychosomatics. 1997;38:433–9.
26. Girard TD, Pandharipande PP, Ely EW. Delirium in the intensive care unit. Crit Care. 2008;12(suppl 3):S3.
27. Han JH, Zimmerman EE, Cutler N, Schnelle J, Morandi A, Dittus RS, et al. Delirium in older emergency department patients: recognition, risk factors, and psychomotor subtypes. Acad Emerg Med. 2009;16:193–200.
28. Inouye SK, Bogardus ST, Charpentier PA, et al. A multicomponent intervention to prevent delirium in hospitalized older patients. N Engl J Med. 1999;340:669–76.
29. Andrew MK, Freter SH, Rockwood K. Prevalence and outcomes of delirium in community and non-acute care settings in people without dementia: a report from the Canadian Study of Health and Aging. BMC Med. 2006;23:15.
30. Gutiérrez Segura JC, Posada Guzmán AD, Osorio Tabares E, Jaramillo Vásquez J, Cuartas Cuartas SM, Corredor MM. Delirium en pacientes con fractura de cadera. Hospital Universitario San Jorge, Pereira-2005. Rev Médica Risaralda. 2006;12:34–9.
31. Toro AC, Escobar LM, Franco JG, et al. [Spanish version of the CAM-ICU (Confusion Assessment Method for the Intensive Care Unit). Pilot study of validation] Medicina intensiva/Sociedad Española de Medicina Intensiva y Unidades Coronarias.2010; 34(1):14–21.

Metabolic Association Between the Gut–Brain Axis in Autism Spectrum Disorders

33

María Andrea Delgado, Adriana Fochesato, Luis Isaías Juncos, and Pascual Ángel Gargiulo

Abstract

Autism spectrum disorder (ASD) is a severe, complex neurodevelopmental disorder, characterized by impairments in social interaction and communication with restricted and stereotyped behavior patterns. ASD symptoms result from a complex interaction between genetic and environment factors. Food intolerances, allergies, altered intestinal permeability (leaky gut), immune dysregulation, neuroinflammation and oxidative stress may trigger ASD symptoms. ASD patients have shown increased urinary levels of β-casomorphin and gliadorphin peptides produced by incomplete digestion of gluten proteins and milk casein. "Leaky gut" may facilitate the transport of these peptides into the central nervous system (CNS) inducing direct "opioid activity" and thus affecting neurotransmission. ASD patients on gluten and/or casein-free diet have shown improvement in most behavior and cognitive scores. Immune dysregulation leads to a neuroinflammatory response that correlates between immune dysfunction with behavioral and cognitive impairments in ASD patients. Genetic variants of the *MET* gene (7q31.2) are risk factors for ASD. The MET receptor participates in brain cortex and cerebellum development and in gastrointestinal and immunological functions. A high percentage of ASD children have shown non-celiac gluten sensitivity, an immune reaction against gluten in subjects not affected with celiac disease with prominent mucosal eosinophil infiltration and increased blood eosinophilia. ASD patients have shown alterations in

M.A. Delgado, PhD (✉) • A. Fochesato
L.I. Juncos
Fundación J Robert Cade,
Pedro de Oñate 253, Córdoba X5003DDZ, Argentina
e-mail: delgadoandrea73@gmail.com

P.Á Gargiulo
Laboratorio de Neurociencias y Psicología Experimental, Área de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, CONICET, Mendoza, Argentina

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_33

brain anatomy involved in language and social interaction skills, correlating with specific aspects of ASD symptoms. ASD behavior results from abnormal interactions between the opioid system and various pathways together with anatomical alterations in the CNS. Individualized diagnosis and prognostic predictions should provide effective personalized therapies in ASD patients.

Keywords

Autism spectrum disorders • Opioid peptides • Leaky gut • Genetics Immune dysregulation

Introduction

Autism spectrum disorders (ASDs) (Online Mendelian Inheritance in Man—(OMIM)—209850) is increasingly recognized as a systemic and complex disease process whose main features are displayed by a severe and complex neurodevelopmental disorder. Indeed, this state is characterized by impairment in reciprocal social interaction and communication, and restricted and stereotyped patterns of interests and behaviors [1].

The latest studies, according to the Centers for Disease Control and Prevention and Autism and Developmental Disabilities Monitoring (ADDM) Network, indicated that the prevalence was 1 in 68 children, aged up to 8 years from 11 communities within the United States, who had been identified with ASD in 2012 [2].

ASD is a highly heritable disorder, with onset usually after the 3rd year of age. Estimations of ASD prevalence diverge by sex and ethnicity. It affects predominantly males, with a sex ratio of approximately 4.5:1. Indeed, approximately 1 in 42 boys and 1 in 189 girls living in the ADDM Network communities were identified as having ASD. All 11 sites reported higher prevalence among white children than among black (1.2%) or Hispanic children (1.5%), while ASD was more prevalent in black children than in Hispanic children (1.3%) [2]. The intellectual ability of children with ASD was classified in different proportions: 31% of the children fell within the range of intellectual disability (IQ ≤70), 23% had borderline disability (IQ = 71–85), and 46% were in the average or higher range of intellectual ability (IQ >85). In addition, there were differences between ethnic groups in the rate of intellectual disability: 48% of non-Hispanic black children, 38% of Hispanic children, and 25% of non-Hispanic white children were classified with intellectual disability [2].

This recent and rapid increase in ASD prevalence highlights the importance of furthering research, including risk factors, etiology, biomarkers, and effective interventions [2].

ASD refers to a broader definition of autism that includes classical and atypical autism, Asperger syndrome, and pervasive developmental disorder [3]. Although the etiology of autism is not yet understood, patients with ASD show manifestations of a systemic and complex disease process, and symptoms persisting throughout life [4].

The symptoms of autism are thought to result from a complex and variable interaction between genetic and environmental factors [5]. Food intolerances or allergies, altered intestinal permeability (leaky gut), dietary opioid peptides in bloodstream, neuroinflammation, immune dysregulation, and oxidative stress by mitochondrial dysfunction may trigger ASD symptoms [6–10]. (Fig. 33.1). Scientific evidence has confirmed the improvement of ASD symptoms after early interventions to prevent common biomedical abnormalities believed to lead to harmful consequences on behavior and neurological functioning [11–13].

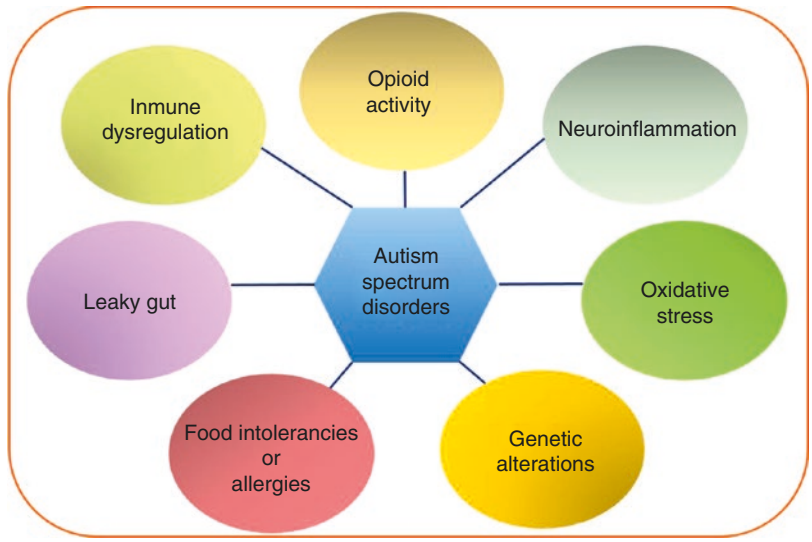


Fig. 33.1 Schematic representation of different mechanisms thought to be implicated in the pathogenesis of autism

Metabolic Insights in ASD

In 1966, Dohan postulated that schizophrenia is the cause of an overload of peptides derived from dietary gluten [14]. This hypothesis was later extended to include autism [15, 16].

The normal dietary protein digestion entails their breakdown to smaller molecules until reaching the basic unit (amino acid). Therefore, an incomplete protein breakdown generates bigger structural compounds, namely peptides, composed by 2–8 amino acids. Indeed, increased urinary β-casomorphin and gliadorphin peptides have been reported in ASD patients as a result of an incomplete digestion of casein and gluten respectively [6]. Both of these peptides show structural similarities in fragment tyrosine–proline (tyr–pro) in position *1–2* and proline (pro) amino acid in positions *4* and *6* (Fig. 33.2).

Altered intestinal permeability has been implicated in ASD patients, and is thought to be the link between the gut and brain in autism pathogenesis [17–19]. Supporting this hypothesis, gastrointestinal disorders, such as diarrhea, chronic constipation, abdominal pain, ulcerative colitis, and others, have been reported in ASD patients [20–23].

	1	**2**	3	**4**	5	**6**	7
B-Casomorphin	**tyr**	**pro**	phe	**pro**	gly	**pro**	Ile
Gliadorphin	**tyr**	**pro**	gln	**pro**	gln	**pro**	phe

Fig. 33.2 Amino acid sequence for β-casomorphin-7 and gliadorphin-7 peptides. Amino acids: tyrosine *(tyr),* proline *(pro),* phenylalanine *(phe),* glycine *(gly),* isoleucine *(ile),* glutamine *(gln)*

ASD patients are four times more likely to suffer from gastrointestinal problems than the rest of the population [24]. This is a fact known since the first clinical description of autism in the 1940s [25]. Moreover, there is evidence of a strong correlation between gastrointestinal symptoms and autism severity that could be explained by three different mechanisms: (1) an increased permeability of the intestinal mucosa not related to inflammatory processes, such as tight junction defects or alterations in the intestinal flora composition (gut microbiome). (2) a possible second mechanism involving inadequate or deficient enzymes for either intestinal or blood breakdown of β-casomorphins or gliadorphins which allows exorphins in large amounts to gain access to the blood and circulate until taken up by the brain, (3) a possible

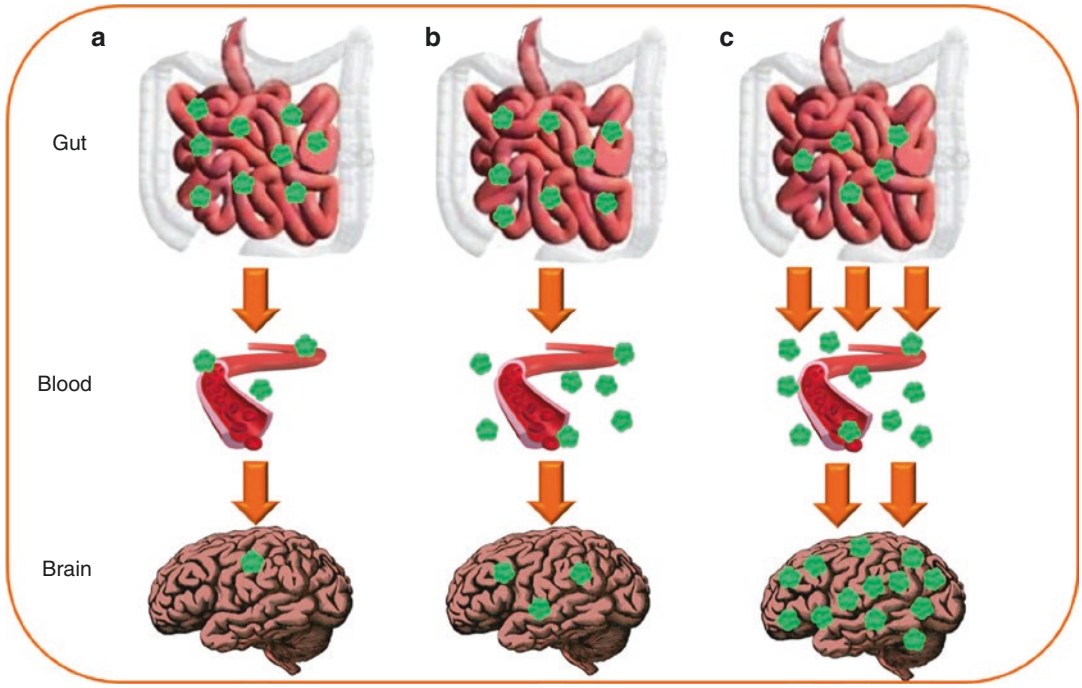


Fig. 33.3 Passage of opioid peptides through gut, blood, and central nervous system (CNS). (**a**) Normal gut–brain transport of peptides. In healthy subjects, with no major problems with intestinal wall permeability, a portion of peptides (*green image*) normally crosses from the intestine into the bloodstream. A portion of these peptides bloodstream will cross through the blood–brain barrier (BBB), accessing the brain. (**b**) Excessive peptide production in the gut. Subjects with abnormal intestinal conditions (gut pH alterations or enzyme system deficiencies) may show increased intestinal peptide levels that will go through the bloodstream and later the BBB reaching the brain. (**c**) Excessive permeability of the intestinal membrane. Subjects with normal gut peptide levels with a flaw in intestinal membrane permeability. This situation allows greater amounts of biologically active peptides to enter the brain through the bloodstream

third mechanism involving an inflammatory process causing increased intestinal wall permeability [6, 17, 18, 26] (Fig. 33.3).

Both peptides, β-casomorphin and gliadorphin, induce direct "opioid activity" [27, 28]. The "opioid excess" theory suggests that ASD symptoms are a consequence of the action of exogenous peptides affecting neurotransmission within the central nervous system (CNS) [15, 29–32].

In the absence of an established pathogenesis for autism, some authors have proposed explanations and treatments based on diet and nutrients. Reports on the use of a gluten-and casein-free diet have shown improvement of ASD symptoms, such as social isolation, and overall ability to communicate and interact [33]. Moreover, Cade et al. showed that a gluten- and casein-free diet was accompanied by improvement in 81% of autistic children within 3 months in most of the behavior categories [6]. Other researchers have shown similar results [34–39].

Genetic Insights in ASD

Direct Brain Impacts Genetic heterogeneity and gene–environment interactions have been found in patients with ASD. Many ASD risk genes encode regulatory proteins of the glutamatergic system involved in the synaptic process and excitatory/inhibitory brain networks, namely: *NRXN1*, *PTEN, SHANK3, UBE3a, NF1, NLGN3/4, CNTNAP2, SYNGAP1*, and *FMR1* [40–48]. Mutations in these genes during CNS development trigger disruptions in excitatory/inhibitory brain circuits [45, 47, 48].

Actually, before synaptogenesis and brain network formation, critical developmental events such as neurogenesis, migration, cellular differentiation, and polarization take place [49, 50]. Therefore, alterations in genes involved in these early stages of CNS development may contribute to a serious damage in brain function, particularly in relation to genes responsible for posterior synaptic function [47, 51].

The International Molecular Genetic Study of Autism Consortium (IMGSAC) was the first to identify a genome linkage with the autism susceptibility locus1 (AUTS1) on the chromosome 7q region [52, 53]. Moreover, evidence of genetic association with autism has been reported for genes located in three different regions of chromosome 7q; 7q21 (*RELN, SERPINE1*), 7q31 (*MET*), and 7q35–36 (*CNTNAP2, EN2*). Each of these chromosome 7q genes, and possibly others, may contribute to autism risk [43, 54–56].

The human gene *MET* (proto-oncogene hepatocyte growth factor receptor, OMIM 164860) that is located on chromosome 7q31.2 and covers approximately 126 kb, encodes a high-affinity transmembrane receptor tyrosine kinase that binds to the hepatocyte growth factor (HGF) [57, 58]. Protein receptor tyrosine kinases (RTKs) are cell-membrane receptors that participate in key stages of CNS development, such as neurogenesis, neuronal differentiation, migration, connectivity, and plasticity [59].

Different studies have reported this gene as a risk factor for ASD based on genetic variants overrepresented in individuals with ASD compared with control populations [60–64].

Furthermore, the expression of the MET receptor and HGF protein was reduced in postmortem brains of individuals with ASD [65]. MET and HGF have been involved in neuronal development and maturation of functional circuits, particularly in cerebral cortex, cerebellum, and hippocampus [59, 66–68]. With distinct spatial and temporal profiles, these brain regions may be altered in autism [69, 70]. Cellular signaling through the MET receptor contributes to neuronal migration and synaptogenesis, among other developmental processes. Therefore, alterations in MET/HGF would stimulate disrupted behavioral and cognitive functions in ASD [59].

Interestingly, MET also contributes to gastrointestinal and immunological functions [71–74]. Alterations in both systems frequently co-occur in ASD patients [18, 19, 22, 60].

Several studies have found a relationship between *MET* promoter variants and the risk for developing ASD. Furthermore, functional imaging and animal studies tie alterations in *MET* gene to morphological and functional disruptions in brain regions related with ASD [59, 75].

MET gene polymorphisms "*rs1858830*" and "*rs38845*" have frequently been found in individuals with ASD in Europe and USA, and may contribute to ASD susceptibility [63, 76]. *MET* gene promoter *"rs1858830*" is a common guanine (G) to cytosine (C) single nucleotide polymorphism, with significant association in ASD families reported in Italian and USA cohorts. The "C allele" is more common in individuals with ASD than in the general population [59]. Expression studies have shown that "C allele" results in decreased *MET* promoter activity, thus reducing transcription of the *MET* gene as well as the specific transcription factor complexes binding "SP1" [60]. The functional ASD risk variant *rs1858830* ("C allele"), which reduces MET protein expression, specifically impacts on the network of connections of different areas of the brain involved in social behavior, including recognizing emotions shown on people's faces [75]. Moreover, *rs1858830* has shown diminished functional and structural connectivity in temporo-parietal lobes brain areas that present a large MET expression, which leads to decreased MET protein in the brains of individuals with ASD [59, 75].

Sousa et al. described an association of another polymorphism located in intron 1 of *MET* gene "*rs38845"* with a potential risk of autism susceptibility [63]. These authors suggested that *rs38845* "A allele" may regulate gene expression interfering in the transcription of *MET* gene by a transcriptional activator "IRF1". Thereby, IRF1 would bind to *MET* promoter, thus altering MET/HGF signaling in ASD patients [63].

Gastrointestinal Impacts As stated before, high rates of gastrointestinal conditions in individuals with ASD have been reported in several studies [77, 78]. *MET* gene polymorphism *rs1858830* "C allele" has been associated with ASD patients with gastrointestinal dysfunctions. Moreover, the transmission of the "C allele" might be enhanced in families with co-occurring ASD and gastrointestinal conditions [60].

MET signaling system alteration can lead to brain and gastrointestinal dysfunctions, and would explain the pathophysiology of ASD and GI comorbidities [60].

Immune Dysregulation and Neuroinflammation in ASD

There is strong evidence of an association between immune dysfunction with ASD development and behavioral symptoms severity, because there are critical interactions between CNS and the immune system [79, 80]. Patients with ASD often exhibit alterations in cytokine levels, lymphocytes T helper type 2 (Th2), abnormal immune cell function, mast cell activation, and the presence of autoantibodies, supporting the association between chronic inflammation and immune dysregulation [7, 79].

General alterations of immune mechanisms trigger chronic inflammation and immune dysregulation in CNS, leading to a neuroinflammatory response [79–81].

Several in-vivo and postmortem studies have found chronic inflammatory processes in multiple areas of the brain, highlighting the correlation between immune dysfunction with behavioral and cognitive impairments in ASD patients [8, 82, 83]. Moreover, some studies have shown immune system dysregulation during a critical period of development in children with ASD [79]. These findings suggested that inflammation plays an important role in the pathogenesis of ASD, and lead to research to elucidate innate immunity pathways associated with neuronal activity in ASD [82, 84].

The improvement of immunological alterations in patients with ASD can alleviate some symptoms of the disorder and enhance overall functioning of affected individuals [85, 86].

Association Between Allergies and ASD

Food intolerances and allergic diseases are common presentations in ASD patients [87–89].

ASD shares with sufferers from IgE and non-IgE-mediated allergic reactions some neuropsychiatric symptoms and mood disorders, such as anxiety, hyperactivity, irritability, tics, sleep disturbance, incoordination, and learning disabilities that ameliorate after anti-allergic treatments. Allergic diseases are associated with pain and discomfort that exacerbate behavioral symptoms in ASD patients [90, 91].

Idiopathic ASD patients frequently show increased mast cell activation in many body organs, or "mastocytosis" [92]. Mast cell stimulation may be triggered by stress, environment, immune reaction, and toxics impacting on brain areas associated with behavior and language [93]. Mast cells release pro-inflammatory molecules and histamine that stimulate the hypothalamus–pituitary–adrenal and sympathetic axes, influencing behavior and cognition [94–96].

Allergic diseases potentially impact on behavioral symptoms and cognitive activity in ASD children. Thus, treatment of allergies may improve symptoms such as anxiety, hyperactivity, and irritability, ameliorating ASD behavior [20, 97].

Non-celiac Food Sensitivity and ASD

Non-celiac gluten sensitivity (NCGS) is a heterogeneous condition regarded as a distinct clinical entity, unrelated to celiac disease, and whose symptoms are triggered by gluten ingestion in the absence of celiac-specific antibodies and of classical celiac villous atrophy [98, 99]. NCGS is an immune reaction against gluten in subjects not affected with celiac disease (CD) or wheat allergy (WA). It does not involve

autoimmune mechanisms. In fact, this entity is characterized by intestinal and extra-intestinal symptoms triggered by the ingestion of gluten-containing food [99, 100].

There is strong evidence of comorbidity in ASD with intestinal pathology, and susceptibility to suffer from allergies [101, 102]. IgA antibodies to gluten and casein were found in ASD patients, suggesting a possible mechanism for the increased intestinal permeability and for some of the symptoms in ASD patients. The presence of IgG antibodies to gluten and casein is another indication that the foreign peptides reach the blood stream intact and in sufficient amounts to stimulate a vigorous IgG response, and are therefore available for uptake by the brain [6, 103]. Several studies have described clinical, serological, and histological characteristics with eosinophil infiltration in the duodenal and colon mucosa in NCGS [103]. Prominent mucosal eosinophil infiltration and increased prevalence of blood eosinophilia have been found in a high percentage of children with ASD. These features are improved in children following a gluten-free diet [104, 105].

Identification and specific medical treatment of GI pathologies in patients with ASD could diminish discomfort, thereby improving behavior problems [105].

Neuroanatomy and Brain Chemistry in ASD

Magnetic resonance images and brain scan studies in ASD patients have shown alterations in brain anatomy and white matter connections in the frontal lobe, the arcuate bundle, temporal cortices, amygdala, and the anterior cingulate cortex. Moreover, post-mortem studies of ASD patient's brains have shown abnormal number of Purkinje cells in cerebellum, as well as dendritic arborization in hippocampus [106]. These brain regions are involved in language and social interaction skills, attention control, empathy, motivation, emotions, error-monitoring, pain perception, behavioral adaptation to a changing environment, and consciousness dissociation. All these facts correlate with specific aspects of ASD symptoms [107–110].

Opioid-active peptides have shown high affinity for the same brain tissues as other related compounds, such as endorphins or morphine [110, 111]. Thus, opioid peptides may cause excitatory and inhibitory actions in CNS, modulating presynaptic release of neurotransmitters. This is associated with the excitatory/inhibitory imbalance hypothesis (neuronal homeostasis) in ASD [112]. These peptides are involved directly or indirectly, firing neural action potentials, and/or releasing intracellular calcium [113–116]. Opioid peptides can inhibit glutamatergic (excitatory) signaling at spinal and supraspinal level, or disinhibit GABAergic signaling (inhibition) [116]. Opioid-receptor binding is involved in cellular proliferation, migration, and differentiation during CNS development [117, 118].

There are three main classes of opioid receptors—mu, delta, and kappa—which belong to the G protein-coupled family. These opioid receptors exhibit different functions and binding characteristics. A specific opioid peptide is able to bind with more than one type of receptor [119, 120]. The binding of opioid peptides to these receptors leads to analgesia and euphoric response [119].

Beta casomorphine-7 (BCM-7) interacts with opioid receptors and affects brain regions including the nucleus accumbens, caudate–putamen, ventral tegmental and median raphe nucleus, and orbitofrontal, prefrontal, parietal, temporal, occipital, and entorhinal cortex. Most of these brain areas have been found to be altered in ASD. Some of these brain regions are integrated by dopaminergic, serotoninergic, and GABAergic systems, suggesting that BCM-7 may interfere with all of these pathways. BCM-7 may disturb cortical association or functional connections, and could be implicated in emotions and motivated behavior, social adaptation, hallucinations, and delusions, typical manifestations of patients with ASD [28, 112, 121].

In brief, ASD behavior results from abnormal interactions between the endogenous opioid system and various pathways, together with anatomical alterations in the CNS. Biochemical, molecular, neurophysiological, and neuroimaging

studies should provide further insights into the pathogenesis of ASD. Due to etiologic and phenotypic heterogeneity of ASD, individualized diagnosis and prognostic predictions should provide effective personalized therapies in ASD patients [109, 122, 123].

References

1. Lord C, Risi S, Lambrecht L, Cook Jr EH, Leventhal BL, DiLavore PC, Pickles A, Rutter M. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord. 2000;30(3):205–23.
2. Christensen DL, Baio J, Van Naarden BK, Bilder D, Charles J, Constantino JN, Daniels J, Durkin MS, Fitzgerald RT, Kurzius-Spencer M, Lee LC, Pettygrove S, Robinson C, Schulz E, Wells C, Wingate MS, Zahorodny W, Yeargin-Allsopp M. Centers for disease control and prevention (CDC). Prevalence and characteristics of autism spectrum disorder among children aged 8 years-Autism and Developmental isabilities onitoring Network, 11 sites, United States, 2012. MMWR Surveill Summ. 2016;65(3):1–23.
3. Volkmar F, Chawarska K, Klin A. Autism in infancy and early childhood. Annu Rev Psychol. 2005;56:315–36.
4. Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M. Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med. 1995;25:63–77.
5. Grafodatskaya D, Chung B, Szatmari P, Weksberg R. Autism spectrum disorders and epigenetics. J Am Acad Child Adolesc Psychiatry. 2010;49:794–809.
6. Cade R, Private FM, Rowland N, Sun Z, Zele V, Wagemaker H, Edelstein C. Autism and schizophrenia: intestinal disorders. Nutr Neurosci. 2000;3:57–72.
7. Molloy CA, Morrow AL, Meinzen-Derr J, Schleifer K, Dienger K, Manning-Courtney P, Altaye M, Wills-Karp M. Elevated cytokine levels in children with autism spectrum disorder. J Neuroimmunol. 2006;172(1):198–205.
8. Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, Courchesne E, Everall IP. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry. 2010;68(4):368–76.
9. Williams BL, Hornig M, Parekh T, Lipkin WI. Application of novel PCR based methods for detection, quantitation, and phylogenetic characterization of Sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. MBio. 2012;3(1):e00261–11.
10. Chauhan A, Audhya T, Chauhan V. Brain region-specific glutathione redox imbalance in autism. Neurochem Res. 2012;37(8):1681–9.
11. Pellicano E. Do autistic symptoms persist across time? Evidence of substantial change in symptomatology over a 3-year period in cognitively able children with autism. Am J Intellect Dev Disabil. 2012;17(2):156–66.
12. Fein D, Barton M, Eigsti IM, Kelley E, Naigles L, Schultz RT, Stevens M, Helt M, Orinstein A, Rosenthal M, Troyb E, Tyson K. Optimal outcome in individuals with a history of autism. J Child Psychol Psychiatry. 2013;54(2):195–205.
13. Eriksson MA, Westerlund J, Hedvall Å, Åmark P, Gillberg C, Fernell E. Medical conditions affect the outcome of early intervention in preschool children with autism spectrum disorders. Eur Child Adolesc Psychiatry. 2013;22(1):23–33.
14. Dohan FC. Cereals and schizophrenia data and hypothesis. Acta Psychiatr Scand. 1966;42:125–52.
15. Reichelt KL, Hole K, Hamberger A, Saelid G, Edmminson PD, Braestrup CB, Lingjaerde O, Ledall P, Orbeck H. Biological active peptide containing fractions in schizophrenia and childhood autism. Adv Biochem Psychopharmacol. 1981;28:627–43.
16. Travé Rodriguez AL, Barreiro Marin P, Gálvez Borrero IM, del Olmo R-NF, Díaz ÁA. Association between autism and schizophrenia. J Nerv Ment Dis. 1994;182(8):478–9.
17. Gardner MLG. Absorption of intact proteins and peptides. In: Johnson LR, editor. Physiology of the gastro-intestinal tract. New York: Raven Press; 1994. p. 1795–820.
18. D'Eufemia P, Celli M, Finocchiaro R, Pacifico L, Viozzi L, Zaccagnini M, Cardi E, Giardini O. Abnormal intestinal permeability in children with autism. Acta Paediatr. 1996;85:1076–9.
19. de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, Carteni M, De Rosa M, Francavilla R, Riegler G, Militerni R, Bravaccio C. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr. 2010;51(4):418–24.
20. Goodwin MS, Cowen MA, Goodwin TC. Malabsorption and cerebral dysfunction. A multivariate and comparative study of autistic children. J Autism Child Schizophr. 1971;1:48–62.
21. Coleman M. Calcium studies and their relationship to coeliac disease in autistic patients. In: Colema M, editor. The autistic syndromes. Amsterdam: North Holland Publishing Corp; 1976. p. 197–205.
22. Buie T, Fuchs 3rd GJ, Furuta GT, Kooros K, Levy J, Lewis JD, Wershil BK, Winter H. Recommendations for evaluation and treatment of common gastrointestinal problems in children with ASDs. Pediatrics. 2010;125(Suppl 1):S19–29.
23. Gorrindo P, Williams KC, Lee EB, Walker LS, McGrew SG, Levitt P. Gastrointestinal dysfunction in autism: parental report, clinical evaluation, and associated factors. Autism Res. 2012;5(2):101–8.
24. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum

disorder: a meta-analysis. Pediatrics. 2014;133(5): 872–83.
25. Kanner L. Follow-up study of eleven autistic children originally reported in 1943. J Autism Child Schizophr. 1971;1(2):119–45.
26. Adams JB, Audhya T, McDonough-Means S, Rubin RA, Quig D, Geis E, Gehn E, Loresto M, Mitchell J, Atwood S, Barnhouse S, Lee W. Effect of a vitamin/mineral supplement on children and adults with autism. BMC Pediatr. 2011;11:111.
27. Sun Z, Cade JR, Fregly MJ, Privette RM. β-Casomorphin induces fos-like immunoreactivity in discrete brain regions relevant to schizophrenia and autism. Autism. 1999;3(1):67–83.
28. Sun Z, Cade R. A peptide found in schizophrenia and autism causes behavioral changes in rats. Autism. 1999;3(1):85–95.
29. Panksepp J. A neurochemical theory of autism. Trends Neurosci. 1979;2:174–7.
30. Reichelt KL, Knivsberg AM, Nodland M, Lind G. Nature and consequence of hyperpeptiduria and bovine casomorphine found in autistic Syndromes. Brain Dysfunction. 1994;7:71–85.
31. Reichelt KL, Knivsberg AM. Can the pathophysiology of autism be explained by the nature of the discovered urine peptides? Nutr Neurosci. 2003;6:19–28.
32. Reichelt KL, Tveiten D, Knivsberg AM, Brønstad G. Peptides: role in autism with emphasis on exorphins. Microb Ecol Health Dis. 2012;24:23.
33. Millward C, Ferriter M, Calver S, Connell-Jones G. Gluten- and casein-free diets for autistic spectrum disorder. Cochrane Database Syst Rev. 2008;16(2): CD003498.
34. Reichelt KL, Ekrem J, Scott H. Gluten, milk proteins and autism: dietary intervention effects on behavior and peptide secretion. J Appl Nutr. 1990;42(1):1–11.
35. Lucarelli S, Frediani T, Zingoni AM, Ferruzzi F, Giardini O, Quintieri F, Barbato M, D'Eufemia P, Cardi E. Food allergy and infantile autism. Panminerva Med. 1995;37(3):137–41.
36. Whiteley P, Rodgersa J. Gluten-free diet as an intervention for autism and associated spectrum disorders: preliminary findings. Autism. 1999;3(1):45.
37. Whiteley P, Shattock P. Biochemical aspects in autism spectrum disorders: updating the opioid-excess theory and presenting new opportunities for biomedical intervention. Expert Opin Ther Targets. 2002;6(2):175–83.
38. Knivsberg AM, Reichelt KL, Høien T, Nødland M. A randomised, controlled study of dietary intervention in autistic syndromes. Nutr Neurosci. 2002;5(4):251–61.
39. Pedersen L, Parlar S, Kvist K, Whiteley P, Shattock P. Data mining the ScanBrit study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders: behavioural and psychometric measures of dietary response. Nutr Neurosci. 2014;17(5):207–13.
40. Alarcón M, Abrahams BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, Sebat J, Wigler M, Martin CL, Ledbetter DH, Nelson SF, Cantor RM, Geschwind DH. Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. Am J Hum Genet. 2008;82(1):150–9.
41. Clement JP, Aceti M, Creson TK, Ozkan ED, Shi Y, Reish NJ, Almonte AG, Miller BH, Wiltgen BJ, Miller CA, Xu X, Rumbaugh G. Pathogenic SYNGAP1 mutations impair cognitive development by disrupting maturation of dendritic spine synapses. Cell. 2012;151(4):709–23.
42. Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, Nygren G, Rastam M, Gillberg IC, Anckarsäter H, Sponheim E, Goubran-Botros H, Delorme R, Chabane N, Mouren-Simeoni MC, de Mas P, Bieth E, Rogé B, Héron D, Burglen L, Gillberg C, Leboyer M, Bourgeron T. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet. 2007;39(1):25–7.
43. Peñagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, Sonnenblick LI, Gruver R, Almajano J, Bragin A, Golshani P, Trachtenberg JT, Peles E, Geschwind DH. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. Cell. 2011;147(1):235–46.
44. Piggot J, Shirinyan D, Shemmassian S, Vazirian S, Alarcón M. Neural systems approaches to the neurogenetics of autism spectrum disorders. Neuroscience. 2009;164(1):247–56.
45. Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, Südhof TC. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science. 2007;318(5847): 71–6.
46. Yashiro K, Riday TT, Condon KH, Roberts AC, Bernardo DR, Prakash R, Weinberg RJ, Ehlers MD, Philpot BD. Ube3a is required for experience-dependent maturation of the neocortex. Nat Neurosci. 2009;12(6):777–83.
47. Ebert DH, Greenberg ME. Activity-dependent neuronal signalling and autism spectrum disorder. Nature. 2013;493(7432):327–37.
48. Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav. 2003;2(5):255–67.
49. Bradke F, Dotti CG. Establishment of neuronal polarity: lessons from cultured hippocampal neurons. Curr Opin Neurobiol. 2000;10(5):574–81.
50. Mueller BK. Growth cone guidance: first steps towards a deeper understanding. Annu Rev Neurosci. 1999;22:351–88.
51. Qui S, Aldinger KA, Levitt P. Modeling of autism genetic variations in mice: focusing on synaptic and microcircuit dysfunctions. Dev Neurosci. 2012; 34(2–3):88–100.
52. IMGSAC. A full genome screen for autism with evidence for linkage to a region on chromosome 7q. International molecular genetic study of autism consortium. Hum Mol Genet. 1998;7(3):571–8.

53. IMGSAC. Further characterization of the autism susceptibility locus AUTS1 on chromosome 7q. Hum Mol Genet. 2001;10(9):973–82.
54. Zhang H, Liu X, Zhang C, Mundo E, Macciardi F, Grayson DR, Guidotti AR, Holden JJ. Reelin gene alleles and susceptibility to autism spectrum disorders. Mol Psychiatry. 2002;7:1012–7.
55. Yonan AL, Alarcón M, Cheng R, Magnusson PKE, Spence SJ, Palmer AA, Grunn A, Juo SHH, Terwilliger JD, Liu J, Cantor RM, Geschwind DH, Gilliam TC. A genomewide screen of 345 families for autism-susceptibility loci. Am J Hum Genet. 2003;73(4):886–97.
56. Persico AM, Napolioni V. Autism genetics. Behav Brain Res. 2013;15:95–112.
57. Cooper CS, Park M, Blair DG, Tainsky MA, Huebner K, Croce CM, Vande Woude GF. Molecular cloning of a new transforming gene from a chemically transformed human cell line. Nature. 1984;311(5981):29–33.
58. Naldini L, Weidner KM, Vigna E, Gaudino G, Bardelli A, Ponzetto C, Narsimhan RP, Hartmann G, Zarnegar R, Michalopoulos GK, Birchmeierl W, Comoglio PM. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. EMBO J. 1991;10(10):2867–78.
59. Peng Y, Huentelman M, Smith C, Qiu S. MET receptor tyrosine kinase as an autism genetic risk factor. Int Rev Neurobiol. 2013;113:135–65.
60. Campbell DB, Buie TM, Winter H, Bauman M, Sutcliffe JS, Perrin JM, Levitt P. Distinct genetic risk based on association of MET in families with co-occurring autism and gastrointestinal conditions. Pediatrics. 2009;123(3):1018–24.
61. Campbell DB. When linkage signal for autism MET candidate gene. Eur J Hum Genet. 2009;17(6): 699–700.
62. Jackson PB, Boccuto L, Skinner C, Collins JS, Neri G, Gurrieri F, Schwartz CE. Further evidence that the rs1858830 C variant in the promoter region of the MET gene is associated with autistic disorder. Autism Res. 2009;2(4):232–6.
63. Sousa I, Clark TG, Toma C, Kobayashi K, Choma M, Holt R, Sykes NH, Lamb JA, Bailey AJ, Battaglia A, Maestrini E, Monaco AP. MET and autism susceptibility: family and case-control studies. Eur J Hum Genet. 2009;17(6):749–58.
64. Thanseem I, Nakamura K, Miyachi T, Toyota T, Yamada S, Tsujii M, Tsuchiya KJ, Anitha A, Iwayama Y, Yamada K, Hattori E, Matsuzaki H, Matsumoto K, Iwata Y, Suzuki K, Suda S, Kawai M, Sugihara G, Takebayashi K, Takei N, Ichikawa H, Sugiyama T, Yoshikawa T, Mori N. Further evidence for the role of MET in autism susceptibility. Neurosci Res. 2010;68(2):137–41.
65. Campbell DB, D'Oronzio R, Garbett K, Ebert PJ, Mirnics K, Levitt P. Persico. Disruption of cerebral cortex MET signaling in autism spectrum disorder. AM. Ann Neurol. 2007;62(3):243–50.
66. Birchmeier C, Gherardi E. Developmental roles of HGF/SF and its receptor, the c-Met tyrosine kinase. Trends Cell Biol. 1998;8:404–10.
67. Yang XM, Park M. Expression of the MET/hepatocyte growth factor/scatter factor receptor and its ligand during differentiation of murine P19 embryonal carcinoma cells. Dev Biol. 1993;157:308–20.
68. Leraci A, Forni PE, Ponzetto C. Viable hypomorphic signaling mutant of the Met receptor reveals a role for hepatocyte growth factor in postnatal cerebellar development. Proc Natl Acad Sci U S A. 2002;99: 15200–5.
69. Judson MC, Bergman MY, Campbell DB, Eagleson KL, Levitt P. Dynamic gene and protein expression patterns of the autism-associated met receptor tyrosine kinase in the developing mouse forebrain. J Comp Neurol. 2009;513(5):511–31.
70. Judson MC, Eagleson KL, Levitt P. A new synaptic player leading to autism risk: met receptor tyrosine kinase. J Neurodev Disord. 2011;3(3):282–92.
71. Beilmann M, Odenthal M, Jung W, Vande Woude GF, Dienes HP, Schirmacher P. Neoexpression of the c-met/hepatocyte growth factor-scatter factor receptor gene in activated monocytes. Blood. 1997;90(11): 4450–8.
72. Tahara Y, Ido A, Yamamoto S, Miyata Y, Uto H, Hori T, Hayashi K, Tsubouchi H. Hepatocyte growth factor facilitates colonic mucosal repair in experimental ulcerative colitis in rats. J Pharmacol Exp Ther. 2003;307(1):146–15.
73. Ido A, Numata M, Kodama M, Tsubouchi H. Mucosal repair and growth factors: recombinant human hepatocyte growth factor as an innovative therapy for inflammatory bowel disease. J Gastroenterol. 2005;40(10):925–31.
74. Okunishi K, Dohi M, Nakagome K, Tanaka R, Mizuno S, Matsumoto K, Miyazaki J, Nakamura T, Yamamoto K. A novel role of hepatocyte growth factor as an immune regulator through suppressing dendritic cell function. J Immunol. 2005;175(7):4745–53.
75. Rudie JD, Hernandez LM, Brown JA, Beck-Pancer D, Colich NL, Gorrindo P, Thompson PM, Geschwind DH, Bookheimer SY, Levitt P, Dapretto M. Autism-associated promoter variant in MET impacts functional and structural brain networks. Neuron. 2012;75(5):904–15.
76. Campbell DB, Sutcliffe JS, Ebert PJ, Militerni R, Bravaccio C, Trillo S, Elia M, Schneider C, Melmed R, Sacco R, Persico AM, Levitt P. A genetic variant that disrupts MET transcription is associated with autism. Proc Natl Acad Sci U S A. 2006; 103(45):16834–9.
77. Quigley EM, Hurley D. Autism and the gastrointestinal tract. Am J Gastroenterol. 2000;95(9):2154–6.
78. Levy SE, Souders MC, Ittenbach RF, Giarelli E, Mulberg AE, Pinto-Martin JA. Relationship of dietary intake to gastrointestinal symptoms in children with autistic spectrum disorders. Biol Psychiatry. 2007;61(4):492–7.

79. Heuer L, Ashwood P, Schauer J, Goines P, Krakowiak P, Hertz-Picciotto I, Hansen R, Croen LA, Pessah IN, Van de Water J. Reduced levels of immunoglobulin in children with autism correlates with behavioral symptoms. Autism Res. 2008;1(5):275–83.
80. Enstrom A, Krakowiak P, Onore C, Pessah IN, Hertz-Picciotto I, Hansen RL, Van de Water JA, Ashwood P. Increased IgG4 levels in children with autism disorder. Brain Behav Immun. 2009;23(3):389–95.
81. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain, Behav Immu. 2011;25(1):40–5.
82. Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, Ji L, Brown T, Malik M. Elevated immune response in the brain of autistic patients. J Neuroimmunol. 2009;207(1):111–6.
83. Wei H, Zou H, Sheikh AM, Malik M, Dobkin C, Brown WT, Li X. IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. J Neuroinflammation. 2011;8(1):52.
84. Gupta S, Ellis SE, Ashar FN, Moes A, Bader JS, Zhan J, West AB, Arking DE. Transcriptome analysis reveals dysregulation of innate immune response genes and neuronal activity-dependent genes in autism. Nat Commun. 2014;5:5748.
85. Boris M, Kaiser CC, Goldblatt A, Elice MW, Edelson SM, Adams JB, Feinstein DL. Effect of pioglitazone treatment on behavioral symptoms in autistic children. J Neuroinflammation. 2007;4:3.
86. Sharma A, Gokulchandran N, Chopra G, Kulkarni P, Lohia M, Badhe P, Jacob VC. Administration of autologous bone marrow-derived mononuclear cells in children with incurable neurological disorders and injury is safe and improves their quality of life. Cell Transplant. 2012;21(Supplement 1):S79–90.
87. Schmitt J, Romanos M, Pfennig A, Leopold K, Meurer M. Psychiatric comorbidity in adult eczema. Br J Dermatol. 2009;161(4):878–83.
88. Kohane IS, McMurry A, Weber G, MacFadden D, Rappaport L, Kunkel L, Bickel J, Wattanasin N, Spence S, Murphy S, Churchill S. The co-morbidity burden of children and young adults with autism spectrum disorders. PLoS One. 2012;7(4):e33224.
89. Fasmer OB, Riise T, Eagan TM, Lund A, Dilsaver SC, Hundal O, Oedegaard KJ. Comorbidity of asthma with ADHD. J Atten Disord. 2011;15(7):564–71.
90. Millman M, Campbell MB, Wright KL, Johnston A. Allergy and learning disabilities in children. Ann Allergy. 1976;36(3):149–60.
91. Price CE, Rona RJ, Chinn S. Associations of excessive irritability with common illnesses and food intolerance. Paediatr Perinat Epidemiol. 1990;4(2):156–60.
92. Angelidou A, Alysandratos KD, Asadi S, Zhang B, Francis K, Vasiadi M, Kalogeromitros D, Theoharides TC. Brief report: "allergic symptoms" in children with autism spectrum disorders. More than meets the eye? J Autism Dev Disord. 2011;41(11):1579–85.
93. Theoharides TC, Enakuaa S, Sismanopoulos N, Asadi S, Papadimas EC, Angelidou A, Alysandratos KD. Contribution of stress to asthma worsening through mast cell activation. Ann Allergy Asthma Immunol. 2012;109(1):14–9.
94. Scaccianoce S, Lombardo K, Nicolai R, Affricano D, Angelucci L. Studies on the involvement of histamine in the hypothalamic–pituitary–adrenal axis activation induced by nerve growth factor. Life Sci. 2000;67(26):3143–52.
95. Kalogeromitros D, Syrigou EK, Makris M, Kempuraj D, Stavrianeas NG, Vasiadi M, Theoharides TC. Nasal provocation of patients with allergic rhinitis and the hypothalamic–pituitary–adrenal axis. Ann Allergy Asthma Immunol. 2007;98(3):269–73.
96. Liezmann C, Klapp B, Peters EM. Stress, atopy and allergy: a re-evaluation from a psychoneuroimmunologic persepective. Dermatoendocrinol. 2011;3(1):37–40.
97. Jyonouchi H. Autism spectrum disorders and allergy: observation from a pediatric allergy/immunology clinic. Expert Rev Clin Immunol. 2010;6(3):397–411.
98. Catassi C, Bai JC, Bonaz B, Bouma G, Calabrò A, Carroccio A, Castillejo G, Ciacci C, Cristofori F, Dolinsek J, Francavilla R, Elli L, Green P, Holtmeier W, Koehler P, Koletzko S, Meinhold C, Sanders D, Schumann M, Schuppan D, Ullrich R, Vécsei A, Volta U, Zevallos V, Sapone A, Fasano A. Nonceliac gluten sensitivity: the new frontier of gluten-related disorders. Nutrients. 2013;5(10):3839–53.
99. Fasano A, Sapone A, Zevallos V, Schuppan D. Nonceliac gluten sensitivity. Gastroenterology. 2015;148(6):1195–204.
100. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M, Kaukinen K, Rostami K, Sanders DS, Schumann M, Ullrich R, Villalta D, Volta U, Catassi C, Fasano A. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Med. 2012;10:13.
101. Sandler RH, Finegold SM, Bolte ER, Buchanan CP, Maxwell AP, Väisänen ML, Nelson MN, Wexler HM. Short-term benefit from oral vancomycin treatment of regressive-onset autism. J Child Neurol. 2000;15(7):429–35.
102. Schieve LA, Gonzalez V, Boulet SL, Visser SN, Rice CE, Van Naarden BK, Boyle CA. Concurrent medical conditions and health care use and needs among children with learning and behavioral developmental disabilities, National Health Interview Survey, 2006-2010. Res Dev Disabil. 2011;33(2):467–76.
103. Carroccio A, Mansueto P, Iacono G, Soresi M, D'Alcamo A, Cavataio F, Brusca I, Florena AM,

Ambrosiano G, Seidita A, Pirrone G, Rini GB. Non-celiac wheat sensitivity diagnosed by double-blind placebo-controlled challenge: exploring a new clinical entity. Am J Gastroenterol. 2012;107(12): 1898–906.
104. Ashwood P, Anthony A, Pellicer AA, Torrente F, Walker-Smith JA, Wakefield AJ. Intestinal lymphocyte populations in children with regressive autism: evidence for extensive mucosal immunopathology. J ClinImmunol. 2003;23(6):504–17.
105. Chen B, Girgis S, El-Matary W. Childhood autism and eosinophilic colitis. Digestion. 2010;81(2):127–9.
106. Courchesne E, Campbell K, Solso S. Brain growth across the life span in autism: age-specific changes in anatomical pathology. Brain Res. 2011;1380: 138–45.
107. Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, Morgan J. Mapping early brain development in autism. Neuron. 2007;56(2):399–413.
108. Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. Trends Neurosci. 2008;31(3):137–45.
109. Catani M, Dell'Acqua F, Budisavljevic S, Howells H, Thiebaut de Schotten M, Froudist-Walsh S, D'Anna L, Thompson A, Sandrone S, Bullmore ET, Suckling J, Baron-Cohen S, Lombardo MV, Wheelwright SJ, Chakrabarti B, Lai MC, Ruigrok AN, Leemans A, Ecker C, Consortium MA, Craig MC, Murphy DG. Frontal networks in adults with autism spectrum disorder. Brain. 2016;139(Pt 2): 616–30.
110. Zioudrou C, Streaty RA, Klee WA. Opioid peptides derived from food proteins The exorphins. J Biol Chem. 1979;254(7):2446–9.
111. Huebner FR, Lieberman KW, Rubino RP, Wall JS. Demonstration of high opioid-like activity in isolated peptides from wheat gluten hydrolysates. Peptides. 1984;5(6):1139–47.
112. van Elst LT, Maier S, Fangmeier T, Endres D, Mueller GT, Nickel K, Ebert D, Lange T, Hennig J, Biscaldi M, Riedel A, Perlov E. Magnetic resonance spectroscopy comparing adults with high functioning autism and above average IQ. Mol Psychiatry. 2014;19(12):1251.
113. Siggins GR, Henriksen SJ, Chavkin C, Gruol D. Opioid peptides and epileptogenesis in the limbic system: cellular mechanisms. Adv Neurol. 1986; 44:501–12.
114. Wagner R, DeLeo JA, Coombs DW, Willenbring S, Fromm C. Spinal dynorphin immunoreactivity increases bilaterally in a neuropathic pain model. Brain Res. 1993;629(2):323–6.
115. Simmons ML, Chavkin C. Endogenous opioid regulation of hippocampal function. Int Rev Neurobiol. 1996;39:145–96.
116. Henriksen G, Willoch F. Imaging of opioid receptors in the central nervous system. Brain. 2008;131(Pt 5):1171–96.
117. Zagon IS, Gibo DM, McLaughlin PJ. Adult and developing human cerebella exhibit different profiles of opioid binding sites. Brain Res. 1990;523:62–8.
118. Zagon IS, McLaughlin PJ. Opioid growth factor receptor in the developing nervous system: laboratory findings. In: Zagon IS, McLaughlin PJ, editors. Receptors and the developing nervous system, Growth Factors and Hormones, vol. L. London: Chapman and Hall; 1993. p. 39–62.
119. Froehlich JC. Opioid peptides. Alcohol Health Res World. 1997;21(2):132–6.
120. Teschemacher H. Opioid receptor ligands derived from food proteins. Curr Pharm Des. 2003;9(16): 1331–44.
121. Doty RW. Schizophrenia: a disease of interhemispheric processes at forebrain and brainstem levels? Behav Brain Res. 1989;34(1–2):1–33.
122. Sher L. Autistic disorder and the endogenous opioid system. Med Hypotheses. 1997;48(5):413–4.
123. Lajonchere C, Jones N, Coury DL, Perrin JM. Leadership in health care, research, and quality improvement for children and adolescents with autism spectrum disorders: autism treatment network and autism intervention research network on physical health. Pediatrics. 2012;130(Suppl 2): S62–8.

34 Update on Dementia. Pathophysiology, Diagnosis, and Treatment. DSM-IV versus DSM-V

Rose E. Nina-Estrella

Abstract

Dementia is frequent in the elderly, and advancing age is the strongest risk factor. It includes Alzheimer's disease (AD), Vascular dementia (VaD), and other neurogenerative disorders such as Lewy body dementia (LBD), and other less-common neurodegenerative dementing diseases, such as frontotemporal dementia (FTD). All this acquired disorder of cognition and the related behavioral impairment interferes with social and occupational functioning. The fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) present differences in the description of AD and VaD. The new DSM recognizes the acceptable alternative "neurocognitive disorder" as a newly preferred and more scientific term than "dementia". This new diagnosis includes both the dementia and amnesic disorder diagnoses from DSM-IV. Furthermore, DSM-V recognizes specific etiologic subtypes of neurocognitive dysfunction, such as Alzheimer's disease, Parkinson's disease, HIV infection, Lewy body disease, and Vascular disease. This is a review based on scientific evidence and information concerning the most common dementia, Alzheimer's disease (AD) and the second most important, Vascular dementia (VaD), and the main differences between the classifications of DSM-IV and DSM-V for both diseases.

Keywords

Dementia • Alzheimer's disease • Vascular dementia • Major and mild neurocognitive disorder • DSM–IV • DSM-V

R.E. Nina-Estrella
Department of Pharmacology, School of Physiological Sciences, Faculty of Health Sciences, University Autónoma of Santo Domingo, Santo Domingo, Dominican Republic
e-mail: roseninaestrella@gmail.com

Introduction

According to many specific references such as the World Alzheimer Report 2015, the number of people living with dementia globally is expected

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_34

to rise from the current 46 million to 131.5 million by 2050. Global costs to treat dementia, estimated at about US$818 billion in 2015, are expected to soar to $1 trillion by 2018 and to $2 trillion by 2030 [1]. Dementia is most common in the elderly. Multiple neuropathologic processes may underlie dementia, including both neurodegenerative diseases and vascular disease. In addition, comorbidity (the presence of more than one disease process) is more common than dementia in elderly persons [2–5].

There are two most important dementias. Alzheimer's disease (AD) is the most common neurodegenerative disease responsible for dementia. About half of dementia cases result from AD [2, 3]. Many measurable AD pathologic changes occur in most cognitively intact elderly individuals who undergo autopsy. This indicates that AD is a chronic disease with latent and prodromal stages. It suggests that individuals may have varying abilities to compensate, either biologically or functionally, for the presence of pathological changes underlying AD [6].

Vascular dementia is the second most common form of dementia after AD. The condition is not a single disease. It is a group of syndromes related to different vascular mechanisms. Vascular dementia is preventable, but in this dementia early detection and an accurate diagnosis are also important [7].

It is clinically important to use the Hachinski Ischemic Score (HIS) which aims to distinguish Vascular dementia from Alzheimer's disease [8]. Hachinski's ischemic scale seems to be reliable approximately in 90% of cases in the differential diagnosis between Vascular and Alzheimer dementias, especially in the multi-infarct group [9]. The presence of 13 clinical symptoms comprises the HIS. It assigns two points to each of the following symptoms: abrupt onset, fluctuating course, history of stroke, focal neurologic signs, and focal neurologic symptoms. It also assigns additional points for stepwise deterioration, nocturnal confusion, preservation of personality, depression, somatic complaints, emotional incontinence, hypertension, and associated atherosclerosis. A score of 7 or higher suggests Vascular dementia, and a score of 4 or less suggests AD.

As has been mentioned, dementia includes a group of neurodegenerative disorders characterized by progressive loss of cognitive function and a decrease in the ability to perform daily living activities [10].

There are two American mental disorder classifications that could be used at present for diagnosis criteria of mental disorders: the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), and the fifth edition (DSM-5). We are at a transitional point, discontinuing the use of the DSM-IV and starting use of the new DSM- V. It is true that some doctors have a strong resistance to the use of the DSM-V in respect of the new mental disorders classification. When the DSM-V was published, it led to many controversial medical and psychiatric opinions.

DSM-IV was published in 1994 and DSM-5 was published in 2013. The DSM-V is now the standard classification of mental disorders used by mental health professionals in the United States. It is intended to be used in all clinical settings by clinicians of different theoretical orientations. It can be used by mental health and other health professionals, including psychiatrists and other physicians, psychologists, social workers, nurses, occupational and rehabilitation therapists, and counselors. It can also be used in research in clinical and community populations [11]. We see great differences in the diagnosis of AD and Vascular dementia between the two classifications, and it is the purpose of this chapter to clarify these differences.

Alzheimer's Disease

Let's start with the history background of AD.This dementia was first described in 1901 by a German psychiatrist named Alois Alzheimer. He observed a patient at the Frankfurt Asylum named Mrs. Auguste D. This 51-year-old woman suffered from a loss of short-term memory, among other behavioral symptoms that puzzled Dr. Alzheimer [12]. After 5 years, in April 1906, the patient died, and Dr. Alzheimer sent her brain and her medical records to Munich, where he was

working in the lab of Dr. Emil Kraepelin. By staining sections of her brain in the laboratory, he was able to identify amyloid plaques and neurofibrillary tangles [12]. The important seminar given by Dr. Alzheimer on November 3, 1906, was the first time that the pathology and the clinical symptoms of the disorder had been presented together. The nosological entity was termed presenile dementia. Alzheimer published his findings in 1907 [13].

In the past 20 years, an effort has been made to understand the neurogenetics and pathophysiology of AD. Four different genes are definitively associated with AD. Other genes that may have a probable role have been identified. The mechanisms by which altered amyloid and tau protein metabolism, inflammation, oxidative stress, and hormonal changes may produce neuronal degeneration in AD are being elucidated, and rational pharmacologic interventions based on these discoveries are being developed [14].

Etiology

The cause of AD is unknown. But there are many possible risk factors to be considered. Many investigators now believe that converging environmental and genetic risk factors trigger a pathophysiologic cascade that, acting over decades, leads to Alzheimer pathology and dementia [15]. A group of risk factors for Alzheimer-type dementia have been identified [16–19]:

(a) Advancing age
(b) Family history
(c) APOE 4 genotype[1]
(d) Obesity
(e) Insulin resistance
(f) Vascular factors
(g) Dyslipidemia
(h) Hypertension
(i) Traumatic brain injury
(j) Inflammatory markers
(k) Down syndrome

Based on evidence, there are some other possible risk factors, like depression. Other important risk factors to consider are the genetic risk factors, which are described below in detail. However, there are also some protective factors, such education and long-term use of nonsteroidal anti-inflammatory drugs [22–24].

With regard to genetic factors, it has been described that in some families an autosomal dominant AD has been observed. It accounts for less than 5% of cases, and is almost exclusively early-onset AD. These cases occur in at least three individuals in two or more generations, with two of the individuals being first-degree relatives [25]. If we follow familial clustering, it represents approximately 15–25% of late-onset AD cases, and most often involves late-onset AD. In familial clustering, at least two of the affected individuals are third-degree relatives or closer [25].

Mutations in the following genes unequivocally cause early-onset autosomal-dominant AD:

1. Amyloid precursor protein (APP*)* gene on chromosome 21
2. Presenilin-1 (PS1) gene on chromosome 14
3. Presenilin-2 (PS2) gene on chromosome 1

All three of these genes lead to a relative excess in the production of the stickier 42-amino acid form of the Ab peptide over the less sticky 40-amino-acid form [25].

It has been postulated that beta-pleated peptide has neurotoxic properties, and that it leads to a cascade of events. These events are not well understood, and result in neuronal death, synapse loss, and the formation of neurofibrillary tangles (NFTs) and senile plaques (SPs), between other lesions. However, mutations that have been found to date only make it possible to explain less than half of the cases of early-onset AD [26]. Familial Alzheimer's disease is caused by any one of a number of different single-gene mutations, such as mutations on chromosome 21, which cause the

[1]The *APOE* gene (located on chromosome 19) is the only gene identified related to early-onset and late-onset of AD. APOE ε4 is called a risk-factor gene because it increases a person's risk of developing the disease; however, inheriting an APOE ε4 allele does not necessarily mean that a person will develop AD [20, 21].

formation of abnormal amyloid precursor protein (APP). Afterwards, several mis-sense genetic mutations within the APP gene were identified in these familial AD kindreds. These mutations resulted in amino acid substitutions in APP that appear to alter the previously described proteolytic processing of APP, generating amyloidogenic forms of Ab [26]. Approximately 50–70% of early-onset autosomal-dominant AD cases appear to be associated with a locus (AD3) mapped by genetic linkage to the long arm of chromosome 14 (14q24.3). Numerous mis-sense mutations have been identified on a strong candidate gene called PS1 [26].

There is another important gene. The gene encoding the cholesterol-carrying apolipoprotein E (APOE*)* on chromosome 19 has been linked to increased risk for AD, principally late-onset but also some early-onset cases. This gene is inherited as an autosomal codominant trait with three alleles. The APOE E2 allele, the least prevalent of the three common APOE alleles, is associated with the lowest risk of developing AD, with a lower rate of annual hippocampal atrophy, higher cerebrospinal fluid Aβ and lower phosphor-tau, suggesting less AD pathology [27, 28].

APOE E4 gene "dose" is correlated with increased risk and earlier onset of AD [29]. Blood pressure is very important in those individuals who are genetically predisposed to AD. They are advised to closely control their blood pressure. Hypertension has been shown to interact with APOE E4 genotype to increase amyloid deposition in cognitively healthy middle-aged and older adults. Controlling hypertension may significantly decrease the risk of developing amyloid deposits, even in those with genetic risk [30, 31].

Although research supports the relationship between the APOE ε4 variant and the occurrence of late-onset AD, the full mechanism of action and the pathophysiology are not known [20, 21].

There are also other genome-wide association studies that have identified additional susceptibility loci. They are the following: clusterin (CLU*)* gene, phosphatidylinositol-binding clathrin assembly protein *(*PICALM*)* gene, complement receptor 1 (CR1*)* gene, ATP-binding cassette sub-family A member 7 gene *(*ABCA7), membrane-spanning gene cluster (MS4A6A/MS4A4E), ephrin receptor A1 *(* EPHA1*),* CD33*,* CD2AP [26].

It is important to note that many APOE E4 carriers do not develop AD, and many patients with AD do not have this allele. The presence of an APOE E4 allele does not secure the diagnosis of AD, but instead, the APOE E4 allele acts as a biologic risk factor for the disease, especially in those younger than 70 years [14].

Other risk factor to describe is depression. Depression has been identified as a risk factor for AD and other dementias. Recent Framingham data have helped to bolster the epidemiological association. The study showed a 50% increase in AD and dementia in those who were depressed at baseline. During a 17-year follow-up period, a total of 21.6% of participants who were depressed at baseline developed dementia, as compared with 16.6% of those who were not depressed [32].

Pathophysiology

In the pathophysiology of normal aging and in AD, the pathologic hallmarks of AD are the same that occur in the brains of cognitively intact persons. In AD, tau is changed chemically. If we describes what happen it begins to pair with other threads of tau, which become tangled together. When this happens, the microtubules disintegrate, collapsing the neuron transport system. The formation of these neurofibrillary tangles (NFTs) may result first in communication malfunctions between neurons and later in the death of the cells. This is called apoptosis. In addition to NFTs, the anatomic pathology of AD includes senile plaques (SPs), also known as beta-amyloid plaques. They may be observed at the microscopic level, and cerebrocortical atrophy at the macroscopic level. The hippocampus and medial temporal lobe are the initial sites of tangle deposition and structure atrophy. This can be seen on brain magnetic resonance imaging early in AD and helps supporting a clinical diagnosis [33].

SPs and NFTs were described by Alois Alzheimer in his original report on the disorder in 1907 [13]. They are now universally accepted as the pathological hallmark of the disease.

Although NFTs and SPs are characteristic of AD, they are not pathognomonic. NFTs are found in several other neurodegenerative disorders. SPs may occur in normal aging. The only presence of these lesions is not sufficient to support the diagnosis of AD. It is important that symptoms and lesions must be present together in sufficient numbers and in a characteristic topographic distribution to fulfill the current histopathologic criteria for AD.

For example, in a study in which neuropathologists were blinded to clinical data, they identified 76% of brains of cognitively intact elderly patients as demonstrating AD [33]. The accumulation of SPs primarily precedes the clinical onset of AD. NFTs, loss of neurons, and loss of synapses accompany the progression of cognitive decline [34].

Diagnosis

Patients with Alzheimer's disease (AD) most commonly present insidiously progressive memory loss. Other spheres of cognitive impairment are added over several years. This loss may be associated with slowly progressive behavioral changes. After memory loss occurs, there are others symptoms that appear: language disorders (e.g., anomia) and impairment in their visuospatial skills and executive functions [14].

The diagnosis of Alzheimer's disease should include: signs and symptoms, with the diagnosis criteria as guidelines, biomarkers which confirm the diagnosis, blood test, imaging, neuoropsychological test and pathophysiology.

The symptoms of AD can be classified into the following stages:

(a) Preclinical
(b) Mild
(c) Moderate
(d) Severe

Preclinical Alzheimer's Disease

The pathologic changes begin in the entorhinal cortex, which is near the hippocampus and directly connected to it. AD then proceeds to the hippocampus, which is the structure that is essential to the formation of short-term and long-term memories. Affected regions begin to atrophy [14]. These brain changes probably start 10–20 years before any visible signs or symptoms appear. They could start in a silent way after 40 years of age. Memory loss, the first visible sign, is the main feature of amnestic mild cognitive impairment (MCI). Many scientists think MCI is often an initial, transitional clinical phase between normal brain aging and AD. A patient with preclinical AD may appear completely normal on physical examination and mental status testing. At this stage, there is normally no alteration in judgment or the ability to perform activities of daily living [14].

Mild Alzheimer's Disease

In the mild stage we can observe that the cerebral cortex is affected, memory loss continues and impairment of other cognitive abilities are also present. Later in the disease, physical abilities decline. The clinical diagnosis of AD is usually made during this stage. Signs and symptoms of mild AD can include the following:

Memory loss
Confusion about the location of familiar places (getting lost begins to occur)
Compromised judgment often leading to bad decisions
Taking longer to accomplish normal daily tasks
Trouble handling money and paying bills
Compromised judgment often leading to bad decisions
Loss of spontaneity and sense of initiative
Mood and personality changes
Increased anxiety

The growing number of plaques and tangles first damage areas of the brain that control memory, language, and reasoning. In mild AD, a person seems to be healthy but is actually having more and more trouble making sense of the world around him or her. The realization that something is wrong often comes gradually, because the early signs can be confused with changes that can

happen normally with aging. For example: in many cases, the family has a more difficult time handling the diagnosis than the patient does, some patients do not seem emotionally affected, probably because of the sense of apathy, a feeling which occurs in AD. In other cases, following the initial diagnosis, patients should be carefully monitored for a depressed mood. Although it is common for patients with early AD to be depressed about the diagnosis, they rarely become suicidal [14].

Moderate Alzheimer's Disease

After the mild stage, the moderate stage starts; damage continues to affect the cerebral cortex that controls language, reasoning, sensory processing, and conscious thought. Affected regions continue to atrophy, and signs and symptoms of the disease become more pronounced. Behavioral symptoms, such as wandering and agitation, can occur. More intensive supervision and care become necessary, and this can be difficult for many spouses and families.

The symptoms of this stage can include the following:

Increasing memory loss, confusion, and shortened attention span
Problems recognizing friends and family members
Repetitive statements or movement; occasional muscle twitches
Hallucinations, delusions, suspiciousness or paranoia, irritability
Difficulty with language; problems with reading, writing, working with numbers
Difficulty organizing thoughts and thinking logically
Inability to learn new things or to cope with new or unexpected situations
Restlessness, agitation, anxiety, tearfulness, wandering, especially in the late afternoon or at night
Loss of impulse control (shown through behavior, such as undressing at inappropriate times or places, or vulgar language)
Perceptual-motor problems (such as trouble getting out of a chair or setting the table)

Anger is a primary emotion that can mask underlying confusion and anxiety. Also, the risk of violent and homicidal behavior is highest at this stage of disease progression. Patients should be carefully monitored for any behavior that may compromise the safety of those around them. Since it is the case of a person who cannot remember the past or anticipate the future, the world around them can be strange and frightening. Staying close to a trusted and familiar caregiver may be the only thing that makes sense and provides security. The individual may constantly follow his or her caregiver and feel lost when the person is out of sight. Judgment and impulse control continue to decline at this stage [14].

Severe Alzheimer's Disease

In the last stage, illness severity is perceived. Plaques and tangles are widespread throughout the brain, and areas of the brain have been atrophied. Patients cannot recognize family and loved ones or communicate in any way. This is a burden for the families. They are completely dependent on others for care. All sense of self seems to disappear.

There are other symptoms:

Weight loss
Seizures, skin infections, difficulty swallowing
Groaning, moaning, or grunting
Increased sleeping
Lack of bladder and bowel control

In end-stage AD, patients may be in bed much or all of the time. Death is often the result of other illnesses, frequently aspiration pneumonia.

Clinical guidelines for the diagnosis of AD have been formulated by the National Institutes of Health–Alzheimer's Disease and Related Disorders Association (NIH-ADRDA); the American Psychiatric Association, in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V)*;* and the Consortium to Establish a Registry in Alzheimer's disease (CERAD). In 2011, the National Institute on Aging (NIA) and the Alzheimer's Association (AA) workgroup released new research and clinical diagnostic

criteria for AD [35]. The NIH–ADRDA criteria for the diagnosis of AD require the finding of a slowly progressive memory loss of insidious onset in a fully conscious patient. AD cannot be diagnosed in patients with clouded consciousness or delirium [35]. The focus of the 2011 NIA-AA criteria is the need to create a more accurate diagnosis of preclinical disease so that treatment can begin before neurons are significantly damaged, while they are more likely to respond. The report includes criteria for diagnosis of the following:

Asymptomatic, preclinical AD (for purposes of research, not clinical diagnosis) [36].
Mild cognitive impairment (MCI), an early symptomatic but predementia phase of AD [37]
AD dementia [38]

The diagnosis of AD also needs laboratory tests and biomarkers, imaging and neuropsychological tests. Alzheimer disease (AD) is a clinical diagnosis. But as we have mentioned, imaging studies and laboratory tests may be used. Used imaging studies are computed tomography [CT], magnetic resonance imaging [MRI] and, in selected cases, single-photon emission CT [SPECT] or positron-emission tomography [PET].

These tests help exclude other possible causes for dementia (e.g., cerebrovascular disease, cobalamin [vitamin B_{12}] deficiency, syphilis, thyroid disease [37]). Brain scanning with SPECT or PET is not recommended for the routine workup of patients with typical presentations of AD. These modalities may be useful in atypical cases, or when a form of frontotemporal dementia is a more likely diagnosis [39].

There are two important organizations working in early AD detection. They are the Amyloid Imaging Taskforce (AIT), an assembly of experts from the Alzheimer's Association, and the Society of Nuclear Medicine and Molecular Imaging (SNMMI). They developed guidelines for the use of amyloid β (Aβ) positron emission tomography (PET) imaging to clarify diagnoses of AD or frontotemporal dementia. It described that, amyloid imaging is appropriate in patients with persistent or progressive unexplained mild cognitive impairment, in those satisfying core clinical criteria for possible AD because of unclear clinical presentation, and in patients with progressive dementia and atypically early age of onset. The committee recommends against imaging in asymptomatic individuals and patients with a clear AD diagnosis with typical age of onset. Scanning cannot be used to stage dementia or determine its severity, and it should not be used in lieu of genotyping for suspected autosomal mutation carriers [40].

There are three imaging agents regularly used for diagnostic. The first one is the florbetapir F 18 (AMYViD). This was approved by the FDA in April 2012 as a diagnostic imaging agent. It is indicated for PET brain imaging of beta-amyloid neuritic plagues in adults. It has been evaluated in Alzheimer's disease but also in other cognitive declines [41–43].

The second was approved by the FDA in October 2013. It is the 18F–labeled Pittsburgh compound B (PIB) derivative, flutemetamol F18 injection (Vizamyl), for use with PET brain imaging in adults undergoing evaluation for Alzheimer disease and dementia. Like florbetapir F18, flutemetamol F18 attaches to beta-amyloid in the brain and produces a PET image that can be used to assess its presence. A positive scan indicates that there is likely a moderate or greater amount of amyloid in the brain, but it does not establish a diagnosis of Alzheimer's disease or other dementia. The effectiveness of flutemetamol F18 was established in two clinical studies with 384 participants who had a wide range of cognitive function [44].

The final and third agent, florbetaben F18 (Neuraceq), was approved by the FDA in March 2014. Images may be obtained between 45–130 min following the injected dose. FDA approval was based on safety data from 872 patients who participated in global clinical trials, as well as on three studies that examined images from adults with a range of cognitive function, including 205 end-of-life patients who had agreed to participate in a post-mortem brain donation program. Images were analyzed from 82 subjects with post-mortem confirmation of the presence or absence of beta-amyloid neuritic plaques [45]. Subjects in this study underwent testing of memory and executive function along

with fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) scanning and amyloid deposition with C 11 Pittsburgh Compound B (PiB PET). The researchers found that amyloid burden and lower FDG metabolism (synaptic dysfunction) independently predicted episodic memory performance. Subjects with worse memory performance had higher PiB deposition and lower FDG metabolism in regions of the brain commonly affected in AD [46].

Cerebral spinal fluid (CSF) is a new biomarker. But routine measurement of cerebral spinal fluid tau and amyloid is not recommended except in research settings. Lumbar puncture for measurement of tau and amyloid may become part of the diagnostic workup when effective therapies that slow the rate of progression of AD have been developed, particularly if the therapies are specific for AD and carry significant morbidity [14]. It is observed in the CSF levels of tau and phosphorylated tau that are often elevated in AD, whereas amyloid levels are usually low. The reason for this is not known, but perhaps amyloid levels are low because the amyloid is deposited in the brain rather than the CSF. By measuring both proteins, sensitivity and specificity of at least 80–90% can be achieved [14].

Another research tool is the genotyping for apolipoprotein E (APOE) alleles. It has been helpful in determining the risk of AD in populations, but until recently it was of little, if any, value in making a clinical diagnosis and developing a management plan in individual patients. Numerous consensus statements have recommended against using APOE genotyping for predicting AD risk [25].

One of the neuropsychological tests used in the assessment of AD is the Mini-Mental State Examination (MMSE). It is often used to assess cognitive status. Health providers are increasingly using an alternative mental status test, the Montreal Cognitive Assessment (MoCA) to screen for cognitive impairment [47, 48].

There are many conditions for the differential diagnosis of Alzheimer's disease. One of them is depression. Depression is an important consideration in the differential diagnosis of Alzheimer's disease (AD). The clinical manifestations of depression overlap with those of AD. In addition, an estimated 30–50% of AD patients have comorbid depression [49]. The psychological tests for assessing depression (e.g., the Hamilton Scale for Depression, the Beck Depression Inventory, and the Geriatric Depression Scale) were designed for use in other patient populations, and may be less reliable in patients with AD. Consequently, the National Institute of Mental Health has developed provisional diagnostic criteria for depression in AD [49].

Treatment

The drugs approved by the US Food and Drug Administration (FDA) for AD treatment are few. All drugs approved by the FDA for the treatment of AD modulate neurotransmitters, either acetylcholine or glutamate, and these are only symptomatic therapies. The standard medical treatment for AD includes cholinesterase inhibitors (ChEIs) and a partial *N* -methyl-D-aspartate (NMDA) antagonist [50, 51].

Secondary symptoms of AD (e.g., depression, agitation, aggression, hallucinations, delusions, sleep disorders) can be problematic. Behavioral symptoms in particular are common, and can exacerbate cognitive and functional impairment. The following classes of psychotropic medications have been used to treat these secondary symptoms [52]: antidepressants, anxiolytics, antiparkinsonian agents, beta-blockers, antiepileptic drugs (for their effects on behavior), and neuroleptics or antipsychotics.

Most studies of psychotropic drugs for AD have demonstrated null or limited efficacy. Recent pharmacologic research in AD focuses principally on the development of disease-modifying drugs that can slow or reverse the progression of AD. Targets of these investigational agents have included beta-amyloid production, aggregation, and clearance, as well as tau phosphorylation and assembly. To date, none of these drugs has demonstrated efficacy in phase III trials [46].

There are many experimental therapies that have been proposed for AD. These include anti-amyloid therapy, reversal of excess tau phosphorylation, estrogen therapy, vitamin E therapy, and

free-radical scavenger therapy. Based on the evidence, the results are contradictory and disappointing. In the past 10 years, numerous antiamyloid therapy studies have been conducted to decrease toxic amyloid fragments in the brain, including studies of the following:

Vaccination with amyloid species
Administration of monoclonal antiamyloid antibodies
Brain shunting to improve removal of amyloid
Beta-secretase inhibitors to prevent generation of the A-beta amyloid fragment
Administration of intravenous immune globulin that may contain amyloid-binding antibodies
Selective amyloid-lowering agents
Chelating agents to prevent amyloid polymerization

Other therapeutic options such as direct current stimulation are being explored for a possible therapeutic role in AD. However, evidence of therapeutic benefit from these modalities is highly preliminary [53]. Disease-modifying therapies would delay the onset of AD and/or slow the rate of progression. Since brain changes associated with AD probably start decades before dementia becomes clinically apparent, many investigators believe that disease-modifying therapies are much more likely to be effective if they are started in a presymptomatic stage [53].

Neuropsychological, neuroimaging, and genetic methods are identifying patients at increased risk. Although phase III trials for several potential disease-modifying therapies have been completed, none of these agents have shown clear efficacy, and therefore have not yet been approved by the FDA [14]. Prevention could be a good choice. Evidence, largely epidemiologic, suggests that healthy lifestyles can reduce the risk of AD. Physical activity, exercise, cardiorespiratory fitness and Mediterranean diet may be protective [54, 55].

Vascular Dementia

Vascular dementia (VaD) is the second most common cause of dementia. It is observed in the United States and Europe, but it is the most common form in some parts of Asia and Latin America. This is a preventable dementia, but early detection and an accurate diagnosis are important. Patients who have had a stroke are at increased risk for VaD. Recently, vascular lesions also have been thought to play a role in AD [56].

The background history of VaD started early, in 1899. At first, arteriosclerosis and senile dementia were described as different syndromes. In 1969, Mayer-Gross et al. described this syndrome, and reported that hypertension is the cause in approximately 50% of patients. In 1974, Hachinski et al. coined the term multi-infarct dementia. In 1985, Loeb used the broader term vascular dementia. Recently, Bowler and Hachinski introduced a new term, vascular cognitive impairment [56].

If we describe the epidemiology of VaD, the prevalence rate is 1.5% in Western countries and approximately 2.2% in Japan. In Japan, Vascular dementia accounts for 50% of all dementias that occur in individuals older than 65 years. In Europe, Vascular dementia and mixed dementia account for approximately 20% and 40% of cases, respectively. In Latin America, 15% of all dementias are vascular. In community-based studies in Australia, the prevalence rate for vascular and mixed dementia is 13% and 28% respectively [55]. The prevalence rate of dementia is 9 times higher in patients who have had a stroke than in controls. One year after a stroke, 25% of patients develop new-onset dementia. Within 4 years following a stroke, the relative risk of incident dementia is 5.5%. The prevalence of Vascular dementia is higher in men than in women [56].

Etiology

The risk factors for VaD are from vascular causes. They include hypertension, smoking, hypercholesterolemia, diabetes mellitus, and cardiovascular and cerebrovascular disease. Several causes and presentations of VaD have clinical value. Perhaps the most obvious patients are those who meet criteria for dementia and have sustained a clinical stroke, either large artery (usually cortical)

or small artery (lacunes) in subcortical areas. Strokes are usually confirmed by neuroimaging that demonstrates either multiple infarcts or a single strategically placed infarct (e.g., angular gyrus, thalamus, brain forebrain, posterior cerebral artery, or anterior cerebral artery). In this field, MRI is more sensitive than CT [57].

It was mentioned before that the risk factors of Vascular dementia are vascular causes. These may be influenced by many other factors. Some of the most important factors that can lead to the development of dementia are older age, lower education level, family history of dementia, left-sided lesions, larger lesions, larger periventricular white matter ischemic lesions and strokes in thalamic artery territory, inferomedian temporal lobes, hippocampus, and watershed infarcts involving superior frontal and parietal regions [57].

Pathophysiology

VaD has many subtypes. The following subtypes of Vascular dementia have been described to date. The spectrum includes (a) mild vascular cognitive impairment, (b) multi-infarct dementia, (c) vascular dementia due to a strategic single infarct, (d) vascular dementia due to lacunar lesions, (e) vascular dementia due to hemorrhagic lesions, (f) Binswanger disease, (g) subcortical vascular dementia, and (h) mixed dementia (combination of AD and vascular dementia) [56].

The vascular causes of the VaD are vascular diseases. These produce either focal or diffuse effects on the brain and cause cognitive decline. The focal cerebrovascular disease occurs secondary to thrombotic or embolic vascular occlusions. Common areas of the brain associated with cognitive decline are the white matter of the cerebral hemispheres and the deep gray nuclei, especially the striatum and the thalamus. Hypertension is the major cause of diffuse disease, and in many patients, both focal and diffuse diseases are observed together. The three most common mechanisms of Vascular dementia are multiple cortical infarcts, a strategic single infarct, and small vessel disease [56].

Diagnosis

The diagnosis of Vascular dementia may be complemented with the Hachinski Ischemic Score, a clinically useful tool for distinguishing Vascular dementia from Alzheimer's disease [57]. This score was described in the Alzheimer section above, and it was mentioned that a score of 7 or higher suggests Vascular dementia and a score of 4 or less suggests Alzheimer's disease. Patients with VaD commonly have mood and behavioral changes. In some patients with lacunar state and Binswanger disease, such problems may be more prominent than intellectual deficits. Executive functioning deficits are seen prior to severe memory loss in the early stages of subcortical vascular cognitive impairment [58].

For the diagnosis of VaD, several specific diagnostic criteria can be used, including the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5*)* criteria, the International Classification of Diseases, Tenth Edition criteria, the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria, the Alzheimer's Disease Diagnostic and Treatment Center criteria, and the Hachinski Ischemic Score [58].

Patients with Vascular dementia have poorer verbal fluency and more perseverative behavior compared to patients with AD. They may even have other signs of executive dysfunction such as cognitive slowing, difficulty in shifting sets, and problems with abstraction. Commonly used mental status tests include the Folstein Mini-Mental State Examination and the Cognitive Abilities Screening Instrument [59]. Neuropsychological findings vary with the site and severity of cerebrovascular disease.

Laboratory tests should be performed to rule out other causes of dementia. These laboratory tests are very important; they should routinely include a CBC count, erythrocyte sedimentation rate, glucose level, renal and liver function tests, serologic tests for syphilis, vitamin B-12 and red blood cell folate levels, and thyroid function tests [56]. Neuroimaging studies are other important biomarkers to use. They may include CT brain scanning and MRI of the

brain. The absence of cerebrovascular lesions on CT scanning or MRI is evidence against vascular etiology. The features on CT scanning or MRI that are suggestive of Vascular dementia are bilateral multiple infarcts located in the dominant hemisphere and limbic structures, multiple lacunar strokes, or periventricular white matter lesions extending into the deep white matter [56].

Health professionals can perform a Mini-Mental Status Exam (MMSE) [47], depression assessment screening using *DSM-5* criteria [60], the Geriatric Depression Scale (GDS) [61], or the Cornell Scale for Depression in Dementia [62]. They should also assess for suicidal and homicidal risk, if necessary. Health professionals can directly ask patients about suicidal or homicidal ideation (thoughts), intent, or plan.

There is another condition to consider, mild cognitive impairment (MCI). Patients with vascular MCI, which is a prodromal stage for subcortical vascular dementia, have MRI features that differ from patients with amnestic MCI, which is the prodromal stage for AD. Vascular MCI shows more extensive white matter lacunar infarcts and leukoaraiosis and minimal hippocampal and entorhinal cortical atrophies, whereas the opposite is true for amnestic MCI.

Functional imaging may also be used for diagnosis. According to a 2000 study by Nagata et al. [63] in 2000, positron emission tomography may be useful for differentiating Vascular dementia from AD. Hypoperfusion and hypometabolism can be observed in the frontal lobe, including the cingulate and superior frontal gyri, in patients with Vascular dementia. Parietotemporal pattern is observed in patients with AD. Starkstein et al. in 1996 and other authors have demonstrated that single-photon emission CT scanning produce similar findings [64].

Another evaluation that occasionally is performed in VaD is cerebral angiography, but this is performed before carotid artery surgery. It is also useful in cases of possible cerebral vasculitis; cerebral vessels can demonstrate beading. Tests that may be useful for evaluation of stroke and in certain cases of Vascular dementia include the following: echocardiography, Holter monitoring and carotid duplex Doppler scanning.

Treatment

The most important treatment in Vascular dementia is prevention. The prevention of new strokes is an example. The treatment could include administering antiplatelet drugs and controlling major vascular risk factors. Aspirin has also been found to slow the progression of VaD. Treatment of risk factors such as hypertension, hypercholesterolemia, and diabetes mellitus are very important.

The prescription of neuroprotective drugs such as nimodipine, propentofylline, and posatirelin are currently under study and may be useful for Vascular dementia. Nicardipine is a dihydropyridine calcium channel blocker that was studied for the treatment of cognitive deterioration of vascular origin. Preliminary studies showed a decrease in cognitive deterioration in patients with cerebrovascular disease. Increasing evidence supports the involvement of the cholinergic system in Vascular dementia, similar to that seen in Alzheimer dementia. However, no cholinesterase inhibitors have been approved to date for the treatment of Vascular dementia, despite positive results in clinical trials with this medication [64].

The conditions of agitation and psychosis are common in elderly patients with dementia and are challenging to manage. Even if antipsychotics have a "black-box" warning with dementia by FDA, in some countries antipsychotics are prescribed for monitoring psychotic symptoms, with a successful result. Relatively few studies have examined the use of antidepressants for the treatment of agitation and psychosis in dementia. However, the selective serotonin reuptake inhibitors (SSRIs) sertraline and citalopram appear to be associated with a reduction in symptoms of agitation when compared with placebo [65].

Differences DSM IV Versus DSM V

The need for a classification of mental disorders is very important. This has been clear throughout the history of medicine, but until recently there was little agreement on which disorders should be included and the optimal method for their organization [11]. The history of classification is

too extensive to be summarized here. We will not display here those aspects that have led directly to the development of the *Diagnostic and Statistical Manual of Mental Disorders (DSM)* and to the mental disorders sections in the various editions of the *International Classification of Diseases (ICD)* [11], the reason being that the present summary will focus only on the DSM-IV and DSM-V and their descriptions of dementia.

DSM-IV

DSM–IV was published in 1994. It was the culmination of a 6-year effort that involved more than 1,000 individuals and numerous professional organizations. Developers of DSM–IV and the 10th edition of the ICD worked closely to coordinate their efforts, resulting in increased congruence between the two systems and fewer meaningless differences in wording. ICD–10 was published in 1992 [11]. The International Classification of Diseases (ICD-11) will be published in 2017.

Alzheimer's disease dementia, according to the criteria of the DSM-IV, is a syndrome that may be characterized by multiple cognitive deficits. They include memory impairment and at least one of the following: aphasia, apraxia, agnosia, or disturbance in executive functioning. Social or occupational function is also impaired. A diagnosis of dementia should not be made during the course of a delirium. A dementia and a delirium may both be diagnosed if the dementia is present at times when the delirium is not present [65].

DSM-V

At the beginning of 2000, for the fifth major revision of the Diagnostic and Statistical Manual of Mental Disorders (DSM–V), work groups were formed creating a research agenda. These work groups generated hundreds of white papers, monographs, and journal articles, providing the field with a summary of the state of the science relevant to psychiatric diagnosis and indicating where gaps existed in the current research, with hopes that more emphasis would be placed on research within those areas. Afterwards, in 2007, APA formed the DSM–5 Task Force to begin revising the manual, as well as 13 work groups focusing on various disorder areas. In 2013, the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition was released, replacing the term dementia with major neurocognitive disorder and mild neurocognitive disorder. The new terms focus on a decline, rather than a deficit, in function [11].

The first point to consider when the differences are described is the categories criteria. The American Psychiatric Association published DSM-V, and the DSM-IV category "Dementia, Delirium, Amnestic, and Other Cognitive Disorders" has undergone extensive revision. DSM-V has renamed this category as "Neurocognitive Disorders" (NCD), which now covers three entities: delirium, major neurocognitive disorders, and mild neurocognitive disorders. The DSM-IV version of mild NCD resembles the DSM-V version in name only. DSM-IV defined mild NCD based on a single criterion, whereas DSM-5 defines mild NCD by using several cognitive and related criteria. The main difference between mild NCD and the Key International Symposium criteria of mild cognitive impairment (MCI) is that the research work that led to the construct of MCI primarily involved elderly study participants (even though age was not part of the definition of MCI), whereas mild NCD includes acquired cognitive disorders of all age groups. DSM-V essentially discusses the epidemiology and diagnostic markers of mild NCD by drawing congruence between MCI and mild NCD [66].

Another important contribution of DSM-V is its elimination of the obligatory requirement to have memory impairment in the diagnosis of any type of dementia. For example, memory impairment was a necessary criterion for the DSM-IV diagnosis of Vascular dementia, whereas in DSM-V, the obligatory requirement for involvement of the memory domain is eliminated. DSM-V has thus rectified the "Alzheimer's-centric" criteria of DSM-IV. DSM-V also introduced additional cognitive domains that were

not present in DSM-IV: complex attention and social cognition (in addition to the DSM-IV domains of language, memory, executive function, and visuospatial function). DSM-IV used categories that described cortical lesions such as aphasia, apraxia, and agnosia as cognitive disturbances, but DSM-V has eliminated these terms, and instead listed cognitive domains (i.e., complex attention, executive function, learning and memory, language, and perceptual-motor and social cognition) [67]. DSM-V also described another weakness of DSM-IV, the absence of criteria to objectively assess cognitive decline, by using neuropsychological testing. In DSM-V, the following criterion is added: "A substantial impairment in cognitive performance, preferably documented by standardized neuropsychological testing" [67].

There is another major change related to a substantial revision of "cognitive disorder not otherwise specified." This DSM-IV category undergoes marked change in order to further elaborate mild NCD, which also includes MCI [67].

One of the great benefits of the "mild NCD" definition is that it offers a more structured diagnostic approach. First, the clinician needs to decide whether the cognitive impairment is mild or major NCD. The next step is to identify possible etiology, and the last step is to document the presence or absence of behavioral symptoms [66].

In addition, the DSM-V definition of mild NCD is developed on four criteria and two specifiers. The four criteria refer to cognitive changes, functional activities, and exclusion of delirium and competing mental disorders. The two specifiers are the presumed etiologies of mild NCD and the presence or absence of behavioral problems. While the category "mild NCD" may improve reliability of diagnoses, it has yet to withstand scientific scrutiny to be considered a valid construct [67].

When mild cognitive impairment (MCI) is described, it is considered to be a transition state between normal cognition and dementia. The subtypes of MCI are highly heterogeneous in terms of etiology, presentation, and prognosis. Patients with the amnestic subtype of MCI are at high risk of progression to Alzheimer's disease (AD). This subtype may represent the prodromal stage of AD. Moreover, patients with MCI who are not aware of their memory deficits, and in whom practice effects are not observed, exhibit parietotemporal hypoperfusion on single photon emission CT, indicating that these findings are predictors of progression to AD.

In this review, one source of debate and argument to be considered is age. Some people argue that one of the main reasons for replacing the terms "dementia" and "MCI" with "major NCD" and "mild NCD" is that both dementia and MCI are associated with acquired geriatric disorders, whereas major and mild NCD are acquired cognitive disorders of all age groups. This classification, however, may potentially lead to "lumping" together different diseases. For example, a 20-year-old football player with concussion and cognitive problems could be diagnosed with mild NCD (due to traumatic brain injury). A person aged 80 years with insidious onset and gradually progressing cognitive decline, and who has minimal loss of independence, could also be diagnosed with mild NCD (due to AD) [68].

By definition, mild cognitive impairment (MCI) is considered to be a transition state between normal cognition and dementia. The subtypes of MCI are highly heterogeneous in terms of etiology, presentation, and prognosis. Patients with the amnestic subtype of MCI are at a high risk of progression to Alzheimer's disease (AD). This subtype may represent the prodromal stage of AD [69]. In order to meet the DSM-V criteria for AD, the individual must meet the criteria for major or mild neurocognitive disorder, and there should be insidious onset and gradual progression of impairment in one or more cognitive domains (for major neurocognitive disorder, at least two domains must be impaired). The individual must also meet criteria for either probable or possible AD as outlined in DSM-V [70].

This new diagnosis includes both the dementia and amnestic disorder diagnoses from DSM-IV. Also, DSM-5 recognizes specific etiologic subtypes of neurocognitive dysfunction, such as Alzheimer's disease, Parkinson's disease, HIV infection, Lewy body disease, and Vascular disease. Each subgroup can be further divided into

mild or major degrees of cognitive impairment on the basis of cognitive decline, especially the inability to perform functions of daily living independently. In addition, a subspecifier "with" or "without behavioral disturbances" is available [70].

With regard to Vascular dementia, DSM-V categorizes it as an etiological subtype of either major or mild neurocognitive disorder. A summary of the DSM-V diagnostic criteria is as follows [58]: evidence of modest (mild) or significant (major) cognitive decline from a previous level of performance in one or more cognitive domains (complex attention, executive function, learning and memory, language, and perceptual-motor or social cognition). It may be based on: (1) concern of the individual, a knowledgeable informant, or the clinician that there has been a decline in cognitive function, and (2) an impairment in cognitive performance (modest or significant) documented by standardized testing or another qualified assessment. The symptoms are not better explained by another brain disease or systemic disorder.

Probable vascular neurocognitive disorder is diagnosed if one of the following is present: (1) clinical criteria are supported by neuroimaging evidence of significant parenchymal injury attributed to cerebrovascular disease, (2) the neurocognitive syndrome is temporarily related to one or more documented cerebrovascular events, or (3) both clinical and genetic evidence of cerebrovascular disease is present.

The clinical features are consistent with a vascular etiology as suggested by either of the following: (1) onset of the cognitive deficits is temporally related to one or more cerebrovascular events, or (2) evidence for decline is prominent in complex attention (including processing speed) and frontal executive functions. There is evidence of the presence of cerebrovascular disease from history, physical examination, and/or neuroimaging considered sufficient to account for the neurocognitive deficits.

Possible vascular neurocognitive disorder is diagnosed if the clinical criteria are met but neuroimaging is not available, and the temporal relationship of the neurocognitive syndrome with one or more cerebrovascular events is not established.

The nosologic distinctions between varying dementia etiologies should prove helpful in determining prognosis and therapeutic course. These nosologic distinctions are important so that the clinicians will be able to more clearly determine whether the cognitive decline alone should be the focus of concern and intervention, or whether behavioral disturbances should also be considered and addressed [71]. In addition, a mild degree of cognitive impairment is consistent with recent research suggesting that treatments for declining cognition may be phase-specific, with certain medications and approaches possibly only working early in the course of the disease. DSM-V gives an objective distinction between mild and major impairment, and this is very helpful for the clinician.

Mild neurocognitive disorder requires "modest" cognitive decline which does not interfere with "capacity for independence in everyday activities" such as paying bills or taking medications correctly. Cognitive decline meets the "major" criteria when "significant" impairment is evident or reported, and when it does interfere with a patient's independence to the point that assistance is required. The diagnostic distinction depends heavily on observable behaviors [71]. It is important to mention that mild neurocognitive disorder goes beyond normal issues of aging. It describes a level of cognitive decline that requires compensatory strategies and accommodations to provide help in maintaining independence and performing activities of daily living. When it is diagnosed as a disorder, there must be changes that impact cognitive functioning. These symptoms are usually observed by the individual, a close relative, or other knowledgeable informant, such as a friend, colleague, or clinician, or they are detected through objective testing [60].

There is a great clinical need to recognize individuals who need care for cognitive issues that go beyond normal aging. The impact of these problems is evident, but clinicians have lacked a reliable diagnosis by which to assess symptoms or understand the most appropriate treatment or services. Recent studies suggest that identifying mild neurocognitive disorder as early as possible may allow interventions to be more effective [71].

Optimistically, this new classification system will stimulate in many areas. One of the important areas is research, research in the areas of prevention and early intervention of neurocognitive disorders with physicians and mental health professionals.

Conclusion

Alzheimer's disease and Vascular dementia are the first and second most common dementias worldwide. Dementia diagnosis describes the biomarkers for screening and treatment. The new DSM-V classification provides new features and concepts, such as major and mild neurocognitive disorder instead of dementia as described by the DSM-IV. It is very important for physicians and mental health professionals to know the differences between the two classifications when aiming to improve their assessment, knowledge, and clinical practice with dementing elderly patients in their clinical settings. Additionally, the new classification stimulates research in the prevention and early intervention of neurocognitive disorders. Prevention is the best choice for treatment right now.

References

1. WHO Outlines Global Dementia Research Priorities. [Internet] Medscape. [updated 2016; cited 2016 Sep 2] Available from: www.medscape.com/viewarticle/866984.
2. Sonnen JA, Larson EB, Crane PK, et al. Pathological correlates of dementia in a longitudinal, population-based sample of aging. Ann Neurol. 2007;62(4):406–13. [Medline].
3. White L, Petrovitch H, Hardman J, et al. Cerebrovascular pathology and dementia in autopsied Honolulu–Asia Aging Study participants. Ann N Y Acad Sci. 2002;977:9–23. [Medline].
4. Bennett DA, Schneider JA, Arvanitakis Z, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. Neurology. 2006;66(12):1837–44. [Medline].
5. Knopman DS, Parisi JE, Salviati A, et al. Neuropathology of cognitively normal elderly. J Neuropathol Exp Neurol. 2003;62(11):1087–95. [Medline].
6. Stern Y. Cognitive reserve and Alzheimer disease. Alzheimer Dis Assoc Disord. 2006;20(3 Suppl 2): S69–74. [Medline].
7. Roman GC. The epidemiology of vascular dementia. Handb Clin Neurol. 2008;89(3rd series):639–58.
8. Hachinski VC, Iliff LD, Zilhka E, et al. Cerebral blood flow in dementia. Arch Neurol. 1975;32:632–7.
9. Leel-Ossy L. Data on the clinical pathology of vascular dementia. Orv Hetil. 1995;136(31):1651–7. Review. Hungarian.
10. Arevalo-Rodriguez I, Segura O, Solà I, Bonfill X, Sanchez E, Alonso-Coello P. Diagnostic tools for Alzheimer's disease dementia and other dementias. BMC Neurol. 2014;14:183.
11. American Psychiatry Association [Internet]. Arlington: [updated 23 Aug 2016; cited 2 Sep 2016]. Available from: https://www.psychiatry.org/psychiatrists/practice/dsm/dsm-5.
12. Maurer K, Maurer U. Alzheimer: the life of a physician and career of a disease. New York: Columbia University Press; 2003.
13. Alzheimer A. Uber eine eigenartige Erkangkung der Hirnrinde. Allgemeine Zeitschrift fur Psychiatrie und Psychisch–Gerichtliche Medizin. 1907;64:146–8.
14. Lakhan SE, Chawla J. Alzheimer Disease. Medscape Medical News. August 16, 2016.
15. Xu W, Tan L, Wang HF, Jiang T, Tan MS, Tan L, et al. Meta-analysis of modifiable risk factors for Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2015;86(12):1299–306. [Medline].
16. Rocchi A, Orsucci D, Tognoni G, Ceravolo R, Siciliano G. The role of vascular factors in late-onset sporadic Alzheimer's disease. Genetic and molecular aspects. Curr Alzheimer Res. 2009;6(3):224–37. [Medline].
17. S Roriz-Filho J, Sá-Roriz TM, Rosset I, Camozzato AL, Santos AC, Chaves ML, et al. (Pre) diabetes, brain aging, and cognition. Biochim Biophys Acta. 2009;1792(5):432–43. [Medline].
18. Naderali EK, Ratcliffe SH, Dale MC. Obesity and Alzheimer's disease: a link between body weight and cognitive function in old age. Am J Alzheimers Dis Other Demen. 2009;24(6):445–9. [Medline].
19. de la Monte SM. Insulin resistance and Alzheimer's disease. BMB Rep. 2009;42(8):475–81. [Medline].
20. Breitner JC, Wyse BW, Anthony JC, Welsh-Bohmer KA, Steffens DC, Norton MC, et al. APOE-epsilon4 count predicts age when prevalence of AD increases, then declines: the Cache County Study. Neurology. 1999;53(2):321–31. [Medline].
21. Vemuri P, Wiste HJ, Weigand SD, Knopman DS, Shaw LM, Trojanowski JQ, et al. Effect of apolipoprotein E on biomarkers of amyloid load and neuronal pathology in Alzheimer disease. Ann Neurol. 2010;67(3):308–16. [Medline]. [Full Text].
22. Goldbourt U, Schnaider-Beeri M, Davidson M. Socioeconomic status in relationship to death of vascular disease and late-life dementia. J Neurol Sci. 2007;257(1–2):177–81. [Medline].
23. McDowell I, Xi G, Lindsay J, Tierney M. Mapping the connections between education and dementia. J Clin Exp Neuropsychol. 2007;29(2):127–41. [Medline].
24. Szekely CA, Zandi PP. Non-steroidal anti-inflammatory drugs and Alzheimer's disease: the epidemiological evidence. CNS Neurol Disord Drug Targets. 2010;9(2):132–9. [Medline].

25. Goldman JS, Hahn SE, Catania JW, LaRusse-Eckert S, Butson MB, Rumbaugh M, et al. Genetic counseling and testing for Alzheimer disease: joint practice guidelines of the American College of Medical Genetics and the National Society of Genetic Counselors. Genet Med. 2011;13(6):597–605. [Medline]. [Full Text].
26. Hollingworth P, Harold D, Sims R, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011;43(5):429–35. [Medline]. [Full Text].
27. Caselli RJ, Dueck AC. APOE varepsilon2 and presymptomatic stage Alzheimer disease: how much is not enough? Neurology. 2010;75(22):1952–3. [Medline].
28. Chiang GC, Insel PS, Tosun D, et al. Hippocampal atrophy rates and CSF biomarkers in elderly APOE2 normal subjects. Neurology. 2010;75(22):1976–81. [Medline].
29. Finch CE, Morgan TE. Systemic inflammation, infection, ApoE alleles, and Alzheimer disease: a position paper. Curr Alzheimer Res. 2007;4(2):185–9. [Medline].
30. Anderson P. Hypertension interacts with APOE epsilon 4 to increase amyloid load. Available at http://www.medscape.com/viewarticle/781430. Accessed 8 Apr 2013.
31. Rodrigue KM, Rieck JR, Kennedy KM, Devous MD, Diaz-Arrastia R, Park DC. Risk factors for ß-amyloid deposition in healthy aging: vascular and genetic effects. JAMA Neurol. 2013:1–7. [Medline].
32. Saczynski JS, Beiser A, Seshadri S, Auerbach S, Wolf PA, Au R. Depressive symptoms and risk of dementia: the Framingham Heart Study. Neurology. 2010;75(1):35–41. [Medline]. [Full Text].
33. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82(4):239–59. [Medline].
34. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Biol. 2011;3(9):a006189. [Medline].
35. Jack Jr CR, Albert MS, Knopman DS, McKhann GM, Sperling RA, Carrillo MC, et al. Introduction to the recommendations from the National Institute on Aging–Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):257–62. [Medline]. [Full Text].
36. Stages of Alzheimer's disease: recommendations from the National Institute on Aging–Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):280–292. [Medline].
37. Albert MS, Dekosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging–Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):270–9. [Medline].
38. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack Jr CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging–Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):263–9. [Medline].
39. Rabinovici GD, Furst AJ, O'Neil JP, Racine CA, Mormino EC, Baker SL, et al. 11C-PIB PET imaging in Alzheimer disease and frontotemporal lobar degeneration. Neurology. 2007;68(15):1205–12. [Medline].
40. Anderson P. New guidelines for amyloid PET imaging. Available at http://www.medscape.com/viewarticle/778274. Accessed 5 Feb 2013.
41. Choi SR, Schneider JA, Bennett DA, Beach TG, Bedell BJ, Zehntner SP, et al. Correlation of amyloid PET ligand florbetapir F 18 binding with Aß aggregation and neuritic plaque deposition in postmortem brain tissue. Alzheimer Dis Assoc Disord. 2012;26(1):8–16. [Medline]. [Full Text].
42. Clark CM, Schneider JA, Bedell BJ, Beach TG, Bilker WB, Mintun MA, et al. Use of florbetapir–PET for imaging beta-amyloid pathology. JAMA. 2011;305(3):275–83. [Medline].
43. Fleisher AS, Chen K, Liu X, Roontiva A, Thiyyagura P, Ayutyanont N, et al. Using positron emission tomography and florbetapir F18 to image cortical amyloid in patients with mild cognitive impairment or dementia due to Alzheimer disease. Arch Neurol. 2011;68(11):1404–11. [Medline].
44. US Food and Drug Administration (FDA). FDA approves second brain imaging drug to help evaluate patients for Alzheimer's disease, dementia [press release]. 2013. Available at http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm372261.htm. Accessed 25 Oct 2013.
45. Neuraceq (florbetaben F 18) prescribing information. [package insert]. Matran: Piramal Imaging, S.A; 2014.
46. Brooks M. Memory tests plus brain scans detect earliest stages of AD. Medscape Medical News. 2013. [Full Text].
47. Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189–98. [Medline].
48. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: A brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53:695–9.
49. Teng E, Ringman JM, Ross LK, et al. Diagnosing depression in Alzheimer disease with the national institute of mental health provisional criteria. Am J Geriatr Psychiatry. 2008;16(6):469–77. [Medline]. [Full Text].
50. Winslow BT, Onysko MK, Stob CM, Hazlewood KA. Treatment of Alzheimer disease. Am Fam Physician. 2011;83(12):1403–12. [Medline].
51. Massoud F, Léger GC. Pharmacological treatment of Alzheimer disease. Can J Psychiatry. 2011;56(10):579–88. [Medline].
52. Madhusoodanan S, Shah P, Brenner R, Gupta S. Pharmacological treatment of the psychosis of

Alzheimer's disease: what is the best approach? CNS Drugs. 2007;21(2):101–15. [Medline].
53. Freitas C, Mondragón-Llorca H, Pascual-Leone A. Noninvasive brain stimulation in Alzheimer's disease: systematic review and perspectives for the future. Exp Gerontol. 2011;46(8):611–27. [Medline].
54. Rolland Y, Abellan van Kan G, Vellas B. Healthy brain aging: role of exercise and physical activity. Clin Geriatr Med. 2010;26(1):75–87. [Medline].
55. Honea RA, Thomas GP, Harsha A, Anderson HS, Donnelly JE, Brooks WM, et al. Cardiorespiratory fitness and preserved medial temporal lobe volume in Alzheimer disease. Alzheimer Dis Assoc Disord. 2009;23(3):188–97. [Medline]. [Full Text].
56. Alagiakrishnan K, Ahmed I. Medscape Medical News. January 12, 2015.
57. Reuben DB. What is Vascular Dementia? Medscape Medical News. July 15, 2015.
58. Zhang-Nunes SX, Maat-Schieman ML, van Duinen SG, Roos RA, Frosch MP, Greenberg SM. The cerebral beta-amyloid angiopathies: hereditary and sporadic. Brain Pathol. 2006;16(1):30–9. [Medline].
59. Price CC, Jefferson AL, Merino JG, Heilman KM, Libon DJ. Subcortical vascular dementia: integrating neuropsychological and neuroradiologic data. Neurology. 2005;65(3):376–82. [Medline]. [Full Text].
60. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Washington, DC: American Psychiatric Association; 2013.
61. Yesavage JA, Brink TL, Rose TL, et al. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res. 1983;17:37–49.
62. Alexopoulos GS, Abrams RC, Young RC, Shamoian CA. Cornell scale for depression in dementia. Biol Psychiatry. 1988;23(3):271–84. [Medline].
63. Nagata K, Maruya H, Yuya H, et al. Can PET data differentiate Alzheimer's disease from vascular dementia? Ann N Y Acad Sci. 2000;903:252–61. [Medline].
64. Starkstein SE, Sabe L, Vazquez S, et al. Neuropsychological, psychiatric, and cerebral blood flow findings in vascular dementia and Alzheimer's disease. Stroke. 1996;27(3):408–14. [Medline].
65. American Psychiatric Association. Practice guideline for the treatment of patients with Alzheimer's disease and other dementias of late life. American Psychiatric Association. Am J Psychiatry. 1997;154(5 Suppl):1–39. [Medline].
66. Stokin GB, Krell-Roesch J, Petersen R, Geda YE. Mild neurocognitive disorder: an old wine in a new bottle. Harv Rev Psychiatry. 2015;23(5):368–76.
67. Rabins PV, Lyketsos CG. A commentary on the proposed DSM revision regarding the classification of cognitive disorders. Am J Geriatr Psychiatry. 2011;19:201–4. [PubMed].
68. Ganguli M. Can the DSM-5 framework enhance the diagnosis of MCI? Neurology. 2013;81:2045–50. [PMC free article] [PubMed].
69. Shimizu S, Hanyu H. Mild cognitive impairment. Nihon Rinsho. 2016;74(3):455–8.
70. American Psychiatric Association. Neurocognitive disorders. Diagnostic and statistical manual of mental disorders. 5th ed. Washington, DC: American Psychiatric Association; 2013. p. 611–4.
71. Stetka BS, Christoph U, Correll MD. A guide to DSM-5. Medscape. 2013;167:1–3.

Nutrition and Central Nervous System

35

Silvina Monica Alvarez, Nidia N. Gomez, Lorena Navigatore Fonzo, Emilse S. Sanchez, and María Sofía Giménez

Abstract

Clinical studies have revealed that depression is accompanied by impaired brain function and cognitive performances or neurodegenerative processes. Moreover, accumulation of oxidative damage has been implicated in aging and various neurological disorders. This chapter aims to integrate the current knowledge on the relation between brain and diverse alterations in nutrition. The mammalian brain is a lipid-rich organ, where lipids content in gray matter is 36–40% lipid. However, the regulation of cholesterol transport from astrocytes to neurons still remains unclear, among other things. In addition to that, micronutrient status can affect cognitive function at all ages. Vitamin deficiency could influence memory function, and might contribute to cognitive impairment and dementia.

Deficiency of vitamin A, folate, vitamins B6, B12, and minerals such as Fe and Zn are associated with prevalence of depressive symptoms according to several epidemiological studies. Experimental evidence suggests that resveratrol, vitamins A, C, E, D and folate may block oxidative stress and promote clearance of Aβ peptides. An adequate intake of fruit, nuts, vegetables, cereals, legumes, or fish can prevent the depletion. High dietary intake of saturated fat and low intake of vegetables may be associated with increased risk of Alzheimer's disease. Supplementation of diets with omega-3 has been shown to have positive effects on cognitive function. The biochemical and molecular mechanism of these alterations of normal brain function has been described. Future studies should also examine how DNA repair deficiency occurs and affects the nervous system, because this could provide a rational basis for therapies in neurodegenerative diseases.

S.M. Alvarez, PhD (✉) • N.N. Gomez
L. Navigatore Fonzo • E.S. Sanchez • M.S. Giménez
Universidad Nacional de San Luis, IMIBIO SL,
CONICET, Bioquimica y Ciencias Biologicas,
Avenida Ejercito de los Andes 950,
San Luis 5700, Argentina
e-mail: salvarez@unsl.edu.ar; marisofigime44@gmail.com

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_35

Keywords
Central nervous system • Vitamins • Alzheimer's disease • Dementia • Zinc • Fatty acids • Glucose • Insulin

Introduction

It is well known that the normal functioning of the body, including the central nervous system, requires a balanced diet in quality and quantity of nutrients, at all stages of life, beginning at the embryonic period to old age. The nervous system is made up of millions of cells: neurons and the glial cells that surround them. The two cell types interact, allowing the normal process of transmission of nerve impulses.

Considerable efforts are being devoted to understanding how lifestyle can influence the brain and body. Research results show that noninvasive approaches such as diet can have profound consequences for increasing resilience of the central nervous system (CNS) to injuries, and for maintaining cognitive abilities. Diet, a very important part of lifestyle and daily routine, can influence the capability of the brain to fight disease and to react to challenges [1].

Healthy diets, such as those high in omega-3 fatty acids and curcumin, contained in foods such as salmon and the plant turmeric, respectively, can stimulate molecular systems that serve neuronal function and plasticity in the brain and spinal cord. Conversely, unhealthy diets that consist of high amounts of saturated fats and sugars, as prevalent in "junk food," do the opposite. The consumption of high-calorie diets is garnering special recognition as a risk factor for impaired cognitive function and emotional health [1]. Although more research is needed to fully integrate these approaches as treatment for specific neurologic disorders, results accumulated so far are substantial enough to provide a general framework to guide therapeutic applications.

Most of the published research with regard to the effects of foods in human neurologic function has been related to the use of whole foods. A growing body of existing evidence shows the effects of select nutrients or food derivatives in several animal models of neurologic disorders. This information is forming the concept that dietary factors can be used as biologics-based therapies. The capacity of nutrients to influence the CNS may be incorporated through management of whole foods or food-derivative supplements in the diet.

The impact of nutrition on cognition and behavior, especially in women of reproductive age, is not very well known. Well-designed studies are needed to determine the effects of nutrition status on later nervous development of the child, in order to find out what, if any, critical periods exist during gestation with respect to these outcomes, and the severity of nutrient deficiency associated with negative outcomes. Many factors involved in brain aging have been identified. Given the multifactorial and progressive aspects of this process, the early triggering causative events deserve specific investigation.

Nutrition and Myelination

This neuroscientific framing of development holds that early experiences inscribe themselves on a child's brain, and it is this organ that carries childhood influences forward to adulthood. This evidence has diffused widely through public discussion and reveals the concerns of the most critical issues on child's development.

The development of the brain involves the coordination of multiple and complex processes. The synthesis of myelin is known to be influenced by multiple hormonal and growth factor signals as well as complex cell–cell interactions [2], making this a most vulnerable and critical process during nervous system development. The rapid period of brain myelination occurs during the first 2 years of human life, and thus determines the future cognitive and social skills of the individual.

Adverse early life experiences affect the formation of myelin sheath and exert long-lasting effects on neural function. Stressful environments in early life may induce permanent rather than transient consequences in animals. Previous studies have indicated that early unfavorable events augment the risk of behavioral disorders in adulthood, including neuropsychiatric disorders such as depression [3] and psychosis [4]. Moreover, disruption or abnormal myelination has been associated with different psychiatric disorders during adult life such as bipolar depression, schizophrenia, and the autism spectrum.

It is impossible to determine whether a particular nutrient deficiency affects brain development in a free-living human in a specific manner, because the impact and the extension of the effects are subject to multiple concurrent factors such as timing and degree of nutrient deprivation, as well as a child's experiences and environmental input. In addition, there is evidence that myelination is specifically affected in children by food deprivation. It is well known that fatty acids are necessary for the synthesis of myelin and that docosahexaenoic acid (DHA) is critical for the third trimester and the first year after birth for the brainstem auditory pathway [5].

Undernutrition and fatty acid deficiency in rodents not only causes hipomyelination but has also been shown to alter the composition of myelin proteins [6, 7]. Iron depletion plays a particular role in decreasing the amount of myelination and affecting the physiopathology of oligodendrocytes in the CNS [8, 9]. Interestingly, patients diagnosed with schizophrenia showed not only iron deficiency, but also anemia and altered myelin genes, pointing out that developmental iron deprivation may be an animal model for the study of psychiatric disorders, including depressive disorder, bipolar disorder, anxiety disorder, and autism [10, 11].

Iodine is an essential element for proper brain development, and its gestational deficiency leads to reduced myelination [12, 13]. The majority of children with iodine deficiency disorders are brought to psychiatric outpatient units by their families or referred by their teachers with complaints of lack of attention. In a substantial portion of these children, attention deficit/hyperactivity disorder (ADHD) needs to be considered in the differential diagnosis. ADHD is a disorder in the etiology of which neurological, genetic, environmental, dietary, biological, and psychosocial factors are possibly involved [14]. Interestingly, Sanchez et al. demonstrated the link between the opioid exposure in utero of children and the development of ADHD later in life and abnormality in brain myelination [15].

Nutrient deficiency or intervention may affect some children but not others, depending on the amount and quality of stimulation they receive. Nutrient deficiency and experiential input from the environment may have independent additive effects on brain development. In this regard, the opioid system may be one of the keys, since it is well known for dampening physical pain and is also hypothesized to dampen social pain [16].

Brain development in general, and myelination in particular, is affected by experience. For example, the brain expects visual input through the optic nerve for normal development of the visual cortex. The absence of these expected experiences impairs the neurodevelopmental processes that depend on them. These experience-expectant processes also depend on other types of sensory stimulation (e.g., auditory and tactile) and occur early in life. One of the most critical processes affected by visual input and nutrients intake is the circadian rhythm entrainment. Interestingly, psychiatric disorders such as schizophrenia, bipolar disorder, major depressive disorder, and autism are often accompanied by metabolic dysfunction symptoms. All these disorders are associated with abnormalities in oligodendroglia, and provide initial evidence suggesting a role for epigenetic mechanisms and altered circadian rhythms [17]. Since the circadian system controls important brain systems that regulate affective, cognitive, and metabolic functions, and neuropsychiatric and metabolic diseases are often correlated with disturbances of circadian rhythms, it is logical to hypothesize that dysregulation of circadian clocks plays an important role in metabolic comorbidity in psychiatric disorders [18].

Glucose and the Nervous System

Glucose is the primary energy source of the brain, and is needed to provide precursors for the synthesis of neurotransmitters and ATP. In health, the level of blood glucose remains within certain limits (80–110 mg/dl), which is controlled by the interaction of physiological responses of liver, pancreas, and skeletal muscle. The nervous system is also involved in this control, since glucose is essential to its functions.

Low blood glucose levels lead to alterations in the functioning of the nervous system, and can cause injury or severe and irreversible brain damage. To restore normoglycemia, neurohormonal responses are induced as counter-regulatory mechanisms, which include the cessation of the release of endogenous insulin followed by the release of glucagon, catecholamines (including adrenaline from the adrenal medulla and increased circulation of noradrenaline from sympathetic synapses), growth hormone, and activation of the pituitary–adrenal axis with increase of corticotropin (ACTH) and glucocorticoids.

These changes tend to limit glucose utilization and increase hepatic glucose production, not only through hormonal effects, but also through direct autonomic innervation of the liver. Adrenergic symptoms as sweating, tremors and heart palpitations appear. Hunger is also stimulated, which consequently helps restore and maintain normoglycemia. If the blood glucose is low enough, cognitive function begins to deteriorate, particularly cognitive domains of attention, speed of response, and judgment; neuroglycopenic symptoms appear, such as tingling lips and irritability.

The passage of glucose from the blood to the brain requires transport through the endothelial cells of the blood–brain barrier and subsequent entry into neurons and glial cells. Glucose transporter proteins mediate this process. The major isoform of these transporters is the GLUT1, which occurs in two forms, a glycosylated form present in cells of the blood–brain barrier, and another less glycosylated present in glia. GLUT3 is present in neurons and GLUT5 in microglia. GLUT2, 4, and 7 have also been detected in the brain but at lower levels of expression, more restricted to determined regions. All transporters contribute to cerebral glucose utilization.

Intracellular glucose is phosphorylated by hexokinase I (HKI) to form Glc-6-P, which is metabolized by the glycolytic pathway to generate ATP, but is also the substrate for the synthesis of NADPH through via the pentose phosphate, which allows the synthesis of nucleic acids and provides a reducing cofactor in the oxidative process.

Glucose is also the source of biosynthesis of other compounds required by the brain, including complex carbohydrates such as glycoproteins, glycolipids, and amino acids. It is also a carbon donor for methylation reactions, and supplies neurotransmitter precursors [19].

Glycogen, despite its relatively low level in the CNS compared to that present in peripheral tissues, is the largest energetic reserve of the brain. At the cellular level, glycogen is found located exclusively in astrocytes, in the adult brain [20, 21]. Although neurons express glycogen synthase, recent evidence shows that this machinery is kept inactive in neurons through proteasome-dependent mechanisms [22]. Most striking is that glycogen storage activation in cultured neurons causes apoptosis [22], showing also that glycogen is a specific feature of glucose metabolism in astrocytes. The main reserve of glycogen of the brain is in astrocytes instead of neurons, where the energy is required. This suggests that glycogen metabolism requires metabolic interactions between astrocytes and neurons. On the other hand, astrocyte glycogen seems to be directly relevant for learning [23], and the glycolytic end product lactate appears to play a role in long-term memory formation [24].

Glucose, through the glycolytic via pentose generates NADPH, which is an antioxidant cofactor that places the glutathione to reduced state. This is a major reducing agent in oxidative processes of the cell. Interestingly, it has been shown that NADPH is more abundant in astrocytes than in neurons. Astrocytes have a pentose via which is more active than that in neurons and better able to stimulate this pathway in oxidative stress response [25, 26]. Transcriptome analysis in astrocytes and neurons freshly isolated from mouse brain have also revealed high levels of expression of glucose 6-phosphate

dehydrogenase, the limiting speed enzyme via the pentose, in astrocytes compared to neurons [27, 28]. These features show that astrocytes have a big antioxidant capacity in relation to neurons.

Insulin

Insulin influences every aspect of human physiology. In addition to regulating the homeostasis peripheral glucose, insulin is a neuromodulator that contributes to neurological processes.

Insulin, after its discovery in 1921, was considered a peripheral hormone and therefore it was assumed it would not cross the blood–brain barrier (BBB) [29]. However, in 1967, Margolis and Altszuler demonstrated in dogs that the insulin concentration in the cerebrospinal fluid (CSF) increased after an increase in plasma insulin [30], demonstrating that insulin is able to cross the blood–brain barrier. Insulin is a peptide secreted by the pancreatic beta cells, and it is easily transported into the CNS through the BBB by a saturable receptor [31].

In 1978, Havránková et al. showed the widespread presence of insulin receptors (IR) in the central nervous system (CNS) of rats [32], and also found high levels of insulin present in extracts of rat brain. In 1983, Dorn et al. showed that the human brain contains insulin concentrations much higher than those in the blood, and that it was even higher in the hypothalamus [33].

The activation of the signaling cascade of insulin starts with ligand binding of insulin to IR which belongs to the family of tyrosine kinase receptors, which autophosphorylates. It is very important to start the process of signal transduction. Mammalian brains have specific IRs. There are two types; one of them is abundant in the neuron, both in cell bodies and synapses, while the second type is present in lower concentrations in glial cells. They are present in high concentration particularly in the olfactory bulb, hypothalamus, hippocampus, cerebellum, amygdala, and cerebral cortex. This has been implicated in emotion and higher cognitive functions, particularly learning and the memory. Higher concentrations of IR are found in the hippocampus, which is critically involved in the processing of spatial memory, suggesting the role of insulin in learning and experimental evidence. The higher brain insulin signaling is compatible with best memory processes.

Insulin also possesses neuroprotective properties. The brain is highly sensitive to oxidative stress due to its high content of fatty acids, peroxidizable, high need for oxygen, relative shortage of antioxidants and high iron content.The lipid peroxidation in brain tissue, which is expressed as the malondialdehyde (MDA) level is higher in the hyperglycemic compared to normoglycemic controls (blood glucose around 3.7 mM) and lipid peroxidation has also been increased in severe hypoglycemia. Oxidative stress represents a central pathophysiological mediator of diabetes, and is deeply involved in the development and progression of neurodegenerative diseases. Insulin, by stimulating glucose uptake and pyruvate formation, restores intracellular ATP formation as well as reducing oxidative stress [34].

Epidemiological studies show a strong link between type 2 diabetes and the risk of developing Alzheimer's disease (AD) [35]. The exact mechanism by which this increased risk is conferred remains unclear, but may be associated with increased oxidative stress in both type 2 diabetes and AD. Peroxidation protein signaling pathways of insulin and other proteins in the brain may contribute to the high risk of developing DM2. Epidemiological and biological evidence has shown an increased incidence of cognitive impairment and AD in patients with type 2 diabetes [36]. Chronic inflammatory response and oxidative stress associated with type 2 diabetes, β-amyloid (Aß) accumulation proteins, and mitochondrial dysfunction are associated in DM2 and AD. It has been observed in the brains of post-mortem type II diabetes patients that insulin resistance determined lower content of insulin receptors in the brain, with decreased cognitive ability [37].

Lipids and the Cognitive Function

The mammalian brain is a lipid-rich organ, where gray matter contains 36%–40% lipid, white matter 49–66%, and myelin 78–81% [38]. The major

lipids include phosphatidylethanola mine (PE), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylinositol (PI), sphingomyelin, cerebroside, cerebroside sulfate, and ceramide [38]. Most of the lipids are present in the form of phospholipids comprising the complex array of neural fibers that make up the central nervous system.

Fatty Acids

A unique aspect of the lipid composition of all mammalian neurological tissues is the extraordinarily high concentration of DHA (Docosahexaenoic acid) and arachidonic acid (AA). As the most abundant building block of the brain, DHA represents more than 30% of the fatty acids of the phospholipids in the neuron [39]. DHA is primarily concentrated in the neuronal endings and synaptosomes, and is also associated with the neurite growth cones, where it has been shown to promote neurite outgrowth [40].

Brain development is characterized by specific well-defined stages of growth and maturation. Cerebral membrane phospholipids are not composed of the dietary precursors, LA (Linolenic acid) and ALA (alpha-linolenic acid), but of their longer chain and more unsaturated derivatives [41]. Biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFA) in the brain is very limited, is inherently slow due to the low desaturase activities [42, 43], and can be impaired by many factors, including diabetes and the stress-related hormones adrenaline and cortisol. The brain therefore depends on an exogenous supply; hence, the major sources of the longer-chain LC-PUFA species, such as AA, eicosapentaenoic acid (EPA), and DHA, *are* likely to be dietary. At the same time, with regard to PUFA, the brain is a well-protected organ: it uses dietary fatty acids in a highly specific manner. A very short-term restriction of the ω-3 fatty acids in an otherwise complete diet causes few anomalies in the profile of PUFAs in the brain and its organelles [44]. So, a deficiency of ω-3 fatty acids in the diet will not cause anomalies in the brain, unless extremely prolonged [45]. However, even though the fatty acid composition of brain lipids is known to be relatively constant, not responding as readily to changes in dietary fat composition as do the other tissues [46], a number of findings have demonstrated a dose–effect relationship between the quantity of dietary ALA and the DHA content of cerebral structures [47, 48].

Most humans living on a Western diet will have insufficient in-vivo production of DHA from ALA, and will depend on preformed dietary DHA such as is found in some fish, algae, and animal organ meat (or breast milk for infants) to maintain adequate DHA stores. Cessation of DHA supplementation leads to a gradual decline in tissue and blood concentrations of DHA. When diets are ω-3 restricted, the DHA levels in the brain are the most highly conserved. Furthermore, ω-3 PUFA deficiency early in life may result in irreversible damage to biochemical processes in the central nervous system [49].

Therefore, the composition of fatty acids of food is very important for brain function. Most natural foods have varying compositions of fatty acids; however, meat and dairy products have a higher saturated fatty acid composition. Fruits and vegetables tend to be lower in total fatty acids, and the composition is predominantly unsaturated. Fatty acids stimulate gene expression and neuronal activity, increase synaptogenesis and neurogenesis, and prevent neuroinflammation and apoptosis. There is evidence that provides support for the hypothesis that highly saturated or trans-fatty acids increase the risk of dementia, and that high polyunsaturated or monounsaturated fatty acids decrease this risk [50].

Many clinical and animal studies demonstrate the importance of LC-PUFA in neuronal development and neurodegeneration. EPA, DHA, and AA also participate in cardiovascular health and inflammation. High levels of DHA are found in the gray matter of the cerebral cortex, and in the outer segment membranes of the photoreceptor in the retina. It has been shown that in neuronal development, deficiency of DHA and EPA can lead to serious disorders such as schizophrenia, attention deficit, and hyperactivity disorders.

Essential fatty acids (EFAs) are polyunsaturated fatty acids (PUFA) that are provided by foods

because they cannot be synthesized in the body. However, they are necessary for good health. There are two families of EFAs, omega-3 fatty acids (ω-3) and omega-6 (ω-6). The ω-3 PUFAs are important components of cell membranes throughout the body, as they are incorporated into the phospholipids that form cell membranes. The acyl chains interact with other chains in neighboring phospholipid molecules within the bilayer, and the level of chain interactions determines the biophysical properties of the membranes, including their fluidity and consequently their enzymatic activities, cell–cell interactions, binding between signal molecules and receptors, and nutrient transport. This type of membrane is, for example, in myelinated nerve fibers, whereas metabolically active membranes, such as those found in neuron cell bodies, comprise phospholipids containing unsaturated sn-2 chains.

Recent results have shown that deficiency of alpha-linolenic acid in the diet induces marked abnormalities in certain brain structures, such as the frontal cortex and the pituitary gland, more than others. These selective lesions are accompanied by behavioral disorders, particularly affecting certain tests (habituation, adaptation to new situations). Biochemical and behavioral abnormalities are partially offset by dietary supplements such as egg-yolk extracts, rich in omega-3-fatty acids. A dose–effect study showed that the phospholipids of animal origin are more effective than plant phospholipids in reversing the consequences of the deficiency of alpha-linolenic acid, in part because they provide very long preformed chains. On the other hand the deficiency of alpha-linolenic acid decreases the perception of pleasure, slightly altering the efficacy of sensory organs, and affects certain brain structures related to hearing, vision, and smell. For example, a given perception of a sweet taste level requires a greater amount of sugar in subjects with deficiency of alpha-linolenic acid. Epidemiological studies suggest that there is an inverse association between fish or n-3 PUFA intake and risk of neurological disorders [51].

Chronic dietary LC-PUFA deficiency may lead to changes in neuronal membrane phospholipids of the cortex and hippocampus, and may be linked to impaired central nervous system function. In particular, DHA deficiency appears to be involved in neuropsychiatric disorders [52].

It has been observed that supplementation with LC-PUFA (ω-3) is effective for the treatment of patients with schizophrenia or at high risk for psychosis. There is also evidence that it is relevant in the pathophysiology of depression. Some authors have observed in poblational studies that increased intake of fish/seafood correlates with lower rates of lifetime prevalence of unipolar and bipolar depression. Depression is associated with increased production of proinflammatory cytokines and homocysteine levels in plasma, and (ω- 3) is capable of reducing levels of these species, producing a positive effect on mood, partly due to the high content of brain DHA and its involvement in neurogenesis and neuronal plasticity, and partly because of its anti-inflammatory properties [53].

Phospholipids and CNS

The fat in the brain is in part made of phospholipids. Fatty acids are rarely found as free molecules due to their detergent and cytotoxic effects but are generally esterified in larger molecular species such as phospholipids and triacylglycerols. Phospholipids spontaneously form lipid bilayers, and comprise the bulk of membrane elements within the cell. They are also the predominant source of fatty acids for cell signaling reactions. For both signaling and normal phospholipid turnover, PUFAs need to be released from the phospholipids by enzymes known as phospholipases. The most direct method of release from phospholipids is hydrolysis of the molecule at the sn-2 position (where almost all PUFAs are esterified) by the enzyme phospholipase A2 (PLA2). PLA2 activity is the rate-limiting step in the generation of eicosanoids and docosanoids, derived from AA and EPA respectively. Importantly, PLA2 has equal affinity for both EPA and AA; therefore, the proportion of ω-3/ω-6 fatty acids hydrolyzed by PLA2 is a determinant on the ω-3/ω-6 profile of the tissue. DHA is primarily sequestered into the phospholipid membranes of cells within the brain and central nervous system.

Phosphatidylserine (PS) is an acid phospholipid, and it is a natural component of the neuronal membrane. PS is lower than other phospholipids that comprise the biological membranes percentage, but is especially important in determining the surface potential of the neuronal membrane and the local ionic environment. PS esterifies LC-PUFA as DHA and EPA, which is essential for brain activity.

Within the neuronal membrane, PS participates in the activation of protein kinase C (PKC). PKC activity decreases with age and PS also declines with aging. PS decreasing associated with brain aging may be related to cognitive impairment and disability. Pharmacokinetic studies indicate that PS crosses the blood–brain barrier. The PS used in pharmacology comes from soybeans, to avoid the risk of spongiform disease, if derived from bovine brain. A population-based study revealed that PS could improve memory function of elderly people with memory deficits. It has also been found to improve symptoms in child depression and attention deficit/hyperactivity disorder. This indicates that PS is able to correct altered neuronal function in various conditions [54]. There is increasing evidence that indicates that disturbances of fatty acids and phospholipids metabolism can play a part in a wide range of psychiatric, neurological, and developmental disorders in adults [55].

Another consequence reported in whole brain is the change in lipid composition as a function of age in normal subjects [56, 57]. Thus, in the frontal cortex and hippocampus, PE (phosphatidylethanol amine) and PC (Phosphatidylcholine) concentrations decrease by about 30% in the healthy elderly compared to young adults [56]. Also, DHA contents in the main brain phospholipids (PC and PE) have been reported to be reduced in older compared to young subjects [58].

LC-PUFA, in particular DHA; 22:6n-3, are particularly enriched in cell-membrane phospholipids, especially in neural tissues [59, 60]. Also, LC-PUFAs have the capacity to influence plasma membrane organization and activity by modulating the lipid composition and functionality of lipid raft domains [61, 62]. Lipid rafts are cholesterol- and sphingolipid-enriched membrane microdomains resistant to solubilization by non-ionic detergents at low temperatures**.** **T**hey may serve as platforms for intracellular cell signaling [63]. There is some evidence that lipid rafts may be targets of neurodegenerative diseases, the most common form of dementia, such as Alzheimer's disease [64]. Several studies suggest that lipid rafts are likely molecular targets through which long-chain n-3 polyunsaturated fatty acids modulate biochemical activities, and reduce the incidence and severity of human diseases. In AD, brains show altered lipid raft composition and physicochemical properties, which may explain the abnormal lipid raft signaling processes observed in AD [60, 62, 64].

Cholesterol and Nervous System Function

The brain contains five to ten times more cholesterol than any other organ, and this sterol represents 2–3% of the total weight and 20–30% of all lipids in the brain. There is solid evidence that most if not all of this cholesterol is produced in situ rather than imported from the blood, probably because lipoprotein particles, which mediate the intercellular transport of sterols and other lipids, cannot pass the blood–brain barrier [65, 66]. Nervous tissue is capable of cholesterol synthesis, and the synthesis rate and cholesterol content increase drastically during brain development. It is possible that only specific types of neurons depend on external cholesterol. There is good evidence that cholesterol homeostasis is not uniform throughout the brain, but differs from region to region; the cholesterol content and the expression level of cholesterol-specific enzyme synthesis show strong region-specific variation [67].

HMGCoA synthase was found in hippocampal sensory neurons of rabbit [68]. However, this is not sufficient to establish cholesterol synthesis, because it is also used to form isoprenoids. Anyways, cholesterol synthesis has been detected in cultured neurons derived from embryonic or new-born mice, chicken, and rats.

Apparently, neurons require glia-derived cholesterol to form numerous and efficient synapses [69]. Therefore, there is a hypothesis that

states that during postnatal development, neurons downregulate their cholesterol synthesis and import the component from astrocytes, which differentiate postnatally and release cholesterol-rich lipoproteins. Cholesterol biosynthesis in the brain involves several intermediates and mediating enzymes. The regulation of biosynthesis of cholesterol is believed to involve insulin-induced genes (INSIGs) and sterol regulatory element-binding proteins, in particular SREBP-2 [70], and to be controlled through feedback regulation by sterols, including cholesterol itself [71]. HMG-CoA reductase, the rate-controlling enzyme for cholesterol biosynthesis, is the main target of cholesterol regulation [72].

Minerals and the Nervous System

Large amounts of metals coexist and co-localize in the brain, where metals such as iron, copper, and zinc act as essential cofactors in metalloproteinases. They are required for the normal functioning of the nervous tissue, while heavy metals such as mercury and lead are known neurotoxins. The high metal content of the CNS makes it particularly susceptible to metal-catalyzed oxidative damage, protein aggregation, neurotoxicity, and neurodegeneration.

There is increasing evidence that dysregulation of manganese, iron, copper, and zinc homeostasis contributes to a vast range of neurodegenerative diseases. Manganese, copper, and zinc participate in enzymatic mechanisms that protect against free radicals, toxic derivatives of oxygen. More specifically, the full genetic potential of the child for physical growth and mental development may be compromised due to deficiency (even subclinical) of micronutrients. Popescu and Nichol (2011) [73] describe how synchrotron X-ray fluorescence (XRF) imaging can be used to quantitatively assess how the distribution and chemistry of multiple brain metals in experimental animal models are affected by chelators currently used for the treatment of neurodegenerative disease.

According to Frederickson et al. (2000) [74], three distinct pools of cellular zinc can be found in the central nervous system: the most abundant fraction, accounting for about 80%, is bound to intracellular proteins and is immobile; a second pool (5–15% of cellular totals) is sequestered within the vesicles present at glutamatergic synapses. This vesicular zinc is found colocalized with glutamate, and it has neuromodulatory effects. The third pool of zinc (about 5%) is represented by the free, unbound ions in the cytoplasm.

Moreover, cytochemical estimations of the activity of cytochrome oxidase confirm that mitochondrial dysfunctions play a role in synaptic deterioration. Zinc acts as a physiological neuromodulator at glutamatergic synapses; however, in order to avoid neurotoxic damage, the intracellular free Zn^{2+} concentration ($[Zn^{2+}]i$) must be controlled by: (i) extrusion (Zn^{2+} transporters); (ii) buffering (metallothioneins) and (iii) sequestration (mitochondria) systems. In physiological aging, if any of these systems is impaired and/or not adequately coordinated, the resulting significant rise of ($[Zn^{2+}]i$) may inhibit the cellular energy-providing systems and affect mitochondria as primary targets [75].

Brain diseases during aging can also be due to failure of protective mechanisms, due to dietary deficiencies, for instance in anti-oxidants and nutrients (trace elements, vitamins, non-essential micronutrients such as polyphenols) related to protection against free radicals. Among these, recent evidence suggests that zinc ion dishomeostasis may play a pivotal role. Mild or moderate zinc deficiency is more widespread. It is estimated that 82% of pregnant women worldwide have a zinc intake lower than the recommended dietary intake, and this may approach 100% in developing countries [76]. Zinc deficiency impairs whole-body accumulation of PUFAs [77]; thus, brain supplying could be affected. Consequently, zinc deficit induces behavioral changes [78]. Some psychiatric problems can stem from the reduction in dietary zinc; animal experiments have clearly shown that deficiency (in particular during pregnancy) results in loss of neurons and a reduction in brain volume. In contrast, giving pregnant women zinc supplements has not been proved effective for improving the cognitive performance of their children [79].

Zinc is mainly stored in the synaptic vesicles of excitatory synapses (synaptic terminals of hippocampal mossy fibers). Free zinc is important for myelination, and for the release of the neurotransmitters gamma-aminobutyric acid and glutamate, which are key modulators of neuronal excitability [80]. This element plays a role in cognitive development [81], and the sensory receptors and brain regions that perceive and interpret the pleasures of eating are themselves very rich in zinc, and levels in the taste buds are strongly high, suggesting that zinc is necessary for their function [82]. There is a risk for a vicious circle to be established which, unfortunately, is often met in elderly people: the low zinc level leads to reduced appreciation of taste and increases zinc deficiency. Thus the circle becomes even more vicious [81, 82].

The developing nervous system is disturbed by zinc deficiency, especially when the brain undergoes its most rapid period of maturation during fetal life. Studies have shown a correlation between maternal zinc status and neonatal and infant behavior and cognitive function [83]. Few intervention studies in human populations suggested that improving maternal zinc status through prenatal supplementation might improve fetal neurobehavioral development [83]. However, the limited studies on the effects of zinc supplementation on cognitive recovery in zinc-deprived (ZD) animal offspring have reported conflicting results [84].

Perinatal omega-3 deficiency induces overexpression of ZnT3 (transporter identified in synaptic vesicles and found in some regions such as cortex and hippocampus) and causes abnormal zinc metabolism in the brain. Also, perinatal omega-3 polyunsaturated fatty acid supply modifies brain zinc homeostasis during adulthood, at least in rat. This is important because neuronal zinc is involved in formation of amyloid plaques, a major characteristic of Alzheimer's disease [85].

Yu et al. (2016) [86] showed that mild zinc deficiency in rats during pregnancy and lactation leads to the impairment of learning and memory function in offspring, and that zinc supplementation can recover the impairment of spatial learning and memory function. Data from animal studies on nutrient supplementation can be transposed to humans only very cautiously due to the obvious metabolic differences. Contestabile et al. (2016) [87] showed a potent amnesic effect of zinc supplementation in adult rats, and linked it to a dysregulated function of glycogen synthase kinases 3β (GSK-3ß) in the hippocampus. While no evidence for unspecific toxic effects of metal supplementation was found, they could not exclude the possibility that other memory-related cell functions are affected by zinc. Relevance of these results for humans, in particular for treatment of post-traumatic stress disorders, is open to future investigation.

RE1-silencing transcription factor (REST) also called neuron-restrictive silencer factor, a zinc-finger transcription factor, is known to repress thousands of possible target genes, many of which are neuron-specific. Current evidence demonstrates its importance in adult neurons: its functional relevance is considerable**. T**herefore, the identification of REST as a master factor, which was initially proposed for differentiating precursors, appears to be appropriate for adult neurons. Among transcription factors, REST exhibits several unique properties. The very low levels of REST which are initially established during differentiation are maintained in adult neurons by controlled transcription of the *Rest* gene, coupled to the very active ubiquitination and ensuing proteolysis of the REST protein. The unusual length and repetitive structure of RE-1, the DNA sequence of REST-binding in many target genes, ensures that the repressor has highly specific actions. The development of studies in the near future anticipates the way to the identification of multiple, highly interesting processes that take place in physiology and neurological pathology, especially in Alzheimer's disease and Huntington's disease, as well as epilepsy [88].

Vitamins and Dementia

Dementia is a neurocognitive disorder that affects the mental abilities, independence, and quality of life of those affected, mainly older adults (American Psychiatric Association, 2013) [89].

The World Health Organization (WHO) estimated in 2012 that 35.6 million individuals worldwide had dementia in 2011, and the prevalence of this disease will double every 20 years, reaching 115.4 million adults by 2050. Alzheimer's disease is the most common cause of dementia in ageing human populations, and is associated with presence of amyloid-beta (Aβ) plaques, neurofibrillary tangles, and neuronal loss [90, 91]. According to the most recent statistics, 44 million people are affected with Alzheimer's, and these numbers are expected to quadruple by 2050 [92]. In the absence of curative treatment, preventing or postponing the onset of dementia is of critical importance [93, 94]. Numerous studies suggest that vitamins, nutrients, and dietary supplements may delay the onset of age-associated cognitive decline and various forms of dementia including AD [95–98].

B-complex vitamins such as B2, B6, B12, and folate have a beneficial effect on cognition. These are necessary for the production of neurotransmitters, phospholipids, and nucleotides in the brain [99–101]. Low levels of these B vitamins have been associated with increased homocysteine (Hcy), a protein that has been associated with cognitive impairment [97]. Folate metabolism, also known as one-carbon metabolism, plays a fundamental role in DNA synthesis and integrity and in chromosome stability. Impairments of this pathway have been often linked to AD risk [102]. Some studies have shown that AD has been associated with lower levels of Hcy, folate, and vitamin B12 compared to age-matched non-AD controls [103–105]. It was also reported that supplementing AD patients with high doses of vitamins B6 and B12 and folate decreases plasma Hcy concentration and is associated with lower brain atrophy [106, 107].

There is evidence that free radicals may cause oxidative damage, which plays a key role in the pathology of dementias including AD [108, 109]. The cognitive decline observed in these neurodegenerative disorders is associated with increased oxidative stress, which ultimately leads to neuronal death and neurodegeneration [109]. Thus a considerable interest has been generated regarding the potential role that the antioxidant properties of vitamins such as E, A, and C, among others, might play in treating these dementias [110].

The Vitamin E family consists of four tocopherols and four tocotrienols: α (alpha), β (beta), γ (gamma) and δ (delta). Alpha-tocopherol is the predominant form of vitamin E in human tissues, possesses a powerful antioxidant function, and protects membranes from being oxidatively damaged by free radicals [111–113]. It is well known that vitamin E deficiency induces anemia, ataxia, and cognitive dysfunction in humans and rodents [114, 115]. It has been demonstrated that diet supplementation with vitamin E in patients with mild and moderate AD showed a delay in cognitive decline [116, 117]. Shah et al. (2013) [118] observed that subjects with high plasma levels of tocopherols, tocotrienols, or vitamin E had a reduced risk of developing AD, in comparison to persons with lower levels. In addition, the preventive effect of vitamin E with respect to developing AD symptoms was reported by the same group, 2 years later [117].

Apoptosis also plays a pivotal role in the pathogenesis of neurodegenerative diseases. The investigations by Osakada et al. (2004) [119] suggest that vitamin E analogs can exert antiapoptotic neuroprotective action independently of their antioxidant property. In addition, the group of Yonguc [120] found similar results in the rat hippocampus. In particular, it was found that alpha-tocopherol and gamma-tocotrienol inhibit apoptosis of astrocyte and stimulate their proliferation [121].

Vitamin A and its active derivatives, retinoids, play a significant role in the regulation of brain functions [122]. The retinoic acid, the active form of vitamin A, is the ligand of a set of receptors (retinoic acid and retinoid × receptors) that act as transcriptional regulators of their target genes [123]. The retinoids play a key role in cognitive function, specifically in hippocampal long-term depression (LTD) and potentiation, both measures of long-lasting synaptic plasticity, and neurogenesis [124, 125]. Some reports showed that vitamin A-deprived rodents exhibit serious defects in spatial learning and memory, and the administration of retinoic acid has been shown to alleviate deficits in memory performance [126–129].

In addition, retinoids modulate the inflammatory response of microglia and astrocytes, implicated in several senile dementias, including Alzheimer's disease [130, 131]. Microglias, the resident macrophages of the central nervous system, are the first line of defense in the brain. In response to pathogen agents, such as lipopolysaccharide (LPS), amyloid protein, and interferon-γ (IFN-γ) [132, 133], microglia becomes activated, resulting in increased production of cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) [134, 135]. Activation of astrocytes can lead to overproduction and accumulation of various proinflammatory and neurotoxic factors that include cytokines TNFα and IL-1β and chemokines, including RANTES, IL-8, and MCP-1 [136, 137]. Thus, a strong inflammatory response may be autotoxic to neurons, contributing to neuronal dysfunction and cell death and exacerbating the fundamental pathology in neurological disorders. Therefore, treatments that suppress the activation of microglia and astrocytes might be a potential therapeutic approach in chronic neurodegenerative diseases. The investigations by Van Neervenet al. (2010) [138] showed that all-trans retinoic acid block lipopolysaccharide induced activation of inflammatory mediators in astrocytes. In addition, reports have indicated that retinoids significantly inhibit the production of chemokines and proinflammatory cytokines in microglia and astrocytes, which are activated in AD [139, 140].

The decline of the antioxidant status is associated with dementia, especially low levels of vitamin C. Ascorbic acid (the reduced form of vitamin C) is a chelating agent with antioxidant properties that protects cells from oxidative stress [141, 142]. A number of studies have found associations between antioxidants such as vitamin C and cognitive function. In fact, it was found that ascorbic acid alone or in combination with vitamin E and β-carotene enhances cognitive function [143, 144]. Masaki et al. (2000) [145] found a similar effect on cognitive performance in the Honolulu–Asia Aging Study, where Vit C or Vit E supplementation was associated with a higher cognitive performance. Recent studies have shown the potential chronic effects of a suboptimal Vit C status in impaired brain development and in neurodegenerative disorders, such as AD [146, 147]. Alzheimer's patients have reduced plasma levels of vitamin C (ascorbic acid; ascorbate) [148, 149]. Recent researches have shown that treatment with ascorbic acid reversed some of the cognitive deficits found in AD transgenic mice [150, 151]. It was also reported that high levels of dietary ascorbate or supplements decrease the risk of developing the disease [152].

Vitamin D deficiency is highly prevalent in older adults, and is associated with cognitive impairment [153]. The active form of vitamin D (1,25-dihydroxyvitamin D) plays a key role in development and adult brain function [154]. Thus, the vitamin D receptors (VDR) were found in the hippocampus, an area crucial for memory formation [155, 156]. Vitamin D contributes to neuroprotection by modulating the synthesis of neurotrophic agents such as nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF) and nitric oxide synthase (NOS) [157]. Also, it has a neuronal protective effect by enhancing antioxidant pathways in areas of the brain responsible for cognition [158]. A recent study reported that vitamin D deficiency has been associated with neurological diseases such as dementia [159].The investigations by Taghizadeh et al. (2011) [160] confirmed that vitamin D deficiency increases spatial learning deficits in a rat model of AD. In addition, epidemiological and clinical data have shown that vitamin D deficiency is found in patients with Alzheimer's disease [161, 162]. $1,25(OH)_2D3$ has a neuroprotective role in AD, since it enhances cerebral clearance of human amyloid beta (Aβ) peptide [163]. Another study has shown its ability to reduce amyloid deposits by stimulating phagocytosis of the Aβ [164]. These findings demonstrate that antioxidants would be a good therapeutic strategy against dementias such as AD.

Resveratrol is a polyphenol present in red wine, and it exhibits antioxidant, anti-inflammatory, and neuroprotective effects [165–167]. Several studies have reported that resveratrol suppresses oxygen free radical formation and up-regulates the activity of antioxidant enzymes such as superoxide dismutase, catalase,

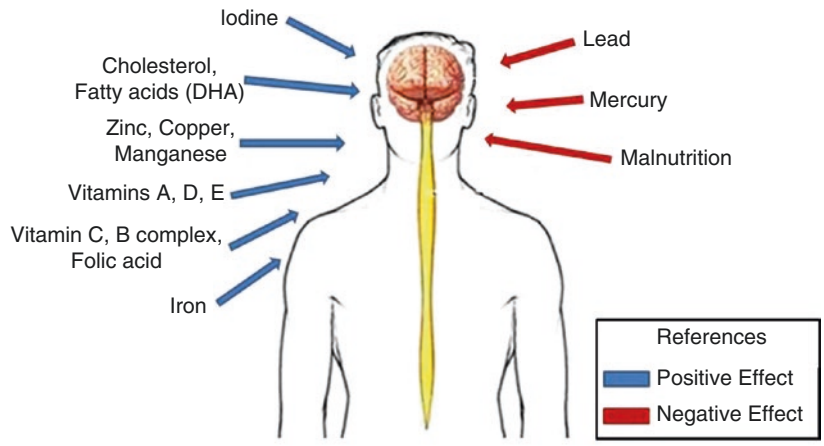


Fig. 35.1 Effect of substances on the central nervous system. The effect of the different substances varies according to the dose and the time of consumption/exposure, as well as with epigenetics, biological rhythm, and the environment. (DHA: docosahexaenoic acid)

and glutathione peroxidase [168, 169]. The inflammatory response at the neuronal level promotes the pathogenesis of several chronic neurodegenerative diseases, including AD. Thus, activation of microglia and astrocytes induces the release of large amounts of pro-inflammatory mediators, including cytokines and chemokines, causing neuronal inflammation and cell death [170]. Findings of Capiralla et al. (2012) [171] showed that oral administration of resveratrol significantly reduced microglial activation in a mouse model of AD.

Resveratrol exhibits strong neuroprotective properties, since it decreases aging-dependent cognitive decline and pathology in AD animal models [172, 173]. In particular, resveratrol inhibits its production of β-amyloid and aggregation and destabilization of the Aβ fibrils [174, 175]. A recent study by Porquet and colleagues reported that dietary resveratrol supplementation reduces amyloid accumulation, tau hyperphosphorylation, and cognitive impairment in a model of AD [176].

Thus, this experimental evidence suggests that resveratrol, vitamins A, C, E, D, and folate may block oxidative stress and promote clearance of Aβ peptides, involved in the pathogenesis of AD, making these dietary supplements a new therapeutic promise to prevent or treat AD.

In conclusion, this review seeks to integrate current knowledge on the relation between brain and nutrition, and some factors have been unclearly identified. Among these, accumulation of oxidative damage has been implicated in aging and various neurological disorders. Recent evidence suggests that zinc ion dishomeostasis may play a pivotal role, being consistent with the reported primary deterioration of synapses.

Most of the lipids are present, forming the complex array of neural fibers that make up the central nervous system (Fig. 35.1). However, questions about the regulation of cholesterol transport from astrocytes to neurons, the cross-talk between neuron and astrocyte, still remain unclear. The understanding of cholesterol metabolism in the brain and its role in disease requires further studies.

Future studies should also examine the deficiencies in other DNA repair processes and the inhibitory effect of diseases linked to metal ions. Understanding how DNA repair deficiency occurs and affects the nervous system could provide a rational basis for therapies in neurodegenerative diseases.

References

1. Gomez-Pinilla F. Brain foods: the effects of nutrients on brain function. Nat Rev Neurosci. 2008;9:568–78.
2. Simons M, Trajkovic K. Neuron–glia communication in the control of oligodendrocyte function and myelin biogenesis. J Cell Sci. 2006;119:4381–9.
3. Kendler KS, Gardner CO, Prescott CA. Toward a comprehensive developmental model for major depression in women. Am J Psychiatry. 2002;159:1133–45.
4. Morgan KD, Dazzan P, Orr KG, Hutchinson G, Chitnis X, Suckling J, Lythgoe D, Pollock SJ, Rossell S,

Shapleske J, Fearon P, Morgan C, David A, McGuire PK, Jones PB, Leff J, Murray RM. Grey matter abnormalities in first-episode schizophrenia and affective psychosis. B J Psych. 2007;191:s111–6.
5. Moore JK, Perazzo LM, Braun A. Time course of axonal myelination in the human brainstem auditory pathway. Hear Res. 1995;87:21–31.
6. Miller SL, Klurfeld DM, Loftus B, Kritchevsky D. Effect of essential fatty acid deficiency on myelin proteins. Lipids. 1984;19:478–80.
7. McKenna MC, Campagnoni AT. Effect of pre- and postnatal essential fatty acid deficiency on brain development and myelination. J Nutr. 1979;109: 1195–204.
8. Silvestroff L, Franco PG, Pasquini JM. Neural and oligodendrocyte progenitor cells: transferrin effects on cell proliferation. ASN Neuro. 2013;5(1):e00107. doi:10.1042/AN20120075.
9. Kwik-Uribe CL, Gietzen D, German JB, Golub MS, Keen CL. Chronic marginal iron intakes during early development in mice result in persistent changes in dopamine metabolism and myelin composition. J Nutr. 2000;130:2821–30.
10. Chen MH, Su TP, Chen YS, Hsu JW, Huang KL, Chang WH, Chen TJ, Bai YM. Association between psychiatric disorders and iron deficiency anemia among children and adolescents: a nationwide population-based study. BMC Psychiatry. 2013;13:161.
11. Nielsen PR, Meyer U, Mortensen PB. Individual and combined effects of maternal anemia and prenatal infection on risk for schizophrenia in offspring. Schizophr Res. 2016;172(1–3):35–40.
12. de Escobar GM, Obregon MJ, del Rey FE. Iodine deficiency and brain development in the first half of pregnancy. Public Health Nutr. 2007;10:1554–70.
13. Dussault JH, Ruel J. Thyroid hormones and brain development. Annu Rev Physiol. 1987;49:321–34.
14. Kanık-Yuksek S, Aycan Z, Oner O. Evaluation of iodine deficiency in children with attention-deficit/ hyperactivity disorder. J Clin Res Pediatr Endicrinol. 2016;8(1):61–6.
15. Sanchez ES, Bigbee JW, Fobbs W, Robinson SE, Sato-Bigbee C. Opioid addiction and pregnancy: perinatal exposure to buprenorphine affects myelination in the developing brain. Glia. 2009;56(9):1017–27.
16. Hsu DT, Sanford BJ, Meyers KK, Love TM, Hazlett KE, Walker SJ, Mickey BJ, Koeppe RA, Langenecker SA, Zubieta JK. It still hurts: altered endogenous opioid activity in the brain during social rejection and acceptance in major depressive disorder. Mol Psychiatry. 2015;20(2):193–200.
17. Aston C, Jiang L, Sokolov BP. Microarray analysis of postmortem temporal cortex from patients with schizophrenia. J Neurosci Res. 2004;77(6):858–66.
18. Barandas R, Landgraf D, McCarthy MJ, Welsh DK. Circadian clocks as modulators of metabolic comorbidity in psychiatric disorders. Curr Psychia Rep. 2015;17(12):98. doi:10.1007/s11920-015-0637-2.
19. Mergenthaler P, Lindauer U, Dienel GA, Meisel A. Sugar for the brain — the role of glucose in physiological and pathological brain function. Trends Neurosci. 2013;36(10):587–97.
20. Magistretti PJ, Sorg O, Yu N, Martin JL, Pellerin L. Neurotransmitter regulates energy metabolism in astrocytes: implications for the metabolic trafficking between neural cells. Dev Neurosci. 1993;15: 306–12.
21. Brown AM, Baltan Tekkök S, Ransom BR. Energy transfer from astrocytes to axons: the role of CNS glycogen. Neurochem Int. 2004;45:529–36.
22. Vilchez D, Ros S, Cifuentes D, Pujadas L, Valles J, Garcia-Fojeda B, Criado-Garcia O, Fernandez-Sanchez E, Medraño-Fernández I, Dominguez J, Garcia-Rocha M, Soriano E, Rodriguez de Cordoba S, Guinovart JJ. Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. Nat Neurosci. 2007;10:1407–13.
23. Hertz L, Gibbs ME. What learning in day-old chickens can teach a neurochemist: focus on astrocyte metabolism. J Neurochem. 2009;109(s1):10–6.
24. Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ, Alberini CM. Astrocyte–neuron lactate transport is required for long term memory formation. Cell. 2011;144:810–23.
25. Garcia-Nogales P, Almeida A, Bolaños JP. Peroxynitrite protects neurons against nitric oxide-mediated apoptosis. A key role for glucose-6-phosphate dehydrogenase activity in neuroprotection. JBC. 2003;278:864–74.
26. Ben-Yoseph O, Boxer PA, Ross BD. Noninvasive assessment of the relative roles of cerebral antioxidant enzymes by quantitation of pentose phosphate pathway activity. Neurochem Res. 1996;21:1005–12.
27. Lovatt D, Sonnewald U, Waagepetersen HS, Schousboe A, He W, Lin JH, Han X, Takano T, Wang S, Sim FJ, Goldman SA, Nedergaard M. The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. J Neurosci. 2007;27:12255–66.
28. Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Kireg PA, Krupenko SA, Thompson WJ, Barres BA. A transcriptome database for astrocytes, neurons and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci. 2008;28:264–78.
29. Banting FG, Best CH. The internal secretion of the pancreas. J Lab Clin Med. 1922;7:251–66.
30. Margolis RU, Altszuler N. Insulin in the cerebrospinal fluid. Nature. 1967;215:1375–6.
31. Banks WA, Owen JB, Erickson MA. Insulin in the brain: there and back again. Pharmacol Ther. 2012;136:82–93.
32. Havrankova J, Schmechel D, Roth J, Brownstein M. Identification of insulin in rat brain. Proc Natl Acad Sci U S A. 1978;75:5737–41.

33. Dorn A, Bernstein HG, Rinne A, Ziegler M, Hahn HJ, Ansorge S. Insulin- and glucagonlike peptides in the brain. Anat Rec. 1983;207:69–77.
34. Duarte AI, Proenca T, Oliveira CR, Santos MS, Rego AC. Insulin restores metabolic function in cultured cortical neurons subjected to oxidative srtress. Diabetes. 2006;55:2863–70.
35. Bosco D, Fava A, Plastino M, Montalcini T, Pujia A. Possible implications of insulin resistance and glucose metabolism in Alzheimer s disease pathogenesis. J Cell Mol Med. 2011;17:1807–21.
36. Tolpannen AM, Lavikainen P, Solomon A, Kivipelto M, Uusitupa M, Soininen H, Hartikainen S. History of medically treated diabetes and risk of Alzheimer disease in a nationwide case-control study. Diabetes Care. 2013;36:2015–9.
37. Blazquez E, Velasquez E, Hurtado-Carneiro V, Ruiz-Albusac JM. Insulin in the brain: its pathophisiological implications for states related with central insulin resistance, type 2 diabetes and Alzheimer s disease. Front Endocrinol (Lausanne). 2014;5:161. doi:10.3389/fendo.2014.00161.
38. O'Brien JS, Sampson EL. Lipid composition of normal human brain: gray matter, white matter, and myelin. J Lipid Res. 1965;6:537–44.
39. Salem Jr N, Kim H-Y, Yergey JA. Docosahexaenoic acid: membrane function and metabolism. In: Simopoulos AP, Kiter RR, Martin RE, editors. Health effects of polyunsaturated fatty acids in seafoods. New York: Academic Press; 1986. p. 263–317.
40. Ikemoto A, Kobayashi T, Watanabe S, Okuyama H. Membrane fatty acid modifications of PC12 cells by arachidonate or docosahexaenoate affect neurite outgrowth but not norepinephrine release. Neurochem Res. 1997;22(6):671–8.
41. Bourre JM. Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during aging. J Nutr Health Aging. 2004;8:163–74.
42. Cook HW. In vitro formation of polyunsaturated fatty acids by desaturation in rat brain: some properties of the enzyme in developing brain and comparison with liver. J Neurochem. 1978;30:1327–34.
43. Purvis M, Clandinin MT, Hacker RR. Chain elongation–desaturation of linoleic acid during the development of the pig: implications for the supply of polyenoic fatty acids to the developing brain. Comp Biochem Physiol. 1983;75B:199–204.
44. Delaš I, Popovic M, Petrovic T, Delaš F, Ivankovic D. Changes in the fatty acid composition of brain and liver phospholipids from rats fed fat-free diet. Food Technol Biotechnol. 2008;3:278–85.
45. Bourre J-M, Durand G, Pascal G, Youyou A. Brain cell and tissuerecovery in rats made deficient in n-3 fatty acids by alteration of dietary fat. J Nutr. 1989;119:15–22.
46. Salem N, Moriguchi T, Greiner RS, et al. Alterations in brain function after loss of docosahexaenoate due to dietary restriction of n-3 fatty acids. J Mol Neurosci. 2001;16:299–307.
47. Bourre JM, Francois M, Youyou A, Dumont O, Piciotti M, Pascal G. The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. J Nutr. 1989b;119:1880–92.
48. Bourre JM, Dumont O, Pascal G, Durand G. Dietary alpha linolenic acid at 1.3 g/kg maintains maximal docosahexaenoic acid concentration in brain, heart and liver of adult rats. J Nutr. 1993;123:1313–9.
49. Uauy R, Mena P, Rojas C. Essential fatty acids in early life: structural and functional role. Proc Nutr Soc. 2000;59:3–15.
50. Morris MC, Tangney CC. Dietary fat composition and dementia risk. Neurobiol Aging. 2014;35(suppl 2):S59–64.
51. Appleton KM, Rogers PJ, Ness AR. Updated systematic review and meta-analysis of the effects of n-3 long-chain polyunsaturated fatty acids on depressed mood. Am J Clin Nutr. 2010;91:757–70.
52. Ferreira CF, Bernardi JR, da Silva DC, de Sa C-PN, de Souza MC, Krolow R, Weis SN, Pettenuzzo L, Kapczinski F, Silveira PP, Dalmaz O. Mitochondrial and oxidative stress aspects in hippocampus of rats submitted to dietary n-3 polyunsaturated fatty acid deficiency after exposure to early stress. Neurochem Res. 2015;40:1870–81.
53. Grosso G, Galvano F, Marventano S, Malaquarnera M, Bucolo C, Drago F, Caraci F. Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. Oxidative Med Cell Longev. 2014;2014:313570. doi:10.1155/2014/313570.
54. Glade MJ, Smith K. Phosphatidylserine and the human brain. Nutrition. 2015;31:781–6.
55. Perica MM, Delas I. Essential fatty acids and psychiatric disorders. Nutr Clin Pract. 2011;26:409–25.
56. Soderberg M, Edlund C, Kristensson K, Dallner G. Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. Lipids. 1991;26:421–5.
57. Plourde M, Fortier M, Vandal M, Tremblay-Mercier J, Freemantle E, Begin M, Pifferi F, Cunnane SC. Unresolved issues in the link between docosahexaenoic acid and Alzheimer's disease. Prostaglandins Leukot Essent Fat Acids. 2007;77:301–8.
58. Horrocks LA, Farooqui AA. Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. Prostaglandins Leukot Essent Fat Acids. 2004;70:361–72.
59. Uauy R, Hoffman DR, Peirano P, Birch DG, Birch EE. Essential fatty acids in visual and brain development. Lipids. 2001;36:885–95.
60. Lane RM, Farlow MR. Lipid homeostasis and apolipoprotein E in the development and progression of Alzheimer's disease. J Lipid Res. 2005;46:949–68.

61. Fan YY, McMurray DN, Ly LH, Chapkin RS. Dietary (n-3) polyunsaturated fatty acids remodel mouse T-cell lipid rafts. J Nutr. 2003;133:1913–2190.
62. Ma DW, Seo J, Switzer KC, Fan YY, McMurray DN, Lupton JR, Chapkin RS. n-3 PUFA and membrane microdomains: a new frontier in bioactive lipid research. J Nutr Biochem. 2004;15:700–6.
63. Brown DA, London E. Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J Biol Chem. 2000;275:17221–4.
64. Michel V, Bakovic M. Lipid rafts in health and disease. Biol Cell. 2007;99:129–40.
65. Pfrieger FW. Cholesterol homeostasis and function in neurons of the central nervous system. Cell Mol Life Sci. 2003;60:1158–71.
66. Björkhem I, Lütjohann D, Diczfalusy U, Stahle L, Ahiborg G, Wahren J. Cholesterol homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation. J Lipid Res. 1998;39:1594–600.
67. Segatto M, Di Giovanni A, Marino M, Pallottini V. Analysis of the protein network of cholesterol homeostasis in different brain regions: an age and sex dependent perspective. J Cell Physiol. 2013;228:1561–7.
68. Swanson LW, Simmons DM, Hofmann SL, Goldstein JL, Brown MS. Localization of mRNA for low density lipoprotein receptor and a cholesterol synthetic enzyme in rabbit nervous system by in situ hybridization. Proc Natl Acad Sci U S A. 1988;85:9821–5.
69. Mauch DH, Nägler K, Schumacher S, Göritz C, Müller EC, Otto A, et al. CNS synaptogenesis promoted by glia-derived cholesterol. Science. 2001;294:1354–7.
70. DeBose-Boyd RA. Feedback regulation of cholesterol synthesis: sterol-accelerated ubiquitination and degradation of HMG coA reductase. Cell Res. 2008;18:609–21.
71. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell. 1997;89:331–40.
72. Wang Y, Muneton S, Sjövall J, Jovanovic JN, Griffiths WJ. The effect of 24S-hydroxycholesterol on cholesterol homeostasis in neurons quantitative changes to the cortical neuron proteome. J Proteome Res. 2008;7:1606–14.
73. Popescu BF, Nichol H. Mapping brain metals to evaluate therapies for neuro-degenerative disease. CNS Neurosci Ther. 2011;17(4):256–68.
74. Frederickson CJ, Suh SW, Silva D, Thompson RB. Importance of zinc in the central nervous system: the zinc-containing neuron. J. Nutrition. 2000;130:1471S–81S.
75. Bertoni-Freddari C, Mocchegiani E, Malavolta M, Casoli T, Di Stefano G, Fattoretti P. Synaptic and mitochondria physiopathologic changes in the aging nervous system and the role of zinc ion homeostasis. Mech Ageing Dev. 2006;127:590–6.
76. Caulfield LE, Zavaleta N, Shankar AH, Merialdi M. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. Am J Clin Nutr. 1998;68(2 Suppl):499S–508S.
77. Cunnane SC, Yang J. Zinc deficiency impairs whole-body accumulation of polyunsaturates and increases the utilization of [1-14C] linoleate for de novo lipid synthesis in pregnant rats. Can J Physiol Pharmacol. 1995;73:1246–52.
78. Golub M, Keen C, Gershwin M, Hendrickx A. Developmental zinc deficiency and behaviour. J Nutr. 1995;125:2263S–71S.
79. Tamura T, Goldenberg RL, Ramey SL, Nelson KG, Chapman VR. Effect of zinc supplementation of pregnant women on the mental and psychomotor development of their children at 5 years of age. Am J Clin Nutr. 2003;77:1512–6.
80. Perez-Rosello T, Anderson CT, Schopfer FJ, Zhao Y, Gilad D, Salvatore SR, Freeman BA, Hershfinkel M, Aizenman E, Tzounopoulos T. Synaptic Zn2+ inhibits neurotransmitter release by promoting endocannabinoid synthesis. J Neurosci. 2013;33(22):9259–72.
81. Bhatnagar S, Taneja S. Zinc and cognitive development. Br J Nutr. 2001;85:S139–S45.
82. Chou H, Chien C, Huang H, Lu K. Effects of zinc deficiency on the vallatepapillae and taste buds in rats. J Formos Med Assoc. 2001;100:326–35.
83. Shah D, Sachdev HP. Zinc deficiency in pregnancy and fetal outcome. Nutr Rev. 2006;64(1):15–30.
84. Piechal A, Blecharz-Klin K, Pyrzanowska J, Widy-Tyszkiewicz E. Maternal zinc supplementation improves spatial memory in rat pups. Biol Trace Elem Res. 2012;147:299–308.
85. Jayasooriya AP, Ackland ML, Mathai ML, Sinclair AJ, Weisinger HS, Weisinger RS, Halver JE, Kitajka K, Puskas LG. Perinatal omega-3 polyunsaturated fatty acid supply modifies brain zinc homeostasis during adulthood. Proc Natl Acad Sci U S A. 2005;102:7133–8.
86. Yu X, Chen W, Wei Z, Ren T, Yang X, Yu X. Effects of maternal mild zinc deficiency and different ways of zinc supplementation for offspring on learning and memory. Food Nutr Res. 2016;60:29467. http://dx.doi.org/10.3402/fnr.v60.29467
87. Contestabile A, Peña-Altamira E, Virgili M, Monti B. Zinc supplementation in rats impairs hippocampal dependent memory consolidation and dampens post-traumatic recollection of stressful event. Eur Neuropsychopharmacol. 2016;26(6):1070–82. doi:10.1016/j.euroneuro.2015.12.041. pii: S0924-977X(15)00431-9
88. Pozzi D, Lignani G, Ferrea E, Contestabile A, Paonessa F, D'Alessandro R, Lippiello P, Boido D, Fassio A, Meldolesi J, Valtorta F, Benfenati F, Baldelli P. REST/NRSF-mediated intrinsic homeo-

stasis protects neuronal networks from hyperexcitability. EMBO J. 2013;32:2994–3007.
89. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington: American Psychiatric Association; 2013.
90. Jicha G, Carr S. Conceptual evolution in Alzheimer's disease: implications for understanding the clinical phenotype of progressive neurodegenerative disease. J Alzheimers Dis. 2010;19(1):253–72.
91. Pimplikar S, Nixon R, Robakis N, Shen J, Tsai L. Amyloid-independent mechanisms in Alzheimer's disease pathogenesis. J Neurosci. 2010;30:14946–54.
92. Alzheimer's Association Report. Alzheimer's disease facts and figures Alzheimer's Association. Alzheimers Dement. 2015;11:332–84.
93. Daviglus M, Bell C, Berrettini W. National institutes of health state-of-the-science conference statement: preventing Alzheimer disease and cognitive decline. Ann Intern Med. 2010;153(3):176–81.
94. Larson E, Yaffe K, Langa K. New insights into the dementia epidemic. N Engl J Med. 2013;369(24): 2275–7.
95. Devore E, Grodstein F, van Rooij F, Hofman A, Stampfer M, Witteman J, Breteler M. Dietary antioxidants and long-term risk of dementia. Arch Neurol. 2010;67(7):819–25.
96. Gillette-Guyonnet S, Secher M, Vellas B. Nutrition and neurodegeneration: epidemiological evidence and challenges for future research. Br J Clin Pharmacol. 2013;75(3):738–55.
97. Haan M, Miller J, Aiello A, Whitmer R, Jagust W, Mungas D, Allen L, Green R. Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging. Am J Clin Nutr. 2007; 85(2):511–7.
98. Slinin Y, Paudel M, Taylor B, Fink H, Ishani A, Canales M, Yaffe K, Barrett-Connor E, Orwoll E, Shikany J, Leblanc E, Cauley J, Ensrud K. 25-Hydroxyvitamin D levels and cognitive performance and decline in elderly men. Neurology. 2010;74(1):33–41.
99. Malouf R, Grimley E. Folic acid with or without vitamin B12 for the prevention and treatment of healthy elderly and demented people. Cochrane Database Syst Rev. 2008;4:CD004514.
100. Mooijaart S, Gussqekloo J, Frolich M, Jolles J, Stott DJ, Westendorp RGJ, de Craen A. Homocysteine, vitamin B-12, and folic acid and the risk of cognitive decline in old age: the Leiden 85-Plus study. Am J Clin Nutr. 2005;82:866–71.
101. Ueland P, Hustad S, Schneede J, Refsum H, Vollset S. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci. 2001;22:195–201.
102. Coppedè F. One-carbon metabolism and Alzheimer's disease: focus on epigenetics. Curr Genet. 2010;11:246–60.
103. Aisen P, Egelko S, Andrews H, Diaz-Arrastia R, Weiner M, DeCarli C, Jagust W, Miller J, Green R, Bell K, Sano M. A pilot study of vitamins to lower plasma homocysteine levels in Alzheimer disease. Am J Geriatr Psychiatry. 2003;11:246–9.
104. Faux N, Ellis K, Porter L, Fowler C, Laws S, Martins R, Pertile K, Rembach A, Rowe C, Rumble R, Szoeke C, Taddei K, Taddei T, Trounson B, Villemagne V, Ward V, Ames D, Masters CL, Bush AI. Homocysteine, vitamin B12, and folic acid levels in Alzheimer's disease, mild cognitive impairment, and healthy elderly: baseline characteristics in subjects of the Australian Imaging Biomarker Lifestyle study. J Alzheimers Dis. 2011;27:909–22.
105. Shea T, Lyons-Weiler J, Rogers E. Homocysteine, folate deprivation and Alzheimer neuropathology. J Alzheimers Dis. 2002;4:261–7.
106. Aisen P, Schneider L, Sano M, Diaz-Arrastia R, van Dyck C, Weiner M, Bottiglieri T, Jin S, Stokes K, Thomas R, Thal L. High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial. JAMA. 2008;300: 1774–83.
107. Smith A, Smith S, de Jager C, Whitbread P, Johnston C, Agacinski G, Oulhaj A, Bradley K, Jacoby R, Refsum H. Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial. PLoS One. 2010;5(9):e12244. doi:10.1371/journal.pone.0012244.
108. Gubandru M, Margina D, Tsitsimpikou C, Goutzourelas N, Tsarouhas K, Ilie M, Tsatsakis A, Kouretas D. Alzheimer's disease treated patients showed different patterns for oxidative stress and inflammation markers. Food Chem Toxicol. 2013; 61:209–14.
109. Mariani E, Polidori M, Cherubini A, Mecocci P. Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview. J Chromatogr B Anal Technol Biomed Life Sci. 2005;827:65–75.
110. Heo J, Hyon-Lee LK. The possible role of antioxidant vitamin C in Alzheimer's disease treatment and prevention. Am J Alzheimers Dis Other Demen. 2013;28:120–5.
111. Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radic Biol Med. 2014;72:76–90.
112. Niki E. Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. Free Radic Biol Med. 2014;66:3–12.
113. Rigotti A. Absorption, transport, and tissue delivery of vitamin E. Mol Asp Med. 2007;28:423–36.
114. Fukui K, Nakamura K, Shirai M, Hirano A, Takatsu H, Urano S. Long-term vitamin E-deficient mice exhibit cognitive dysfunction via elevation of brain oxidation. J Nutr Sci Vitaminol (Tokyo). 2015;61:362–8.
115. Niki E, Traber MG. A history of vitamin E. Ann Nutr Metab. 2012;61:207–12.
116. Dysken M, Sano M, Asthana S, Vertrees J, Pallaki M, Llorente M, Love S, Schellenberg G, McCarten

J, Malphurs J, Prieto S, Chen P, Loreck D, Trapp G, Bakshi R, Mintzer J, Heidebrink J, Vidal-Cardona A, Arroyo L, Cruz A, Zachariah S, Kowall N, Chopra M, Craft S, Thielke S, Turvey C, Woodman C, Monnell K, Gordon TJ, Segal Y, Peduzzi P, Guarino P. Effect of vitamin E and memantine on functional decline in Alzheimer disease: the TEAM-AD VA cooperative randomized trial. JAMA. 2014;311: 33–44.
117. Mangialasche F, Xu W, Kivipelto M, Costanzi E, Ercolani S, Pigliautile M, Cecchetti R, Baglioni M, Simmons A, Soininen H, Tsolaki M, Kloszewska I, Vellas B, Lovestone S, Mecocci P, AddNeuroMed Consortium. Tocopherols and tocotrienols plasma levels are associated with cognitive impairment. Neurobiol Aging. 2012;33:2282–90.
118. Shah R. The role of nutrition and diet in Alzheimer disease: a systematic review. J Am Med Dir Assoc. 2013;14(6):398–402.
119. Osakada F, Hashino A, Kume T, Katsuki H, Kaneko S, Akaike A. Alpha-tocotrienol provides the most potent neuroprotection among vitamin E analogs on cultured striatal neurons. Neuropharmacology. 2004;47:904–15.
120. Yonguc G, Dodurga Y, Adiguzel E, Gundogdu G, Kucukatay V, Ozbal S, Yilmaz I, Cankurt U, Yilmaz Y, Akdogan I. Grape seed extract has superior beneficial effects than vitamin E on oxidative stress and apoptosis in the hippocampus of streptozotocin induced diabetic rats. Gene. 2015;555:119–26. doi:10.1016/j.gene.2014.10.052.
121. Mazlan M, Sue Mian T, Mat Top G, Zurinah Wan Ngah W. Comparative effects of alpha-tocopherol and gamma-tocotrienol against hydrogen peroxide induced apoptosis on primary-cultured astrocytes. J Neurol Sci. 2006;243:5–12.
122. Lane M, Bailey S. Role of retinoid signalling in the adult brain. Prog Neurobiol. 2005;75:275–93.
123. Campo-Paysaa F, Marlétaz F, Laudet V, Schubert M. Retinoic acid signaling in development: tissue-specific functions and evolutionary origins. Genesis. 2008;46:640–56.
124. Luo T, Wagner E, Dräger U. Integrating retinoic acid signaling with brain function. Dev Psychol. 2009;45:139–50.
125. Malaspina A, Michael-Titus AT. Is the modulation of retinoid and retinoid-associated signaling a future therapeutic strategy in neurological trauma and neurodegeneration? J Neurochem. 2008;104:584–95.
126. Cocco S, Diaz G, Stancampiano R, Diana A, Carta M, Curreli R, Sarais L, Fadda F. Vitamin A deficiency produces spatial learning and memory impairment in rats. Neuroscience. 2002;115:475–82.
127. Hernández-Pinto A, Puebla-Jiménez L, Arilla-Ferreiro E. A vitamin A-free diet results in impairment of the rat hippocampal somatostatinergic system. Neuroscience. 2006;141:851–61.
128. Mingaud F, Mormede C, Etchamendy N, Mons N, Niedergang B, Wietrzych M, Pallet V, Jaffard R, Krezel W, Higueret P, Marighetto A. Retinoid hyposignaling contributes to aging-related decline in hippocampal function in short-term/working memory organization and long-term declarative memory encoding in mice. J Neurosci. 2008;28:279–91.
129. Misner D, Jacobs S, Shimizu Y, deUrquiza AM, Solomin L, Perlmann T, De Luca LM, Stevens C, Evans R. Vitamin A deprivation results in reversible loss of hippocampal long-term synaptic plasticity. Proc Natl Acad Sci U S A. 2001;98:11714–9.
130. Kuenzli S, Tran C, Saurat J. Retinoid receptors in inflammatory responses: a potential target for pharmacology. Curr Drug Targets Inflamm Allergy. 2004;3:355–60.
131. Kampmann E, Johann S, van Neerven S, Beyer C, Mey J. Anti-inflammatory effect of retinoic acid on prostaglandin synthesis in cultured cortical astrocytes. J Neurochem. 2008;106:320–32.
132. Hashioka S, Han Y, Fujii S, Kato T, Monji A, Utsumi H, Sawada M, Nakanishi H, Kanba S. Phosphatidylserine and phosphatidylcholine-containing liposomes inhibit amyloid and interferon-induced microglial activation. Free Radic Biol Med. 2007;42:945–54.
133. Kao T, Ou Y, Lin S, Pan H, Song P, Raung S, Lai C, Liao S, Lu H, Chen C. Luteolin inhibits cytokine expression in endotoxin/cytokine-stimulated microglia. J Nutr Biochem. 2011;22:612–24.
134. Benveniste EN. Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. J Mol Med. 1997;75:165–73.
135. Cameron B, Landreth GE. Inflammation, microglia, and Alzheimer's disease. Neurobiol Dis. 2010;37: 503–9.
136. De Keyser J, Zeinstra E, Frohman E. Are astrocytes central players in the pathophysiology of multiple sclerosis? Arch Neurol. 2003;60:132–6.
137. Dong Y, Benveniste E. Immune function of astrocytes. Glia. 2001;36:180–90.
138. Van Neerven S, Nemes A, Imholz P, Regen T, Denecke B, Johann S, Beyer C, Hanisch UK, Mey J. Inflammatory cytokine release of astrocytes in vitro is reduced by all-trans retinoic acid. J Neuroimmunol. 2010;229:169–79.
139. Chakrabarti M, McDonald A, Will Reed J, Moss M, Das B, Ray S. Mechanisms of natural and synthetic retinoids for inhibition of pathogenesis in Alzheimer's disease. J Alzheimers Dis. 2015;50(2): 335–52.
140. Wang R, Chen S, Liu Y, Diao S, Xue Y, You X, Park E, Liao F. All-trans-retinoic acid reduces BACE1 expression under inflammatory conditions via modulation of nuclear factor κB (NFκB) signaling. J Biol Chem. 2015;290(37):22532–42.
141. Patra R, Swarup D, Dwivedi S. Antioxidant effects of tocopherol, ascorbic acid and l-methionine on lead-induced oxidative stress to the liver, kidney and brain in rats. Toxicology. 2001;162:81–8.
142. Ramanathan K, Balakumar B, Panneerselvam C. Effects of ascorbic acid and alpha-tocopherol on

arsenic-induced oxidative stress. Hum Exp Toxicol. 2002;21:675–80.
143. Peacock J, Folsom A, Knopman D, Mosley T, Goff Jr D, Szklo M. Dietary antioxidant intake and cognitive performance in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study investigators. Public Health Nutr. 2000;3(3):337–43.
144. Yonghua L, Shumei L, Yigand M, Ning L, Yu Z. Effects of vitamins E and C combined with β-carotene on cognitive function in the elderly. Exp Ther Med. 2015;9(4):1489–93.
145. Masaki KH, Losonczy KG, Izmirlian G, Foley DJ, Ross GW, Petrovitch H, Havlik R, White LR. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. Neurology. 2000;54(6):1265–72.
146. Bowman G, Dodge H, Frei B, Calabrese C, Oken B, Kaye J, Quinn J. Ascorbic acid and rates of cognitive decline in Alzheimer's disease. J Alzheimers Dis. 2009;16:93–8.
147. Mosdol A, Erens B, Brunner E. Estimated prevalence and predictors of vitamin C deficiency within UK's low-income population. J Public Health Dent. 2008;30:456–60.
148. Charlton K, Rabinowitz T, Geffen L, Dhansay M. Lowered plasma vitamin C, but not vitamin E, concentrations in dementia patients. J Nutr Health Aging. 2004;8:99–107.
149. Riviere S, Birlouez-Aragon I, Nourhashemi F, Vellas B. Low plasma vitamin C in alzheimer patients despite an adequate diet. Int J Geriatr Psychopharmacol. 1998;13:749–54.
150. Murakami K, Murata N, Ozawa Y, Kinoshita N, Irie K, Shirasawa T, Shimizu T. Vitamin C restores behavioral deficits and amyloid- β oligomerization without affecting plaque formation in a mouse model of alzheimer's disease. J Alzheimers Dis. 2011; 26:7–18.
151. Kennard J, Harrison F. Intravenous ascorbate improves spatial memory in middle-aged APP/PSEN1 and wild type mice. Behav Brain Res. 2014;264:34–42.
152. Engelhart M, Geerlings M, Ruitenberg A, van Swieten J, Hofman A, Witteman J, Breteler M. Dietary intake of antioxidants and risk of Alzheimer disease. JAMA. 2002;287:3223–9.
153. Daly R, Gagnon C, Lu Z, Magliano D, Dunstan D, Sikaris K, Zimmet P, Ebeling P, Shaw J. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clin Endocrinol. 2012;77:26–35.
154. Garcion E, Sindji L, Leblondel G, Brachet P, Darcy F. 1,25-Dihydroxyvitamin D3 regulates the synthesis of gamma-glutamyltranspeptidase and glutathione levels in rat primary astrocytes. J Neurochem. 1999;73:859–66.
155. Eyles D, Smith S, Kinobe R, Hewison M, McGrath J. Distribution of the vitamin D receptor and 1[alpha]-hydroxylase in human brain. J Chem Neuroanat. 2005;29:21–30.
156. Langub MC, Herman JP, Malluche HH, Koszewski NJ. Evidence of functional vitamin D receptors in rat hippocampus. Neuroscience. 2001;104:49–56.
157. Balion C, Griffith L, Strifler L, Henderson M, Patterson C, Heckman G, Llewellyn D, Raina P. Vitamin D, cognition, and dementia: a systematic review and meta-analysis. Neurology. 2012;79:1397–405.
158. Buell J, Dawson-Hughes B. Vitamin D and neurocognitive dysfunction: preventing "D" ecline? Mol Asp Med. 2008;29:415–22.
159. Littlejohns T, Henley W, Lang I, Annweiler C, Beauchet O, Chaves P, Fried L, Kestenbaum B, Kuller L, Langa K, Lopez O, Kos K, Soni M, Llewellyn D. Vitamin D and the risk of dementia and Alzheimer disease. Neurology. 2014;83:920–8.
160. Taghizadeh M, Djazayery A, Salami M, Eshraghian MR, Zavareh SA. VitaminD-free regimen intensifies the spatial learning deficit in Alzheimer' s disease. Int J Neurol. 2011;121:16–24.
161. Annweiler C, Rolland Y, Schott AM, Blain H, Vellas B, Beauchet O. Serum vitamin D deficiency as a predictor of incident non-Alzheimer dementias: a 7-year longitudinal study. Dement Geriatr Cogn Disord. 2011;32:273–8.
162. Annweiler C, Maby E, Meyerber M, Beauchet O. Hypovitaminosis D and executive dysfunction in older adults with memory complaint: a memory clinic-based study. Dement Geriatr Cogn Disord. 2014;37:286–93.
163. Ito S, Ohtsuki S, Nezu Y, Koitabashi Y, Murata S, Terasaki T. 1alpha,25-Dihydroxyvitamin D3 enhances cerebral clearance of human amyloid-beta peptide(1–40) from mouse brain across the blood–brain barrier. Fluids Barriers CNS. 2011;8:20.
164. Masoumi A, Goldenson B, Set G. 1α25-dihydroxyvitamin D3 interacts with curcuminoids to stimulate amyloid-β clearance by macrophages of Alzheimer's disease patients. J Alzheimer's Dis. 2009;17:703–17.
165. Kim D, Nguyen M, Dobbin M, Fischer A, Sananbenesi F, Rodgers J, Delalle I, Baur J, Sui G, Armour S, Puigserver P, Sinclair D, Tsai L. SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. EMBO J. 2007;26(13):3169–79.
166. Venturini C, Merlo S, Souto A, Fernandes M, Gomez R, Rhoden C. Resveratrol and red wine function as antioxidants in the nervous system without cellular proliferative effects during experimental diabetes. Oxidative Med Cell Longev. 2010;3:434–41.
167. Ye J, Liu Z, Wei J, Lu L, Huang Y, Luo L. Protective effect of SIRT1 on toxicity of microglial-derived factors induced by LPS to PC12 cells via the p53-caspase-3-dependent apoptotic pathway. Neurosci Lett. 2013;553:72–7.
168. Carrizzo A, Forte M, Damato A, Trimarco V, Salzano F, Bartolo M, Maciag A, Puca A, Vecchione

C. Antioxidant effects of resveratrol in cardiovascular, cerebral and metabolic diseases. Food Chem Toxicol. 2013;61:215–26.
169. Wang H, Yang Y, Qian J, Zhang Q, Xu H, Li J. Resveratrol in cardiovascular disease: what is known from current research? Heart Fail. Review. 2012;17:437–48.
170. Liu B, Hong J. Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. J Pharmacol Exp Ther. 2003;304:1–7.
171. Capiralla H, Vingtdeux V, Zhao H, Sankowski R, Al-Abed Y, Davies P, Marambaud P. Resveratrol mitigates lipopolysaccharide- and Aβ-mediated microglial inflammation by inhibiting the TLR4/NF-κB/STAT signaling cascade. J Neurochem. 2012;120(3):461–72.
172. Karuppagounder S, Pinto J, Xu H, Chen H, Beal M, Gibson G. Dietary supplementation with resveratrol reduces plaque pathology in a transgenic mouse model of Alzheimer's disease. Neurochem Int. 2009;28:1393–405.
173. Marambaud P, Zhao H, Davies P. Resveratrol promotes clearance of Alzheimer's disease amyloid-b peptides. J Biol Chem. 2005;280:37377–82.
174. Ono K, Naiki H, Yamada M. The development of preventives and therapeutics for Alzheimer's disease that inhibit the formation of beta-amyloid fibrils (fAbeta), as well as destabilize preformed fAbeta. Curr Pharm Des. 2006;12:4357–75.
175. Vingtdeux V, Giliberto L, Zhao H, Chandakkar P, Wu Q, Simon J. AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-beta peptide metabolism. J Biol Chem. 2010;285:9100–13.
176. Porquet D, Casadesús G, Bayod S, Vicente A, Canudas A, Vilaplana J. Dietary resveratrol prevents Alzheimer's markers and increases life span in SAMP8. Age (Dordr). 2013;35:1851–65.

Cognitive Impairment/Dementia in Chronic Renal Disease

36

Luis Augusto Juncos, Kiran Chandrashekar, and Luis Isaías Juncos

Abstract

Chronic kidney disease (CKD) is a worldwide public health problem that is increasing in prevalence, especially in the elderly. CKD and/or end stage renal disease (ESRD) patients have numerous comorbidities that increase the risk of cognitive impairment and dementia (CI/D). In fact, almost every stage of CKD is associated with an increased risk of CI/D; the risk increases as the severity of CKD increases. The mechanisms responsible for this increased risk are largely due to the accelerated vascular disease of CKD/ESRD that leads to an increase in vascular dementia. However, other factors such as increased risk of thrombotic and hemorrhagic strokes, uremic toxins, and suboptimal aspects of dialytic therapies also contribute to the development and progression of CI/D. The importance of CI/D in CKD/ESRD patients is that it impairs quality of life, and carries with it a greater risk of hospitalization, disability, dialysis withdrawal, and mortality. Despite the magnitude of the problem, CI/D is largely under-recognized in the renal patient, and optimal management strategies are unknown. The aim of this chapter is to provide an overview of the epidemiology, pathogenesis/pathophysiology, diagnostic approaches, and therapeutic considerations for CI/D in patients with CKD/ESRD.

Keywords

Dialysis dementia • Vascular dementia in chronic kidney disease • White matter lesions and kidney disease • Uremic encephalopathy • Cognitive impairment in chronic kidney disease • Dialysis-induced mental disequilibrium

L.A. Juncos
Department of Medicine/Division of Nephrology, University of Mississippi Medical Center, Jackson, MS, USA

Departments of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, USA

K. Chandrashekar
Department of Medicine/Division of Nephrology, University of Mississippi Medical Center, Jackson, MS, USA

L.I. Juncos, MD, PhD (✉)
Fundación J Robert Cade, Pedro de Oñate 253, Córdoba X5003DDZ, Argentina
e-mail: luis.juncos@jrobertcade.org.ar

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_36

Introduction

The kidneys play a vital role in the metabolism and excretion of endogenous and exogenous hormones and toxins, as well as in regulating blood pressure and fluid/electrolyte homeostasis, thereby facilitating appropriate perfusion and function of distant organs such as the lungs, liver, heart, gut, and brain. Consequently, dysfunction of the kidneys results in an abnormal internal milieu (e.g., increased nitrogenous waste products, oxidative stress, and inflammation), which together with the abnormal fluctuations in fluid and electrolyte balance, and the altered vascular function and tissue perfusion, leads to progressive dysfunction of the distant organs [1, 2]. In this respect, the brain may be particularly susceptible not only because of its sensitivity to changes in the internal milieu, but also because renal injury alters the concentrations of neurotransmitters and disrupts the blood–brain barrier [2–4]. Indeed, various neurological disorders are frequently associated with acute and/or chronic renal injury [5–9] (Table 36.1). Of these, cognitive impairment and dementia (CI/D) are now recognized to be of fundamental importance because of the high prevalence in patients with chronic kidney disease (CKD), and also because of the negative impact that they have on patient compliance and outcomes, as well as healthcare costs [10–13] (Table 36.2). Moreover, while the therapeutic approaches used during renal disease (i.e., hemodialysis) improve certain aspects of CKD-induced encephalopathies, they can also exert negative effects on the central nervous system in both the short and long term [14–16]. All in all, the concurrent presence of CKD and CI/D not only affects the patient's quality of life, but also their long-term prognosis [17–19]. Indeed, patients with combined CKD and CI/D have at least a three times greater risk of mortality compared with an age-matched CKD population without CI/D [17, 20, 21]. Thus a detailed understanding of the mechanisms contributing to the CKD-associated CI/D is needed to optimally manage these patients. This is becoming a growing burden on healthcare systems because of our aging population and the associated increase in patients with CKD and/or CI/D. This chapter reviews the associations and potential pathophysiologic mechanisms between CKD (including end-stage renal disease (ESRD)) and CI/D,

Table 36.2 Complications and outcomes of cognitive dysfunction/dementia in patients with renal disease

Increased risk of hospitalizations
Prolongs hospitalizations
Leads to lower levels of compliance
Higher incidence of cerebral vascular disease
Increased costs of care
Increased risk of death
Decreased average life span after beginning dialysis
Impairs the ability the ability to weigh risks vs benefits of initiating dialysis or withdrawing from dialysis once it is initiated

Table 36.1 Neurologic disorders and complications in patients with CKD and ESRD

Central nervous system	Peripheral nervous system
Uremic encephalopathy	Uremic polyneuropathy
Seizures	Autonomic and cranial neuropathy
Cognitive Impairment/dementia/delirium	Mononeuropathies
Cerebrovascular disease	Carpal tunnel syndrome
Intracerebral hemorrhage	Ischemic monomelic neuropathy
Cerebral micro-bleeds	Compressive neuropathies
Cerebral infarction	*Combination pathologies*
Silent cerebral infarction	Restless leg syndrome*
White matter lesions	
Dialysis encephalopathy/dementia	
Dialysis-induced abnormalities	
Dialysis disequilibrium syndrome	
Cognitive dysfunction induced by rapid fluid, electrolyte and blood pressure shifts	

CKD chronic kidney disease, *ESRD* end-stage renal disease
*Unknown etiology and pathophysiology

as well as the diagnostic and therapeutic challenges and questions that have arisen because of the high prevalence of this association.

Epidemiology

While the presence of CI/D in patients with CKD or ESRD is well recognized, its prevalence has been difficult to accurately establish for several reasons. First, relatively few large studies examine their connection, and the available studies vary greatly in their estimation of disease prevalence [22–25]. Moreover, the cohorts studied may not be representative of the general CKD/ESRD populations, and CI/D may be under-diagnosed in cohorts that rely on diagnostic codes and billing data [17, 26]. Second, CI/D is under-diagnosed in these patients because nephrologists and dialysis staff are not adequately trained to identify mild CI/D. Furthermore, when signs and symptoms are spotted, they are often ascribed to normal aging, other medical problems, or side effects of therapy. Thus, only advanced/obvious dementia is typically diagnosed. Third, cognitive assessments are typically achieved using short screening tests [e.g., MiniMental State Examination (MMSE), Modified Mini-Mental State Exam (3MS)] that have limited sensitivity in ESRD patients [11, 27]. In addition, the timing of testing may also introduce substantial variability in the results [11, 28, 29]. Finally, cognitive impairment and dementia are not listed as comorbid conditions in the CMS 2728 form (ESRD Medical Evidence Report), and cognitive assessments are not required at initiation of dialysis, nor during any of the routine assessments of dialysis patients [30]. Together, these limitations raise the possibility that CI/D is being grossly underdiagnosed, and thus its prevalence is underestimated. This supposition is supported by the fact that the prevalence of dementia is only 7% in the US Renal Data System (USRDS), and 4% in the Dialysis Outcomes and Practice Patterns Study, which represent only one-fifth of probable dementia cases, as determined by more rigorous neuropsychological testing [24, 31, 32]. Similarly, Sehgal et al. [25] found that only 15% of the cognitively impaired patients (MMSE score <24) had a medical record diagnosis of cognitive problems. These studies suggest that there are a vast number of patients with CKD and ESRD who are afflicted with CI/D.

Prevalence of CI/D in CKD

CKD is a term that encompasses a wide spectrum of renal dysfunction ranging from mild (which is usually only diagnosed by blood and urine testing) to ESRD, in which kidney function is impaired to such an extent that the patient requires renal replacement therapy in order to prevent potentially fatal complications from the retention of metabolic waste products, salt, and water. This continuum of CKD has been classified into a five-stage system based on the estimated glomerular filtration rate (eGFR) to help assess the severity of the CKD and its potential for progression [11] (Table 36.3). Overall, CKD has increased in prevalence to the point that it is now recognized as a worldwide public health problem [33]. More than 10% of the adult population currently has some degree of CKD [34], and its prevalence increases considerably with advancing age; 20 to 25% in people aged 65 to 74 to nearly 50% in those aged 75 and over [11, 23, 35].

The prevalence of CI/D, like CKD, also increases with age, affecting 10% of the population over 65 [36–38]. Interestingly, CI/D has many similar risk factors to CKD including obesity, diabetes mellitus, hypertension, and dyslipidemia [39–41], suggesting that similar mechanisms may be contributing to its development. Thus, it is not surprising that these two diseases occur concomitantly in many patients, particularly the elderly. However, the prevalence of CI/D in CKD patients far outpaces its prevalence in the non-CKD population [42, 43], suggesting that CKD itself exacerbates the risk of CI/D [10, 44]. Indeed, CKD has been shown to be an independent risk factor for the development and progression of CI/D by both

Table 36.3 Classification of chronic kidney disease

Stage 1: eGFR >90 ml/min with albuminuria, hematuria or abnormal kidney imaging
Stage 2: eGFR 60–90 ml/min
Stage 3: eGFR 30–59 ml/min
Stage 3A: eGFR 45–59 ml/min
Stage 3B: eGFR 30–44 ml/min
Stage 4: eGFR 15–29 ml/min
Stage 5: eGFR <15 ml/min
ESRD: Patient requires renal replacement therapy or transplantation

Abbreviation: *eGFR* estimated glomerular filtration rate, *ESRD* end-stage renal disease

cross-sectional studies and longitudinal studies [22, 24, 39, 40, 44]. Even moderate CKD increases the risk of CI/D by nearly 40% after adjusting for confounders [22, 35, 45, 46]. Moreover, the type of dementia induced by ageing alone compared to ageing plus CKD is different. Alzheimer's Disease is the most common form of dementia in the elderly [47], whereas vascular dementia accounts for the increased prevalence of CI/D in CKD patients [48]. One explanation for this may be the greater prevalence of both cardiovascular and cerebrovascular risk factors among CKD patients, predisposing them to develop cerebrovascular diseases and consequently CI/D [32, 49, 50].

Patients with CKD of any stage have a greater risk of developing CI/D than the general population [22, 51, 52]. As might be expected, the risk of CI/D increases markedly with worsening kidney function. For every 10 ml/min/1.73m^2 drop in eGFR, the risk of developing CI/D increases by 11% [35, 53], and the risk of declining memory, language skills, executive functioning, and global cognition increases by 15–25% [45]. While most studies have found that CKD is associated with increased risks of CI/D, and that the more advanced the CKD the greater the risk, there are a few studies that have not confirmed this connection. Neither the 3C study [48], nor the study by Slinin et al. [54] found a consistent correlation between CKD and dementia. However, the patient cohort in these studies tended to be healthier, had a relatively low incidence of CKD at baseline, and had fewer cardiovascular risk factors, complicating direct comparisons with other studies. Overall, the bulk of the evidence indicates that CKD is associated with an increased risk for the development of CI/D and accelerated decline in cognitive function.

Prevalence of CI/D in ESRD

ESRD, the last stage of CKD, means that the kidneys are functioning below 15% of their normal function and can no longer support a person's day-to-day life. In the US, more than 300,000 people are diagnosed with ESRD, and its prevalence is increasing worldwide [33, 55]. As previously mentioned, the rate of CI/D increases together with the severity of kidney disease, thus patients with ESRD have the highest prevalence. In ESRD, the prevalence rate of CI/D ranges between 16 and 38%. This is nearly 3 times greater than in the age-matched general population [22, 24, 25, 56, 57]. In fact, some studies that used more robust diagnostic criteria report even higher levels, up to 87% [24]. The variability in the reported prevalence may in part be due to the patient population studied, but more importantly to study design and the large variability in diagnostic testing and criteria used in the distinct studies. For instance, early studies reported moderate rates of CI/D that probably underestimated the true prevalence because they often excluded older and sicker patients from analysis, and used screening tests of limited sensitivity. Later studies tended to be more inclusive and used more thorough neuropsychological testing, thus increasing the diagnostic sensitivity [39]. Not surprisingly, they found the prevalence to be much higher. The cross-sectional analysis by Murray et al. highlights not only the high prevalence of CI/D in hemodialysis patients, but also the marked disparity between the documented history of CI/D (only 2.9%) and its presence upon more thorough neuropsychological testing; 12.7% had normal cognitive function, whereas 13.9%, 36.1%, and 37.3% had mild, moderate, and severe cognitive impairment respectively [24]. Thus overall, it is clear that the prevalence of CI/D is much higher in the CKD/ESRD population than in the general population, even after adjusting for age and other common risk factors. This implies that renal disease per se is a strong risk factor for the development of CI/D. It remains to be determined how much of this increase in risk is due to the same mechanisms that catalyze the accelerated vascular disease present during renal disease, and how much is due to other mechanisms, including those brought on by the treatment strategies used in CKD/ESRD.

Pathogenesis and Pathophysiology

Patients with CKD/ESRD develop CI/D in part via similar mechanisms as the general population, with the caveat that while Alzheimer's Disease is the most common form of dementia in the general population, the increased incidence of CI/D in CKD/ESRD patients is mainly due to an increase in vascular dementia; there is little if any change in the

prevalence of Alzheimer's Disease [46, 58]. Moreover, there are many additional factors that contribute to and/or exacerbate the vascular and neurologic injury in patients with renal dysfunction (Table 36.4). In general, it is practical to divide the mechanisms/factors that cause CI/D in CKD/ESRD into the following. First, there is a marked increase in the occurrence of cerebrovascular disease due to the accelerated vascular disease that is a characteristic of CKD/ESRD. Moreover, the brain is highly susceptible to microvascular disease, including subclinical cerebrovascular lesions, and its other manifestations (e.g., white matter lesions, micro bleeds). Finally, there are numerous additional mechanisms that render CKD/ESRD patients very susceptible to brain injury and CI/D. These may be related to the renal dysfunction itself (non-traditional vascular and neuropsychological risk factors), or to its therapy. Table 36.4 provides a partial list of the various factors that may contribute to the development and progression of CI/D in CKD/ESRD patients. All of these factors interact to a varying degree in individual patients, causing vascular injury, endothelial dysfunction, and/or direct neurotoxicity, thereby resulting in CI/D of variable manifestations and severity (Fig. 36.1).

Table 36.4 Pathogenic factors that may contribute to CKD/ESRD-associated dementia

Cardiovascular risk factors		Cerebrovascular neuro-psychological	Treatment-related
Traditional	Non-traditional/uremic		
Older age Hypertension Diabetes Mellitus Dyslipidemia Albuminuria Sex Race Educational status Cardiovascular disease Smoking Atrial fibrillation	Uremic factors Volume overload Hyperhomocysteinemia Hyperparathyroidism Elevated FGF-23 Low vitamin D levels Anemia Hypercoagulation Inflammation Oxidative stress Malnutrition Frailty	Cerebrovascular Stroke Silent stroke Lacunar infarcts Microembolism Microbleeds White matter lesions Cortical atrophy *Psychological* Depression	Polypharmacy Aluminum Dialysis-related Modality? Hemodialysis initiation Hemodynamic instability Fluid and solute shifts

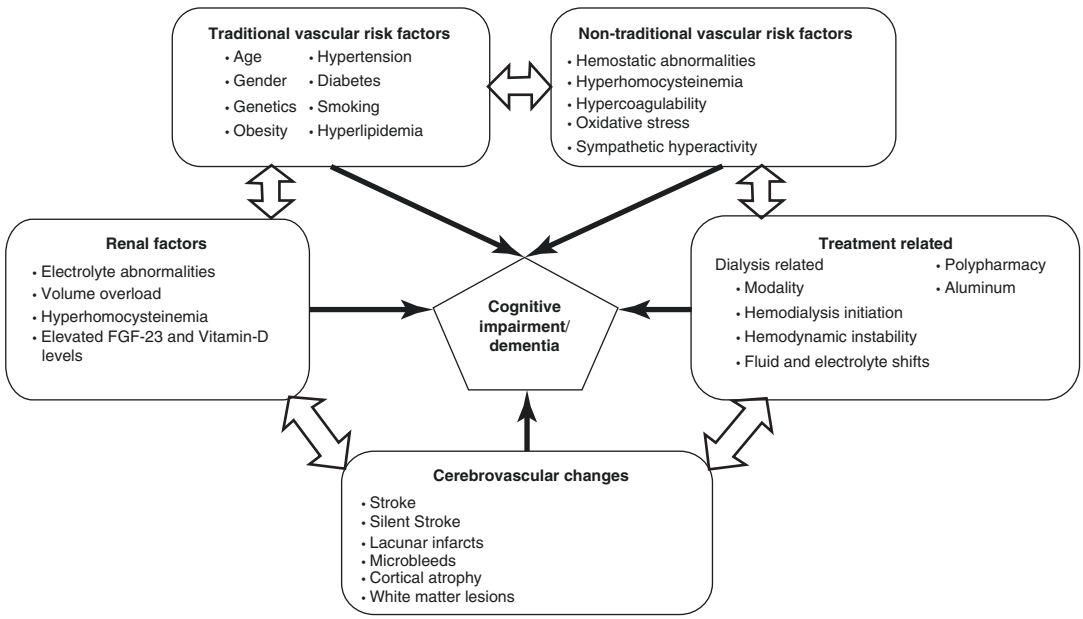


Fig. 36.1 Pathogenesis of cognitive impairment in chronic kidney disease

Cerebrovascular Disease

Thromboembolic Disease Patients with CKD/ESRD frequently have accelerated vascular disease, which is why most of those with advanced disease also have advanced *vasculopathy*, and have high rates of hypertension (80%), diabetes (60%), vascular endothelial dysfunction, carotid atherosclerosis, and cardiovascular events including stroke [19]. Indeed, the prevalence of stroke in the USRDS CKD and ESRD populations are 2.5 and 4 times greater respectively than in the general Medicare population [19]. Moreover, silent strokes occur in nearly 1/3rd of the older population or 5 times more often than symptomatic strokes [59, 60]. They are associated with increased risk of subsequent clinically evident stroke, cognitive and physical decline, and incident dementia [59–62]. Those who develop symptoms have more severe symptoms and worse outcomes; they have a higher morbidity and mortality [63], increased tendency to develop new strokes [64], and suffer 6 to 9 times more strokes when hospitalized than non-CKD/ESRD patients [65]. Atherosclerosis in patients with CKD/ESRD is generally more diffuse and distally located than in the general population, probably because of a combination of traditional atherogenic risk factors (e.g., male gender, age, diabetes mellitus, hypertension, dyslipidemia, and smoking) and factors more specifically related to renal failure and its treatment that impact both cerebrovascular and microvascular disease (discussed below).

The increase in thromboembolic strokes in CKD/ESRD is also related to the high frequency of dilated cardiomyopathy and arrhythmias [66, 67]. In this respect, atrial fibrillation deserves special attention, as it is the most common cardiac dysrhythmia and is associated with increased cardiovascular morbidity and mortality [68, 69]. Patients with advanced CKD and ESRD are especially prone to it because of (a) structural heart disease including left ventricular hypertrophy, coronary artery disease, and degenerative valvular disease (as a result of accelerated calcifications) [69, 70], (b) increased activity of the sympathetic nervous system and the renin angiotensin system, and (c) rapid shifts of fluid and electrolytes [68, 69, 71, 72]. Its prevalence is 10–20 times higher than in the general population; it is 7% and 13% in peritoneal dialysis patients and hemodialysis patients respectively, according to USRDS data [73]. Smaller studies using more sensitive methods suggest much higher rates [74, 75]. The Monitoring in Dialysis Study recorded patients' heart rhythms continuously for up to 30 days, and found that 44% of patients experienced at least one episode of sustained atrial fibrillation (>6 min duration), while 85% developed some period of atrial fibrillation during the 30-day follow-up [76]. Thus, while the risks of atrial fibrillation are well recognized, its impact on CI/D in CKD/ESRD is poorly described and probably grossly under-recognized.

Microvascular Disease

The microvasculature of the brain is highly susceptible to microvascular injury; thus the same mechanisms implicated in the progression of CKD may also affect brain function and morphology, and hence cognition. This parallel injury model is appealing because of the similarities in the microvasculature of the brain and the kidneys, in that both organs have low resistance and are exposed to high-volume blood flow, which make them highly susceptible to microvascular disease (e.g., hypertension, diabetes), which in turn may contribute to dementia. Indeed, there appears to be a positive correlation between hemodynamic impairment and cognitive impairment, suggesting that microvascular damage contributes to CI/D [32, 77].

Microvascular disease may manifest itself as CKD in the kidney, and as white matter lesions, silent brain infarcts, and micro-bleeds in the brain [60, 78]. White matter lesions are caused by the accumulation of degenerating cells. Their prevalence is greatly increased in patients with CKD compared to controls (33% vs 6%) [78, 79]. Indeed, they are independently associated with CKD and albuminuria even after adjusting for hypertension and diabetes mellitus [80], and are associated with deficits in the cognitive domains of executive function and processing speed. Importantly, they are predictors of stroke, dementia, and death [81].

Intracerebral Hemorrhage, Subarachnoid Hemorrhage, and Subdural Hematomas

Advanced CKD/ESRD patients also have a large increase in hemorrhagic strokes including intracerebral, subarachnoid, or subdural hemorrhage, which carry a high morbidity and mortality that reaches up to 60% incidence [82–84]. This increased susceptibility for intracerebral and subarachnoid hemorrhage is multifactorial. First, there are patient-related factors such as hypertension or the presence of cerebral vascular malformations (e.g., saccular aneurysms and dolichoectasia) in certain families with polycystic kidney disease [85, 86]. Second, uremia increases bleeding tendency by altering platelet function and platelet–vessel wall interaction [87–89]. The final and most important risk factors are the use of anticoagulation or platelet antiaggregants [88–92]. These agents, together with hypertension, head trauma, rapid ultrafiltration, and the use of hypertonic dialysate, also increase the risk of acute and chronic subdural hematoma [9]. It is important to note the role of hemodialysis in this risk. On the one hand, uremic bleeding may be largely corrected by dialysis. However, systemic anticoagulation is frequently necessary during hemodialysis to prevent circuit clotting, and thus the hemodialysis procedure is associated with a higher incidence of intracerebral and subarachnoid hemorrhage [93–95], as well as subdural hematomas, all of which impair cognition.

Non-traditional Vascular Factors and Other Factors

CKD/ESRD brings about several changes in the non-traditional vascular factors.This includes retention of some (such as uremic toxins), or abnormal production of others (such as increased oxidant stress and inflammation and decreased erythropoietin), leading to a dysregulation of the internal milieu (e.g., acid base and electrolytes). It is thus easy to imagine how the subsequent metabolic quagmire post-CKD/ESRD can facilitate increased prevalence of CI/D. For instance, retention of uremic factors leads to derangements in glutamine, glycine, aromatic and branched-chain amino acids, and subsequently an imbalance of gamma-aminobutyric acid, serotonin, and dopamine neurotransmitters [40]. The metabolism of these neurotransmitters is additionally impaired by the presence of secondary hyperparathyroidism via abnormally increased cellular uptake of calcium. Moreover, increases in oxidative stress and inflammation, together with hypercoagulability, can cause endothelial dysfunction with subsequent vascular injury including leukoaraiosis, silent strokes, and micro-bleeds or even severe, neurodegenerative stroke [39, 96]. Finally, the episodic electrolyte abnormalities can contribute to CI/D by virtue of their direct effects on cellular function, or via their correction. For instance, an ESRD patient may develop episodic hyponatremia because of bouts of excessive consumption of hypotonic fluids that are then inadvertently corrected too rapidly by hemodialysis, which may result in progressive injury.

Uremic toxins Declining renal function leads to accumulation of a diverse group of uremic retention products including (a) small water-soluble, non-protein-bound compounds (e.g., urea and related carbamylation products, guanidines, uric acid), (b) small, lipid-soluble and/or protein-bound compounds (e.g., homocysteine, indols, phenols), and (c) the middle molecules (parathyroid hormone, advanced glycation end products, β2 microglobulin, FGF-23). These factors individually and/or in combination can effect a variety of cell types that in turn can cause organ dysfunction including cognitive dysfunction [97]. For instance, accrual of guanidino compounds activate N-methyl-d-aspartate receptors while concomitantly inhibiting gamma-aminobutyric acid receptors, causing neurotoxicity [98]. Guanidino compounds can also elevate free levels of homocysteine, which promotes endothelial/vascular injury and thus ultimately contributes to the development of CI/D [99]. *Homocysteine* levels are two- to four-fold elevated in CKD compared to patients without CKD. This exacerbates atherosclerosis by increasing the proliferation of vascular smooth muscle cells, and disrupts vessel wall-related anticoagulant functions, resulting in enhanced thrombogenicity [100, 101]. These vascular effects, when present in cerebral vessels

may contribute to cognitive dysfunction. Indeed, hyperhomocysteinemia is a strong independent factor for the development and progression of CI/D [99]. Other protein-bound uremic factors such as p-cresyl sulfate also contribute to the vascular injury in part by inducing oxidative stress and inflammation, which results in tubular injury, and endothelial injury (increased vascular permeability). In addition, it decreases Klotho, a transmembrane protein that when deficient (as occurs in CKD) is involved in premature aging syndromes and cell senescence [102, 103]. Experimental studies have shown that Klotho-deficient mice have impaired cognition [104], raising the possibility that it may be implicated in CKD/ESRD-induced CI/D. We have enumerated only a few important uremic toxins; indeed, a large variety of other uremic toxins have also been implicated in CKD/ESRD-induced CI/D, but it is outside the scope of this chapter to list them all.

Oxidative stress and inflammation There are numerous mechanisms by which *oxidative stress* is increased in CKD/ESRD [105–107]. It fosters endothelial dysfunction, vascular injury, and direct neurotoxicity, which together may have a causal role for the development of CI/D [44, 108, 109]. One suggested mechanism is via uremia-induced oxidative stress, which upregulates the NMDA receptors, thus inducing neuronal nitric oxide synthase. The ensuing increase in nitric oxide combines with the superoxide, resulting in the formation of peroxynitrite that causes protein nitration leading to structural and functional abnormalities in the brain, and ultimately cognitive dysfunction [110, 111]. In a recent study, experimental CKD induced by subtotal nephrectomy increased oxidative stress levels, and led to cognitive dysfunction. Histological evaluation revealed that the CKD group had increased oxidation in the hippocampal neurons (an important center for learning and memory function) and exacerbated levels of neuronal apoptosis. However, administration of antioxidants attenuated the cognitive dysfunction, suggesting a link between oxidative stress and cognitive decline in these animals [112]. Similarly, *inflammatory* mediators have been associated with dementia in the general population [113]. Moreover, dialysis patients have elevated levels of pro-inflammatory mediators such as prostaglandin D2-synthase, and C-reactive protein, which has been found to induce neuronal apoptosis [114, 115]. Overall, oxidative stress and inflammation work in tandem, inducing endothelial dysfunction and vascular injury causing accelerated atherosclerosis and thus increasing the risk of CI/D in these patients.

Treatment-Related Factors

Aluminum toxicity Aluminum toxicity was a major cause of dialysis dementia in the early days of dialysis [116]. The exact mechanism through which aluminum exerts its neurotoxicity is still unclear. It has been suggested that aluminum increases oxidative stress and inflammatory cytokines. This incites apoptotic cell death, thereby causing neurotoxicity. In the early dialysis set-ups, aluminum contamination in the dialysate fluid, and also the use of phosphate binders with aluminum, had contributed to this toxicity [116]. However, since then, with the advent of more modern water filtering techniques and decreased use of aluminum containing phosphate binders, the prevalence of aluminum related toxicity in dialysis patients has been gradually reducing. Indeed, the Dialysis Outcomes and Practice Study could only detect a prevalence of 0.6–1% of all dialysis patients [31]. Despite concerns about the cost-effectiveness of routine aluminum testing in the United States, the K/DOQI guidelines recommend testing serum aluminum levels at least once a year in all hemodialysis patients, and once every 3 months in patients receiving aluminum containing medications [117].

Dialysis-induced causes While modern renal replacement therapies have numerous benefits and much improved safety profile (e.g., much more accurate ultrafiltration rates and much purer dialysis solutions), the dialysis process itself is still associated with many complications that negatively impact the dialysis patient. For instance, excessive ultrafiltration or the rapid compartmental shifts in fluids, electrolytes, and metabolic products can cause hemodynamic instability, with subsequent alterations in cerebral hemodynamics

and metabolism [32, 118]. Indeed, studies have found evidence of substantially lowered cerebral blood flow velocity, perfusion, and decreased oxygen metabolism after dialysis [118–120]. These changes precipitate the production of cytokines and consequent inflammation, which exacerbates cerebral vascular injury. These acute factors can result in delirium and acute cognitive impairment, which can progress or contribute to chronic dementia. Moreover, hemodynamic instability is often accelerated by the presence of other systemic inflammatory entities such as sepsis and cardiac dysfunction, which may exacerbate the local hypoxemia and thus CI/D. Indeed, dialysis has been linked to cerebrovascular accidents, with the ensuing development and progression of acute and chronic cognitive dysfunction, particularly in the elderly patient [32, 121]. The immediate post-dialytic period appears to be the time when the patients are most susceptible to cerebral ischemia. Indeed, Toyoda et al. found that nearly one third of new onset acute strokes occurred in the first half hour post-dialysis [15]. Interestingly, they reported a greater incidence of infarcts in the vertebrobasilar territory, a region that is more susceptible to hypovolemia, suggesting that hemodynamic changes during the dialytic process may play a central role in instigating the ischemic events.

Diagnosis

Mild to moderate CI/D exhibits subtle signs and symptoms which can easily be missed by an untrained professional. Indeed, most nephrologists and dialysis staff are not trained to recognize cognitive changes in CKD/ESRD patients. However, the presence of CI/D directly impacts their prognosis — this significantly impacts the therapy, overall duration of hospitalization, and health care costs — thus it is crucial for health care professionals to recognize these signs and symptoms [11, 32]. Specifically, CI/D decreases quality of life and increases mortality of all patients, and even more so CKD/ESRD patients. It also interferes with their ability to comprehend and follow the dietary, fluid, and medication regimens, which is of crucial importance because of their impact on outcomes (the average ESRD patient takes 19 pills/day) [122]. Finally, CI/D greatly interferes with their ability to make complex decisions including those related to initiation of dialysis and vascular access placement [11, 32, 123]. Thus, periodic screening is needed to identify patients with CI/D so that proper steps can be taken to improve their clinical care.

Initial Evaluation of CI/D in CKD/ESRD: Ruling Out Depression and Delirium

The first step in evaluating these patients is to elicit a good history when possible. A proper history should optimally include the family/caregivers in addition to the patient, due to the inherent nature of the cognitive defect and also since the caregivers will be the first to notice any overt changes in the patient's cognitive symptoms. The history should include a complete physical examination looking for signs of neurodegenerative diseases such as Parkinson's, tardive dyskinesia, and others, as they can mimic early signs of CI/D [11]. Despite the importance of the history, it is important to recognize that it lacks sensitivity and thus one must have a low threshold to perform additional screening or testing.

The initial evaluation must also assess for the presence of other conditions that can have similar signs and symptoms to those of CI/D, in particular depression and delirium. Indeed, depression is the most common neuropsychiatric disease observed in ESRD patients [13, 124]. The early signs and symptoms of depression are usually indistinguishable from those of early CI/D. It must be ruled out as a reason by performing depression screening tests such as the Beck depression inventory, the five-item Geriatric Depression Scale, or a patient health questionnaire [125, 126]. Similarly, delirium is especially common in older patients who are on multiple medications (such as elderly CKD patients), and must be ruled out by performing the confusion assessment method (a highly sensitive and specific test to detect delirium) [127, 128].

Delirium can be precipitated by a number of causes such as electrolyte disorders (*hyponatremia*, hypercalcemia, or hypoglycemia), medications (opioids, anti-psychotics, anti-cholinergic and anti-histaminergic drugs) infections, alcohol and drug intoxication, or withdrawal among others [129] (Table 36.5). Once delirium is suspected, the nephrologist must assess the likelihood that the metabolic and chemical abnormalities (a measure of dialysis adequacy) may be contributing to the CI/D. Note that because uremia causes encephalopathy, it mistakenly gets blamed for delirium quite frequently. The physician who is following the patient in a longitudinal manner will be able to determine this with more accuracy.

Table 36.5 Differential diagnosis of neurological dysfunction in dialysis

Type	Disorder	Presenting signs and symptoms
Common causes	Delirium	Impaired memory and attention, disorganized thoughts
	Depression	Depressed mood, anhedonia, insomnia/ hypersomnia, psychomotor agitation or retardation
	Alzheimer's	Progressive memory loss, impaired executive function and abnormal visuospatial skills
Metabolic abnormalities	Hypoglycemia	Confusion, lack of concentration, headache, personality changes
	Hypophosphatemia	Ranges from mild paresthesia to severe mental status alterations.
	Hyponatremia	Ranges from mild headache and cramps to severe altered mental status changes and status epilepticus
	Hyperosmolarity	Ranges from mild disorientation to coma
	Hypercalcemia	Anxiety, altered sensorium, depression, insomnia
	Uremia	Depression, delusions, irritability, loss of memory and concentration, psychosis, coma.
	Trace element intoxication	Altered sensorium and confusion
	Aluminum	Altered mental status, seizures, myoclonus, impaired speech
	Vitamin deficiency: Thiamine Vitamin-C Vitamin-B12	Wernicke–Korsakoff, impaired memory, lack of coordination and paralysis Depression, increased arousal, decreased motivation Depression, mania, psychosis
Traumatic brain lesions	Subdural hematoma	Headache, confusion, vomiting, slurred speech, coma
Structural brain lesions	Normal pressure hydrocephalus	Non-specific neurological symptoms
Hypertension induced	Hypertensive encephalopathy	headache, altered consciousness, seizures and impaired vision
	Stroke	confusion, dementia, memory loss, impaired communication
Dialysis induced	Hemodynamic instability	Confusion
	Dialysis delirium syndrome	focal neurological defects, altered consciousness
Alcohol or drug withdrawal		Wide range from mild disorientation to seizures and coma

Screening Tests

After eliminating the reversible causes of cognitive impairment, the patient with suspected CI/D undergoes general screening to detect the presence and type of cognitive dysfunction. Since these patients are followed frequently in CKD/dialysis units, ideally they should undergo these screening tests during their clinic visit or just prior to the dialysis session. However, this may be difficult to accomplish in most renal clinics and dialysis units due to logistical reasons, so early referral to specialized services for evaluation using neuropsychological and neurophysiological tests is warranted.

When screening in CKD clinics and hemodialysis is possible, it normally starts with generalized neuropsychological tests. The ones most commonly used in renal clinics/hemodialysis units include the MMSE, the 3MS, Cognitive Capacity Screening Exam (CCSE) and the Kidney Disease Quality of Life Cognitive Function (KDQOL-CF) scale [11, 130, 131]. Their main drawbacks are that they have suboptimal sensitivity and specificity, and limited efficacy in detecting abnormalities in executive function, a very common finding in vascular dementia and ESRD [39, 131, 132]. Moreover, none of these screening tests (other than the KDQOL-CF) have been validated by clinical trials in CKD/ESRD patients with CI/D [11, 39, 131, 132]. It is also important to point out that CKD/ESRD clinics have a large variability of ethnic groups and tend to have a high proportion of patients with limited education, which further limits the effectiveness of the tests, as some rely on the patient's verbal or mathematical skills [133–135]. The results may also vary depending on when they are administered. For example, they are commonly run on ESRD patients during dialysis (for logistical reasons), yet this is the worst time to run them since cognitive function is at its lowest. One study which looked at the cognitive function 1, 24 and 67 h post-dialysis session in patients on hemodialysis versus peritoneal dialysis found that the hemodialysis patients fared worse after 67 h compared to 1 and 24 h, whereas the peritoneal dialysis patients fared similarly throughout the testing period [136]. Despite these drawbacks, they are valuable in identifying patients that may benefit from referral to a specialist. In our view, any CKD/ESRD patient with abnormal screening or in which the nephrologist has a high suspicion that CI/D may be present, should be referred to a specialist (Fig. 36.2).

Referral to a specialist will trigger a more comprehensive assessment, including in-depth testing to detect specific abnormalities in orientation, memory, intelligence, attention span, depression, and verbal skills (Fig. 36.2). These may include the Wechsler Memory Scale — Third Edition (WAIS-III) to assess personal, temporal, and spatial orientation, estimated verbal IQ test (NAART) to assess intelligence, Simple reaction time (SRT) to assess attention and vigilance, Boston naming test for verbal skills, and Wechsler Memory Scale — Third Edition (WMS-III) for memory [77, 121]. These tests are more sensitive and can thus detect mild to severe cognitive impairment [77, 137]. The specialist can additionally determine whether the patient merits neurophysiological techniques such as EEG and event-related potentials, or specialized imaging.

Imaging Studies

In general, imaging studies are most useful in determining potential etiologic factors that may need to be addressed. For instance, they can confirm the presence of carotid artery stenosis, or suggest the presence of paroxysmal atrial fibrillation because of embolic strokes. Imaging can also not only uncover the presence of white matter lesions, micro-bleeds, lacunar infarcts, and cerebral atrophy, but can also sometimes surprise us with findings consistent with cerebral edema from dialysis disequilibrium syndrome, osmotic demyelination syndrome, posterior reversible encephalopathy syndrome, infections, and sinus vein thrombosis among others [138–140].

As in non-CKD/ESRD patients, neuroimaging is not used to diagnose cognitive impairment or dementia (these are of course clinical diagnoses); rather, it assists the physician to rule out the

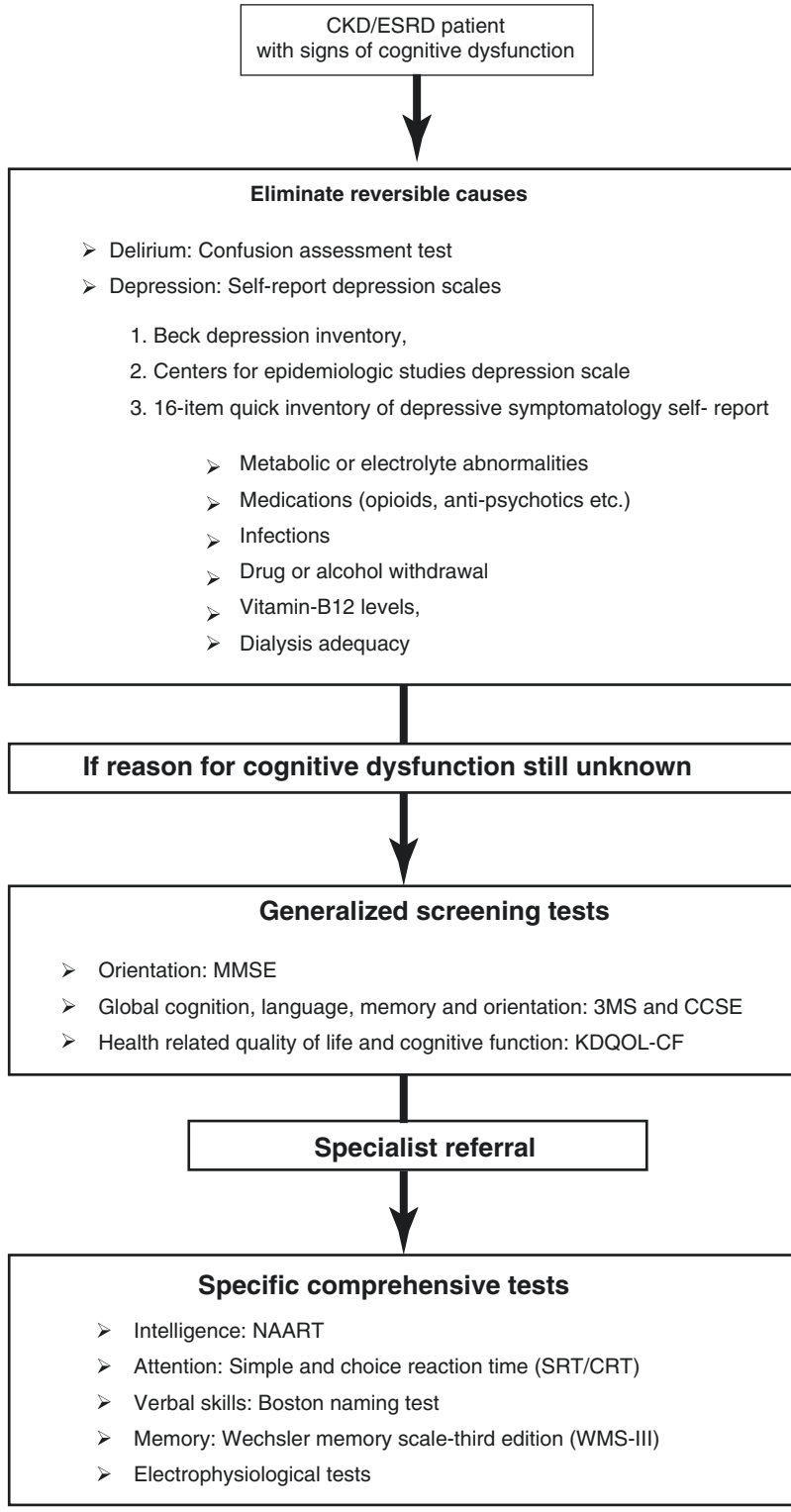


Fig. 36.2 Diagnostic algorithm for cognitive dysfunction in a CKD/ESRD patient

structural and functional causes which can contribute to CI/D. Routinely used imaging techniques include computed tomography (CT) and magnetic resonance imaging (MRI) to detect structural abnormalities, and positron emission tomography (PET) to exclude functional abnormalities. The American Academy of Neurology recommends using CT or MRI imaging to identify structural brain lesions leading to cognitive impairment [141]. CT is usually obtained first in patients presenting with cognitive symptoms because it is widely and quickly available. CT can detect lacunar strokes, periventricular white matter lesions, and infarcts, which when present in the dominant cerebral hemispheres and limbic system suggest the presence of vascular dementia [142]. Moreover, it can also help detect silent brain infarcts, intracerebral hemorrhage, microbleeds, and cerebral atrophy, a very common finding in CKD/ESRD patients [32, 79, 140]. While using intravenous contrast can increase the sensitivity of CT for certain etiologies, it must be used with care in patients with renal dysfunction because it may precipitate or exacerbate acute kidney injury. It should be noted that the presence of renal failure is not a complete contraindication to the use of contrast; the risk of contrast nephropathy is actually quite low if appropriate prophylactic measures are undertaken, particularly ensuring adequate hydration [143, 144].

MRI is more sensitive than CT scans due to the better tissue contrast, flexibility of the image, and also absence of ionizing radiation [145]. MRI studies in CKD/ESRD patients have revealed increased incidence of large and small vessel strokes, along with white matter lesions (risk factors for cognitive dysfunction). Some of the conventional MRI techniques used to detect structural lesions in CKD patients include T2-diffusion weighted imaging and diffusion tensor imaging [146, 147]. However, these conventional MRI techniques are limited in detecting the subtle vascular changes seen in mild to moderate cognitive impairment in some of these CKD/ESRD patients. Advances in MRI techniques, including voxel-based morphometry, have been developed which can be used to better detect these changes. Using gadolinium as a contrast agent improves the sensitivity, but it should not be used in patients with Stage 4 or 5 CKD (an eGFR of <30 ml/min) because it can instigate nephrogenic systemic fibrosis, a catastrophic complication causing systemic fibrosis [148, 149]. If gadolinium has inadvertently been administered to a patient with advanced CKD or ESRD, dialysis should be considered, as it may reduce the contrast load [150]. Various advances to the MRI technique have been developed in order to improve the imaging of the white matter (diffusion tensor imaging) and grey matter (voxel-based morphometry). These methods have been used to demonstrate that CKD/ESRD with CI/D have white matter fiber abnormalities and/or minute microstructural brain atrophy and lower grey matter volume, including in the insular gyrus when compared to healthy controls [14, 151, 152]. These changes may explain the propensity of these patients to develop CI/D [152, 153]. Other advanced techniques include arterial spin-labeled perfusion (ASL) and blood-oxygen-level-determining (BOLD) MRIs. ASL-MRI has been utilized to study the cerebral perfusion changes in hemodialysis patients and the BOLD-functional MRI (fMRI) has the potential to be used to examine the neural mechanisms linked to the pathogenesis of CI/D in ESRD patients [14, 154]. Overall, CT and MRI are very useful in detecting cerebrovascular lesions. In the absence of such lesions, the chances of a vascular etiology for the dementia are low [155, 156].

While CT and MRI reveal structural abnormalities, functional and metabolic abnormalities can be assessed using the PET imaging technique. PET imaging can detect frontal lobe hypoperfusion and hypometabolism in vascular dementia patients, thereby assisting in differentiating it from Alzheimer's Disease, which shows a more parieto-temporal pattern of lowered cerebral perfusion [157–159]. Using PET imaging, Kanai et al. found that hemodialysis patients metabolized less oxygen in the brain and have lower regional blood flow compared to normal individuals [160]. These parameters were greatly diminished post-dialysis. These hemodynamic changes have been postulated to play a role in the cognitive dysfunction observed in these patients.

Therapeutic Strategies

Evidence-based recommendations for managing CI/D in the CKD/ESRD patient are lacking because there are almost no trials targeting these specific populations, and because most clinical trials exclude CKD/ESRD patients due to their complexities brought about by the presence of numerous confounding comorbidities and alterations in drug metabolism. Thus, most approaches are extrapolated from patients with mild to no renal disease, and based on our knowledge of the disease processes causing the CI/D. The initial step is to treat any associated condition such as depression and delirium; in particular, one must ensure appropriate fluid, electrolyte, acid base, and metabolic balance, as well as eliminate non-essential medications. After ruling out or addressing these factors, the therapy should be aimed at the specific causes and risk factors of CI/D. We will not focus on general approaches (N–methyl D-aspartate receptor antagonists and cholinesterase inhibitors) to CI/D caused by Alzheimer's Disease and vascular dementia, since it is largely unchanged (other than dosing the drugs appropriately to adjust for renal function). Rather, we will focus on the components and risk factors that are more directly related to CKD/ESRD that may impact CI/D.

Managing Traditional Cerebrovascular Risk Factors for CI/D in CKD

Vascular dementia in CKD is often interlinked with cerebrovascular events. Hence, medications decreasing stroke risk, such as anti-hypertensives, statins, antiplatelet agents, and anticoagulants may be beneficial in CKD-induced CI/D. In addition, smoking and diabetes should be managed as in patients without CKD. We will discuss three main risks in which there are important considerations in this patient population: hypertension, dyslipidemia, and atrial fibrillation.

Hypertension is highly prevalent in patients with CKD/ESRD, and can be refractory to therapy. While most nephrologists agree that lowering blood pressure is important to slow the progression of CKD and to reduce the risk of cardiovascular complications in CKD /ESRD patients, its effect on CI/D is less clear. Observational studies and clinical trials suggest that antihypertensive therapy reduces CI/D, but longitudinal studies have provided inconsistent results [39, 161]. Moreover, there is controversy as to: (a) what is the optimal blood pressure (cohort studies suggest that aggressive control increases the risk of worsening cognition), (b) what are the best agents for achieving this target, and (c) what is the true risk/benefit ratio for CI/D at different blood pressures [162]. Hence, there are no absolute blood pressure targets, and thus management should be individualized according to the patient's age, severity of albuminuria, and comorbidities [162, 163]. The Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines for the management of blood pressure in CKD suggests a blood pressure threshold of <140/90 mm Hg for CKD patients without proteinuria, and a lower threshold of <130/80 mm Hg in those patients with albuminuria of >30 mg/24 h [164]. The recent SPRINT trial [165] suggests that our target should actually be lower, <120/80 mm Hg in patients with high cardiovascular risk (e.g., CKD patients). However, there are no trials where the main outcome was CI/D (we anxiously await the results of the SPRINT–MIND study). Thus, particular attention must be paid to complaints that may be related to episodic hypotension and changes in neuropsychological symptoms. This is especially true in ESRD patients, who often require more precise adjustments of their antihypertensive regimen because of the additional risk of intradialytic hypotension [166].

Whether there is a preferred class of medication for CI/D in patients with CKD also remains to be determined. Due to the unique microvascular susceptibility seen in both the kidneys and the brain, some studies have suggested that the microvasculature of both organs may benefit from renin–angiotensin system (RAS) blockade, particularly in the presence of albuminuria [138]. Indeed, several studies have shown that RAS blockers prevent renal damage in the kidneys and

also the occurrence or recurrence of stroke in the brain [167, 168]. Interestingly, the protective effects of ACE inhibitors on cognitive decline and dementia do not correlate well with their antihypertensive effects [169]. However it should be mentioned that despite their reported beneficial effects, ACE inhibitors might actually worsen cognitive defects, since they inhibit the conversion of $A\beta_{42}$ (amyloid beta peptide) to the less toxic and amyloidogenic $A\beta_{40}$ [39]. Angiotensin receptor blockers (ARB) do not have this potential negative effect, and thus theoretically may be a better alternative. Calcium channel blockers (CCB) have also been shown to be protective against dementia in a large European trial [170]. Moreover, the combination of a CCB with an ARB was more effective than that of a diuretic and an ARB [138, 171]. Despite these promising results, more work is needed before we can offer strong recommendations.

Hyperlipidemia There are even fewer studies that have examined the efficacy of managing other traditional risk factors on the development and progression of CI/D in CKD/ESRD. Therefore, lipid management is approached as it is for other cardiovascular risk factors following the KDIGO guidelines [172]. The management of hyperlipidemia differs in CKD patients compared to ESRD patients. CKD patients with high risk of cardiovascular disease (Framingham risk >10%) and high LDL levels should be treated with statins. However, statins have not been shown to be effective in patients with ESRD (although study follow-up times were low). Thus, in general ESRD should not be started on statins unless they are young patients with a long life expectancy. If they are already on a statin before they start dialysis, it should be continued. Transplanted patients should be treated like CKD patients. Patients with hypertriglyceridemia are treated using life-style modification; at this time, there are no studies demonstrating that fibrates are effective at reducing mortality in CKD/ESRD patients.

Atrial fibrillation Because atrial fibrillation is widely prevalent in CKD and especially ESRD patients, and they have an increased risk of stroke, it might be assumed that anticoagulation would be especially beneficial in this population. However, the efficacy of anticoagulation therapy appears to be different in CKD vs ESRD patients. Thromboembolic prophylaxis should be implemented in CKD patients, as in the general population [173, 174]. However, it is controversial whether it is advantageous in the ESRD population [175, 176]. Most studies are observational and have found conflicting data, with some suggesting benefit [177–179], but another suggesting a higher risk of stroke [180]. Importantly, most show a significantly increased rate of bleeding risk [178, 181]. Because of this dichotomy, it is not surprising that there are differences between the guidelines. The 2014 American College of Cardiology/American Heart Association guidelines suggest considering warfarin therapy for ESRD patients with non-valvular atrial fibrillation and moderate risk factors [182]. However, the latest KDIGO guidelines state that there is insufficient evidence to recommend routine anticoagulation of ESRD [183]. Our approach is to carefully balance the risk benefit profile of each patient. We are more likely to anticoagulate younger, healthier patients, but not those who have more risks (e.g., elderly patients with moderate risks) or shorter life expectancy. It is important to note that it is more difficult to maintain adequate anticoagulation levels in ESRD patients. They tend to be out of therapeutic range more frequently; thus, the use of novel oral anticoagulants is tempting. However, none have been tested to date in randomized trials. Despite this, apixaban has been approved in the US for prevention of stroke/systemic embolism in the setting of AF among ESRD patients [184].

Managing Other Risk Factors and Uremic Toxins for CI/D in CKD

As mentioned previously, patients with CKD/ESRD have a number of metabolic abnormalities that are not present in other patients with vascular disease that can contribute to the development and/or progression of the vascular disease and subsequent CI/D. Despite the well-known

associations between many of these factors with vascular disease and CI/D, improving or correcting these abnormalities has not had a significant impact on vascular disease and CI/D.

Oxidative Stress and Inflammation Experimental studies have indicated that oxidative stress may also be a strong risk factor for CI/D. Because oxidant stress is very high in CKD/ESRD, anti-oxidant therapy may be particularly beneficial against CI/D associated with CKD/ESRD. Indeed, a recent study by Fujisaki K et al., found that the cognitive decline found in mice with CKD was lessened by administration of tempol, a superoxide dismutase mimetic [112]. Several large longitudinal studies have found an association between the use of antioxidants and protection against cognitive decline and vascular dementia [185, 186], suggesting that oxidant stress may also be beneficial in human CI/D as well. However, the potential usefulness of antioxidant therapy has not been proven in robust clinical trials, and most studies examining the potential benefit of antioxidants on vascular disease have yielded disappointing results. Similarly, although inflammation can contribute to cognitive decline and non-steroidal medications have been found to decrease the dementia risk in the general population [187, 188], there are no studies that have thoroughly examined the benefits of reducing inflammation in attenuating cognitive decline in CKD/ESRD patients. Thus, routine use of antioxidants and/or anti-inflammatory agents for CI/D cannot be recommended at this time.

Hyperhomocysteinemia is a strong independent factor for the development and progression of CI/D [99]. However, therapies that decrease homocysteine levels have not been consistently shown to impact CI/D in the elderly [189, 190], and there is no strong evidence that it is beneficial in CKD/ESRD patients. The VA homocysteine study done on 659 VA patients with advanced CKD/ESRD showed that daily high dose B-vitamin supplementation did not have a beneficial effect on cognition, despite lowering plasma homocysteine levels [191]. Whether homocysteine-lowering therapy is of benefit in transplant recipients is being tested in the FAVORIT Ancillary Cognitive Trial [192]. Hence, at this time there the available evidence does not support routinely treating hyperhomocysteinemia to decrease or prevent progression of CI/D in CKD/ESRD patients.

Anemia, which is exceptionally common in CKD/ESRD, has been associated with dementia in the general population. Leinau et al. [57] in a small cross-sectional study of three dialysis centers found a link between anemia and cognitive impairment in hemodialysis patients. In another cross-sectional study, Murray et al. [24] also noted a similar presence of CI/D in CKD/ESRD patients with anemia. However, longitudinal studies have been unable to confirm these findings. A recent study in a subgroup of 762 adults from the Chronic Renal Insufficiency Cohort (CRIC) was unable to find an independent association between anemia and cognitive dysfunction in older adults [193]. Moreover, the evidence on erythropoietin therapy and cognitive decline is also limited. While one small study reported that erythropoietin improved cognitive function, other recent ones did not. However, comparing these studies is not feasible because the first one lacked controls [194], while the later ones included patients with less severe anemia. Moreover, increasing hemoglobin targets increases cardiovascular mortality [195]. Hence, because of the lack of supporting evidence suggesting that normalizing hemoglobin improves CI/D, and in fact may increase the risk of death, hemoglobin targets should remain at 10–12 g/dl in these patients [195, 196].

Vitamin D While some studies have linked low levels of circulating vitamin-D (25 hydroxy-vitamin-D) to the development and progression of cognitive decline [197, 198], other studies could not find any association [199]. There is presently a large-scale National Institute of Health trial underway which seeks to study the causal relationship between a low levels of vitamin D and CI/D in hemodialysis patients [200].

Renal Replacement Therapies

Once a patient has progressed to ESRD, there are several options for their therapy; conservative care,

dialysis (or similar modality), peritoneal dialysis, or transplant. Most patients end up on hemodialysis. The technological improvements that have made dialysis safer, together with the increasing prevalence of ESRD in the elderly, have led to a substantial increase in elderly patients on dialysis. However, these patients are the most vulnerable to the dialysis-induced complications, and have the highest risk of not benefitting from dialysis. In fact, elderly patients started on dialysis do not have a better survival than those managed with conservative care, and decreased quality of life [201]. Moreover, the concomitant presence of dementia with dialysis further lowers the life-expectancy in ESRD patients [17]. Thus, an individualized approach, based on a thorough assessment of the patient's current status (particularly taking into account the frailty of the patient), should be undertaken when deciding the best approach to treating the patients ESRD treatment options.

Hemodialysis Hemodialysis is a very effective method for removing small, non-protein-bound hydrophilic uremic toxins. However, it is not effective at removing larger substances (the middle molecules), as well as substances that are either protein-bound, lipophilic, or those with large volumes of distribution. The first assumption was that CI/D was maybe occurring as a result of inadequate dialysis with conventional thrice-weekly dialysis [136, 202]. However, increasing either the intensity or frequency of hemodialysis has failed to show any benefit on executive or global function in their patient population [203]. In fact, Murray et al. [204] found that higher intensity of dialysis adequacy correlated with worsening cognitive dysfunction. Kurella et al. [203] in the Frequent Hemodialysis Network study suggested no benefit of frequent hemodialysis on attention, psychomotor speed, memory, or verbal fluency (although there may have been a trend toward improvement in memory and verbal fluency). Attempts to remove middle molecules using other dialysis modalities such as hemodiafiltration, or to achieve slower or more consistent nocturnal hemodialysis, or intensive daily dialysis, to impact CI/D are largely unknown, but may hold promise. The only prospective crossover study to date reported that nocturnal hemodialysis was associated with significant improvements in cognitive symptoms [205], but these promising findings require confirmation. At this time, the most important issues are to adjust the dialysis regimen in a manner that provides adequate clearance and ultrafiltration, while avoiding hemodynamic instability.

Peritoneal Dialysis Peritoneal dialysis can also achieve adequate clearance and ultrafiltration in many, but not all patients. One of its advantages is that it is not commonly associated with the hemodynamic and perfusion alterations that are so prevalent in hemodialysis. Thus, it may be a safer modality than hemodialysis. Indeed, previous studies have reported that cerebrovascular accidents and CI/D are more prevalent in hemodialysis patients than in PD patients [136, 206]. The USRDS found that patients undergoing hemodialysis were nearly twice as likely to suffer from cognitive dysfunction than those on PD [207]. While it is tempting to speculate that the difference may be due to the differences in hemodynamic instability observed with the two modalities, it may be due to selection bias, in that PD patients are younger and healthier, with less confounding factors and co-existing diseases. Indeed, a large, longitudinal study using the National Health Insurance Research Database in Taiwan failed to find any benefit of either modality after adjusting for all pertinent variables [208]. Thus, the scant available data suggest that the prevalence of CI/D might be lower in PD patients, but that this may reflect patient selection bias, rather than because of the dialysis procedure itself [208, 209]. The influence of other PD-related limitations (e.g., fluid overload, secondary metabolic disorders from glucose-based dialysate, and less effective clearance) on CI/D requires further study.

Kidney Transplantation restores kidney function, reverses hyperparathyroidism, and improves quality of life and survival. It is therefore the preferred therapy for many patients with ESRD. Its effects on CI/D are more complex. Most of the available evidence suggests the potential of a biphasic effect. It initially may have detrimental

effects because the ischemia-reperfusion injury causes systemic inflammation and increases neurotoxic cytokines such as IL-1β, IL-6 and TNF-α. However, this period is transient and, as the allograft regains function, gives way to a more long-lasting beneficial phase in which there is reversal of some of the underlying factors contributing to the CI/D. There is consistent removal of uremic toxins, restoration of normal calcium-phosphate homeostasis, and the disappearance of acute fluid and osmotic shifts. Indeed, several studies have reported a beneficial effect of transplantation on CI/D compared to dialysis [210–212]. However, these studies suffer from the same limitations as the PD studies, in that there may be substantial selection bias. There is one cross-sectional study that suggested that transplantation had decreased verbal memory and executive functions when compared to normal controls [213]. The reasons for the opposing results of this study are not known, and are difficult to reconcile. But it does prompt us to remember that transplant can have detrimental effects, particularly related to neurotoxic effects of some immunosuppressive medications and to the increased risk of infections. Overall, however, the available studies suggest that transplantation has a beneficial effect on CI/D [214]. However, all are relatively small, and suffer from inadequate control groups (transplanted patients tend to be younger and healthier), and thus these results should not be extrapolated to all patients with ESRD. It seems prudent to offer transplantation based on the other potential benefits to the patient, while assessing their cognitive function to determine whether a transplant is a viable option for the patient, and perhaps to help prepare for the transplant.

Summary

The prevalence of CKD/ESRD is increasing at an accelerated pace, particularly in the ageing population, and is now considered an epidemic. CKD/ESRD can directly instigate cerebral injury and consequently CI/D, and accelerate it in those populations, such as the elderly, that are particularly susceptible to it. Thus, its impact on health care systems is increasing markedly and becoming a considerable economic burden. Early identification of these patients is essential so that: (a) appropriate decisions can be made regarding choices, goals, and limits of therapy, (b) strategies that may minimize progression of CI/D may be implemented, and (c) measures that assist the patient with compliance of therapy are pursued, with the goal of maintaining the patients' quality of life. This will require a much more aggressive effort that includes a multidisciplinary approach involving nephrologists, renal clinic and dialysis personnel, and mental health professionals.

References

1. Shiao CC, Wu PC, Huang TM, Lai TS, Yang WS, Wu CH, et al. Long-term remote organ consequences following acute kidney injury. Crit Care. 2015;19:438.
2. Nongnuch A, Panorchan K, Davenport A. Brain–kidney crosstalk. Crit Care. 2014;18(3):225.
3. Liu M, Liang Y, Chigurupati S, Lathia JD, Pletnikov M, Sun Z, et al. Acute kidney injury leads to brain inflammation and functional changes. J Am Soc Nephrol JASN. 2008;19(7):1360–70.
4. Mazumder MK, Giri A, Kumar S, Borah A. A highly reproducible mice model of chronic kidney disease: evidences of behavioural abnormalities and blood–brain barrier disruption. Life Sci. 2016;161:27–36.
5. Chillon J-M, Massy ZA, Stengel B. Neurological complications in chronic kidney disease patients. Nephrol Dial Transplant. 2016;31(10):1606–14.
6. Krishnan AV, Kiernan MC. Neurological complications of chronic kidney disease. Nat Rev Neurol. 2009;5(10):542–51.
7. Novak M, Winkelman JW, Unruh M. Restless legs syndrome in patients with chronic kidney disease. Semin Nephrol. 2015;35(4):347–58.
8. Rizzo MA, Frediani F, Granata A, Ravasi B, Cusi D, Gallieni M. Neurological complications of hemodialysis: state of the art. J Nephrol. 2012;25(2):170–82.
9. Brouns R, De Deyn PP. Neurological complications in renal failure: a review. Clin Neurol Neurosurg. 2004;107(1):1–16.
10. Kelley AS, McGarry K, Gorges R, Skinner JS. The burden of health care costs for patients with dementia in the last 5 years of life. Ann Intern Med. 2015;163(10):729–36.
11. Kurella Tamura M, Yaffe K. Dementia and cognitive impairment in ESRD: diagnostic and therapeutic strategies. Kidney Int. 2011;79(1):14–22.
12. Gaxatte C, Daroux M, Bloch J, Puisieux F, Deramecourt V, Boulanger E. Cognitive impairment

and chronic kidney disease: which links? Nephrol Ther. 2011;7(1):10–7.
13. McQuillan R, Jassal SV. Neuropsychiatric complications of chronic kidney disease. Nat Rev Nephrol. 2010;6(8):471–9.
14. Prohovnik I, Post J, Uribarri J, Lee H, Sandu O, Langhoff E. Cerebrovascular effects of hemodialysis in chronic kidney disease. J Cereb Blood Flow Metab. 2007;27(11):1861–9.
15. Toyoda K, Fujii K, Fujimi S, Kumai Y, Tsuchimochi H, Ibayashi S, et al. Stroke in patients on maintenance hemodialysis: a 22-year single-center study. Am J Kidney Dis. 2005;45(6):1058–66.
16. Patel N, Dalal P, Panesar M. Dialysis disequilibrium syndrome: a narrative review. Semin Dial. 2008;21(5):493–8.
17. Rakowski DA, Caillard S, Agodoa LY, Abbott KC. Dementia as a predictor of mortality in dialysis patients. Clin J Am Soc Nephrol. 2006;1(5):1000–5.
18. Ying I, Levitt Z, Jassal SV. Should an elderly patient with stage V CKD and dementia be started on dialysis? Clin J Am Soc Nephrol. 2014;9(5):971–7.
19. System USRD. USRDS 2006 annual data report: atlas of end-stage renal disease in the United States. Bethesda: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2006.
20. Murtagh FE, Marsh JE, Donohoe P, Ekbal NJ, Sheerin NS, Harris FE. Dialysis or not? A comparative survival study of patients over 75 years with chronic kidney disease stage 5. Nephrol Dial Transplant. 2007;22(7):1955–62.
21. Abbott KC, Glanton CW, Trespalacios FC, Oliver DK, Ortiz MI, Agodoa LY, et al. Body mass index, dialysis modality, and survival: analysis of the United States Renal Data System Dialysis Morbidity and Mortality Wave II Study. Kidney Int. 2004;65(2):597–605.
22. Kurella M, Chertow GM, Luan J, Yaffe K. Cognitive impairment in chronic kidney disease. J Am Geriatr Soc. 2004;52(11):1863–9.
23. Kurella Tamura M, Larive B, Unruh ML, Stokes JB, Nissenson A, Mehta RL, et al. Prevalence and correlates of cognitive impairment in hemodialysis patients: the Frequent Hemodialysis Network trials. Clin J Am Soc Nephrol. 2010;5(8):1429–38.
24. Murray AM, Tupper DE, Knopman DS, Gilbertson DT, Pederson SL, Li S, et al. Cognitive impairment in hemodialysis patients is common. Neurology. 2006;67(2):216–23.
25. Sehgal AR, Grey SF, DeOreo PB, Whitehouse PJ. Prevalence, recognition, and implications of mental impairment among hemodialysis patients. Am J Kidney Dis. 1997;30(1):41–9.
26. Jin YP, Gatz M, Johansson B, Pedersen NL. Sensitivity and specificity of dementia coding in two Swedish disease registries. Neurology. 2004;63(4):739–41.
27. Pereira AA, Weiner DE, Scott T, Chandra P, Bluestein R, Griffith J, et al. Subcortical cognitive impairment in dialysis patients. Hemodial Int. 2007;11(3):309–14.
28. Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53(4):695–9.
29. Crum RM, Anthony JC, Bassett SS, Folstein MF. Population-based norms for the Mini-Mental State Examination by age and educational level. JAMA. 1993;269(18):2386–91.
30. Services DoHaHSCfMM. ESRD medical evidence report-CMS2728. Revised on 2006-03-01.
31. Kurella M, Mapes DL, Port FK, Chertow GM. Correlates and outcomes of dementia among dialysis patients: the Dialysis Outcomes and Practice Patterns Study. Nephrol Dial Transplant. 2006;21(9):2543–8.
32. Murray AM. Cognitive impairment in the aging dialysis and chronic kidney disease populations: an occult burden. Adv Chronic Kidney Dis. 2008;15(2):123–32.
33. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. Lancet. 2013;382(9888):260–72.
34. National Center for Chronic Disease Prevention and Health Promotion DoDT, CDC. National Chronic Kidney Disease Fact Sheet, 2014. 2014:1–4.
35. Kurella Tamura M, Wadley V, Yaffe K, McClure LA, Howard G, Go R, et al. Kidney function and cognitive impairment in US adults: the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study. Am J Kidney Dis. 2008;52(2):227–34.
36. Petersen RC. Mild cognitive impairment. N Engl J Med. 2011;364(23):2227–34.
37. Busse A, Hensel A, Guhne U, Angermeyer MC, Riedel-Heller SG. Mild cognitive impairment: long-term course of four clinical subtypes. Neurology. 2006;67(12):2176–85.
38. Lopez OL, Jagust WJ, DeKosky ST, Becker JT, Fitzpatrick A, Dulberg C, et al. Prevalence and classification of mild cognitive impairment in the Cardiovascular Health Study Cognition Study: part 1. Arch Neurol. 2003;60(10):1385–9.
39. Bugnicourt J-M, Godefroy O, Chillon J-M, Choukroun G, Massy ZA. Cognitive disorders and dementia in CKD: the neglected kidney–brain axis. J Am Soc Nephrol. 2013;24(3):353–63.
40. Etgen T. Kidney disease as a determinant of cognitive decline and dementia. Alzheimer's Res Ther. 2015;7(1):29.
41. Lu R, Kiernan MC, Murray A, Rosner MH, Ronco C. Kidney–brain crosstalk in the acute and chronic setting. Nat Rev Nephrol. 2015;11(12):707–19.
42. Weiner DE, Seliger SL. Cognitive and physical function in chronic kidney disease. Curr Opin Nephrol Hypertens. 2014;23(3):291–7.
43. Weiner DE, Sarnak MJ. A decade after the KDOQI CKD guidelines: impact on the cardiovascular disease–CKD paradigm. Am J Kidney Dis. 2012;60(5):710–2.

44. Khatri M, Nickolas T, Moon YP, Paik MC, Rundek T, Elkind MSV, et al. CKD associates with cognitive decline. J Am Soc Nephrol JASN. 2009;20(11): 2427–32.
45. Kurella M, Yaffe K, Shlipak MG, Wenger NK, Chertow GM. Chronic kidney disease and cognitive impairment in menopausal women. Am J Kidney Dis. 2005;45(1):66–76.
46. Seliger SL, Siscovick DS, Stehman-Breen CO, Gillen DL, Fitzpatrick A, Bleyer A, et al. Moderate renal impairment and risk of dementia among older adults: the Cardiovascular Health Cognition Study. J Am Soc Nephrol. 2004;15(7):1904–11.
47. Hugo J, Ganguli M. Dementia and cognitive impairment: epidemiology, diagnosis, and treatment. Clin Geriatr Med. 2014;30(3):421–42.
48. Helmer C, Stengel B, Metzger M, Froissart M, Massy ZA, Tzourio C, et al. Chronic kidney disease, cognitive decline, and incident dementia: the 3C Study. Neurology. 2011;77(23):2043–51.
49. Seliger SL, Longstreth Jr WT, Katz R, Manolio T, Fried LF, Shlipak M, et al. Cystatin C and subclinical brain infarction. J Am Soc Nephrol. 2005;16(12): 3721–7.
50. Seliger SL, Sarnak MJ. Subclinical vascular disease of the brain in dialysis patients. Am J Kidney Dis. 2007;50(1):8–10.
51. Elias MF, Elias PK, Seliger SL, Narsipur SS, Dore GA, Robbins MA. Chronic kidney disease, creatinine and cognitive functioning. Nephrol Dial Transplant. 2009;24(8):2446–52.
52. Madan P, Kalra OP, Agarwal S, Tandon OP. Cognitive impairment in chronic kidney disease. Nephrol Dial Transplant. 2007;22(2):440–4.
53. Etgen T, Sander D, Chonchol M, Briesenick C, Poppert H, Förstl H, et al. Chronic kidney disease is associated with incident cognitive impairment in the elderly: the INVADE study. Nephrol Dial Transplant. 2009;24(10):3144–50.
54. Slinin Y, Paudel ML, Ishani A, Taylor BC, Yaffe K, Murray AM, et al. Kidney function and cognitive performance and decline in older men. J Am Geriatr Soc. 2008;56(11):2082–8.
55. Report UAD. Chapter II: incidence and prevalence of ESRD. 1999.
56. Collins AJ, Kasiske B, Herzog C, Chavers B, Foley R, Gilbertson D, et al. Excerpts from the United States Renal Data System 2006 Annual Data Report. Am J Kidney Dis. 2007;49(1 Suppl 1):A6–7, S1–296.
57. Leinau L, Murphy TE, Bradley E, Fried T. Relationship between conditions addressed by hemodialysis guidelines and non-ESRD-specific conditions affecting quality of life. Clin J Am Soc Nephrol. 2009;4(3):572–8.
58. Fukunishi I, Kitaoka T, Shirai T, Kino K, Kanematsu E, Sato Y. Psychiatric disorders among patients undergoing hemodialysis therapy. Nephron. 2002;91(2):344–7.
59. Bernick C, Kuller L, Dulberg C, Longstreth Jr WT, Manolio T, Beauchamp N, et al. Silent MRI infarcts and the risk of future stroke: the cardiovascular health study. Neurology. 2001;57(7):1222–9.
60. Vermeer SE, Den Heijer T, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM, et al. Incidence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke. 2003;34(2): 392–6.
61. Nakatani T, Naganuma T, Uchida J, Masuda C, Wada S, Sugimura T, et al. Silent cerebral infarction in hemodialysis patients. Am J Nephrol. 2003;23(2): 86–90.
62. Naganuma T, Uchida J, Tsuchida K, Takemoto Y, Tatsumi S, Sugimura K, et al. Silent cerebral infarction predicts vascular events in hemodialysis patients. Kidney Int. 2005;67(6):2434–9.
63. Wetmore JB, Phadnis MA, Ellerbeck EF, Shireman TI, Rigler SK, Mahnken JD. Relationship between stroke and mortality in dialysis patients. Clin J Am Soc Nephrol. 2015;10(1):80–9.
64. Kurella Tamura M, Meyer JB, Saxena AB, Huh JW, Wadley VG, Schiller B. Prevalence and significance of stroke symptoms among patients receiving maintenance dialysis. Neurology. 2012;79(10):981–7.
65. Ovbiagele B. Chronic kidney disease and risk of death during hospitalization for stroke. J Neurol Sci. 2011;301(1–2):46–50.
66. Bozbas H, Atar I, Yildirir A, Ozgul A, Uyar M, Ozdemir N, et al. Prevalence and predictors of arrhythmia in end stage renal disease patients on hemodialysis. Ren Fail. 2007;29(3):331–9.
67. Aoki J, Ikari Y, Nakajima H, Mori M, Sugimoto T, Hatori M, et al. Clinical and pathologic characteristics of dilated cardiomyopathy in hemodialysis patients. Kidney Int. 2005;67(1):333–40.
68. Darby AE, DiMarco JP. Management of atrial fibrillation in patients with structural heart disease. Circulation. 2012;125(7):945–57.
69. Anter E, Jessup M, Callans DJ. Atrial fibrillation and heart failure: treatment considerations for a dual epidemic. Circulation. 2009;119(18):2516–25.
70. Shen CH, Zheng CM, Kiu KT, Chen HA, Wu CC, Lu KC, et al. Increased risk of atrial fibrillation in end-stage renal disease patients on dialysis: a nationwide, population-based study in Taiwan. Medicine (Baltimore). 2016;95(25):e3933.
71. De Vriese AS, Caluwe R, Raggi P. The atrial fibrillation conundrum in dialysis patients. Am Heart J. 2016;174:111–9.
72. Boriani G, Savelieva I, Dan GA, Deharo JC, Ferro C, Israel CW, et al. Chronic kidney disease in patients with cardiac rhythm disturbances or implantable electrical devices: clinical significance and implications for decision making — a position paper of the European Heart Rhythm Association endorsed by the Heart Rhythm Society and the Asia Pacific Heart Rhythm Society. Europace. 2015;17(8):1169–96.

73. System USRD. Chapter 4: cardiovascular disease in patients with CKD. 2015.
74. Wizemann V, Tong L, Satayathum S, Disney A, Akiba T, Fissell RB, et al. Atrial fibrillation in hemodialysis patients: clinical features and associations with anticoagulant therapy. Kidney Int. 2010;77(12):1098–106.
75. Winkelmayer WC, Patrick AR, Liu J, Brookhart MA, Setoguchi S. The increasing prevalence of atrial fibrillation among hemodialysis patients. J Am Soc Nephrol. 2011;22(2):349–57.
76. Charytan DM, Foley R, McCullough PA, Rogers JD, Zimetbaum P, Herzog CA, et al. Arrhythmia and sudden death in hemodialysis patients: protocol and baseline characteristics of the Monitoring in Dialysis Study. Clin J Am Soc Nephrol. 2016;11(4):721–34.
77. Yaffe K, Ackerson L, Kurella Tamura M, Le Blanc P, Kusek JW, Sehgal AR, et al. Chronic kidney disease and cognitive function in older adults: findings from the chronic renal insufficiency cohort cognitive study. J Am Geriatr Soc. 2010;58(2):338–45.
78. Prins ND, van Dijk EJ, den Heijer T, Vermeer SE, Koudstaal PJ, Oudkerk M, et al. Cerebral white matter lesions and the risk of dementia. Arch Neurol. 2004;61(10):1531–4.
79. Toyoda K. Cerebral small vessel disease and chronic kidney disease. J Stroke. 2015;17(1):31–7.
80. Khatri M, Wright CB, Nickolas TL, Yoshita M, Paik MC, Kranwinkel G, et al. Chronic kidney disease is associated with white matter hyperintensity volume: the Northern Manhattan Study (NOMAS). Stroke. 2007;38(12):3121–6.
81. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. BMJ. 2010;341:c3666.
82. Brønnum-Hansen H, Davidsen M, Thorvaldsen P. Long-term survival and causes of death after stroke. Stroke. 2001;32(9):2131–6.
83. Saeed F, Kousar N, Qureshi K, Laurence TN. A review of risk factors for stroke in patients with chronic kidney disease. J Vasc Interv Neurol. 2009;2(1):126–31.
84. Power A. Stroke in dialysis and chronic kidney disease. Blood Purif. 2013;36(3–4):179–83.
85. Seliger SL, Gillen DL, Tirschwell D, Wasse H, Kestenbaum BR, Stehman-Breen CO. Risk factors for incident stroke among patients with end-stage renal disease. J Am Soc Nephrol. 2003;14(10):2623–31.
86. Yoo DJ, Agodoa L, Yuan CM, Abbott KC, Nee R. Risk of intracranial hemorrhage associated with autosomal dominant polycystic kidney disease in patients with end stage renal disease. BMC Nephrol. 2014;15:39.
87. Linthorst GE, Avis HJ, Levi M. Uremic thrombocytopathy is not about urea. J Am Soc Nephrol JASN. 2010;21(5):753–5.
88. Galbusera M, Remuzzi G, Boccardo P. Treatment of bleeding in dialysis patients. Semin Dial. 2009;22(3):279–86.
89. Ho SJ, Gemmell R, Brighton TA. Platelet function testing in uraemic patients. Hematology (Amsterdam, Netherlands). 2008;13(1):49–58.
90. Di Minno G, Martinez J, McKean ML, De La Rosa J, Burke JF, Murphy S. Platelet dysfunction in uremia. Multifaceted defect partially corrected by dialysis. Am J Med. 1985;79(5):552–9.
91. Genovesi S, Santoro A. Warfarin and stroke outcomes in hemodialysis patients with atrial fibrillation. J Am Soc Nephrol. 2009;20(10):2090–2.
92. Harmon JP, Zimmerman DL, Zimmerman DL. Anticoagulant and antiplatelet therapy in patients with chronic kidney disease: risks versus benefits review. Curr Opin Nephrol Hypertens. 2013;22(6):624–8.
93. Iseki K, Kinjo K, Kimura Y, Osawa A, Fukiyama K. Evidence for high risk of cerebral hemorrhage in chronic dialysis patients. Kidney Int. 1993;44(5):1086–90.
94. Ochiai H, Uezono S, Kawano H, Ikeda N, Kodama K, Akiyama H. Factors affecting outcome of intracerebral hemorrhage in patients undergoing chronic hemodialysis. Ren Fail. 2010;32(8):923–7.
95. Kim JK, Shin JJ, Park SK, Hwang YS, Kim TH, Shin HS. Prognostic factors and clinical outcomes of acute intracerebral hemorrhage in patients with chronic kidney disease. J Korean Neurosurg Soc. 2013;54(4):296–301.
96. Schiffrin EL, Lipman ML, Mann JFE. Chronic kidney disease: effects on the cardiovascular system. Circulation. 2007;116(1):85–97.
97. Watanabe K, Watanabe T, Nakayama M. Cerebrorenal interactions: impact of uremic toxins on cognitive function. Neurotoxicology. 2014;44:184–93.
98. De Deyn PP, Vanholder R, Eloot S, Glorieux G. Guanidino compounds as uremic (neuro)toxins. Semin Dial. 2009;22(4):340–5.
99. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med. 2002;346(7):476–83.
100. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med. 1998;338(15):1042–50.
101. Harker LA, Harlan JM, Ross R. Effect of sulfinpyrazone on homocysteine-induced endothelial injury and arteriosclerosis in baboons. Circ Res. 1983;53(6):731–9.
102. Haruna Y, Kashihara N, Satoh M, Tomita N, Namikoshi T, Sasaki T, et al. Amelioration of progressive renal injury by genetic manipulation of Klotho gene. Proc Natl Acad Sci U S A. 2007;104(7):2331–6.
103. Hu MC, Kuro-o M, Moe OW. Secreted Klotho and chronic kidney disease. Adv Exp Med Biol. 2012;728:126–57.
104. Nagai T, Yamada K, Kim HC, Kim YS, Noda Y, Imura A, et al. Cognition impairment in the genetic model of aging Klotho gene mutant mice: a role of oxidative stress. FASEB J. 2003;17(1):50–2.

105. Zachara BA, Wlodarczyk Z, Masztalerz M, Adamowicz A, Gromadzinska J, Wasowicz W. Selenium concentrations and glutathione peroxidase activities in blood of patients before and after allogenic kidney transplantation. Biol Trace Elem Res. 2004;97(1):1–13.
106. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrol Dial Transplant. 2003;18(7):1272–80.
107. Filiopoulos V, Hadjiyannakos D, Takouli L, Metaxaki P, Sideris V, Vlassopoulos D. Inflammation and oxidative stress in end-stage renal disease patients treated with hemodialysis or peritoneal dialysis. Int J Artif Organs. 2009;32(12):872–82.
108. Markesbery WR, Carney JM. Oxidative alterations in Alzheimer's disease. Brain pathol (Zurich, Switzerland). 1999;9(1):133–46.
109. Grammas P, Ovase R. Inflammatory factors are elevated in brain microvessels in Alzheimer's disease. Neurobiol Aging. 2001;22(6):837–42.
110. De Deyn PP, Vanholder R, D'Hooge R. Nitric oxide in uremia: effects of several potentially toxic guanidino compounds. Kidney Int Suppl. 2003;84:S25–8.
111. Bernstein H-G, Bogerts B, Keilhoff G. The many faces of nitric oxide in schizophrenia. A review. Schizophrenia Res. 2005;78(1):69–86.
112. Fujisaki K, Tsuruya K, Yamato M, Toyonaga J, Noguchi H, Nakano T, et al. Cerebral oxidative stress induces spatial working memory dysfunction in uremic mice: neuroprotective effect of tempol. Nephrol Dial Transplant. 2014;29(3):529–38.
113. Sundelof J, Kilander L, Helmersson J, Larsson A, Ronnemaa E, Degerman-Gunnarsson M, et al. Systemic inflammation and the risk of Alzheimer's disease and dementia: a prospective population-based study. J Alzheimers Dis. 2009;18(1):79–87.
114. Amore A, Coppo R. Immunological basis of inflammation in dialysis. Nephrol Dial Transplant. 2002;17(Suppl 8):16–24.
115. Jofré R, Rodriguez-Benitez P, López-Gómez JM, Pérez-Garcia R. Inflammatory syndrome in patients on hemodialysis. J Am Soc Nephrol. 2006;17(12 suppl 3):S274–S80.
116. Rozas VV, Port FK, Easterling RE. An outbreak of dialysis dementia due to aluminum in the dialysate. J Dial. 1978;2(5–6):459–70.
117. Foundation NK. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am J Kidney Dis. 2003;42:S1.
118. Farhoudi M, Abedi Azar S, Abdi R. Brain hemodynamics in patients with end-stage renal disease between hemodialysis sessions. Iran J Kidney Dis. 2012;6(2):110–3.
119. Hata R, Matsumoto M, Handa N, Terakawa H, Sugitani Y, Kamada T. Effects of hemodialysis on cerebral circulation evaluated by transcranial Doppler ultrasonography. Stroke. 1994;25(2):408–12.
120. Skinner H, Mackaness C, Bedforth N, Mahajan R. Cerebral haemodynamics in patients with chronic renal failure: effects of haemodialysis. Br J Anaesth. 2005;94(2):203–5.
121. Pereira AA, Weiner DE, Scott T, Sarnak MJ. Cognitive function in dialysis patients. Am J Kidney Dis. 2005;45(3):448–62.
122. Chiu YW, Teitelbaum I, Misra M, de Leon EM, Adzize T, Mehrotra R. Pill burden, adherence, hyperphosphatemia, and quality of life in maintenance dialysis patients. Clin J Am Soc Nephrol. 2009;4(6):1089–96.
123. Ying I, Levitt Z, Jassal SV. Should an elderly patient with stage V CKD and dementia be started on dialysis? Clin J Am Soc Nephrol CJASN. 2014;9(5):971–7.
124. Amira O. Prevalence of symptoms of depression among patients with chronic kidney disease. Niger J Clin Pract. 2011;14(4):460–3.
125. Cohen SD, Norris L, Acquaviva K, Peterson RA, Kimmel PL. Screening, diagnosis, and treatment of depression in patients with end-stage renal disease. Clin J Am Soc Nephrol. 2007;2(6):1332–42.
126. Hedayati SS, Minhajuddin AT, Toto RD, Morris DW, Rush AJ. Validation of depression screening scales in patients with CKD. Am J Kidney Dis Off J National Kidney Foundation. 2009;54(3):433–9.
127. Thomas C, Kreisel SH, Oster P, Driessen M, Arolt V, Inouye SK. Diagnosing delirium in hospitalized elderly patients with dementia: adapting the confusion assessment method to ICD-10 diagnostic criteria. J Am Geriatr Soc. 2012;60(8):1471–7.
128. Inouye SK, van Dyck CH, Alessi CA, Balkin S, Siegal AP, Horwitz RI. Clarifying confusion: the confusion assessment method. A new method for detection of delirium. Ann Intern Med. 1990;113(12):941–8.
129. Fong TG, Tulebaev SR, Inouye SK. Delirium in elderly adults: diagnosis, prevention and treatment. Nat Rev Neurol. 2009;5(4):210–20.
130. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189–98.
131. Kurella M, Luan J, Yaffe K, Chertow GM. Validation of the Kidney Disease Quality of Life (KDQOL) cognitive function subscale. Kidney Int. 2004;66(6):2361–7.
132. Godefroy O, Fickl A, Roussel M, Auribault C, Bugnicourt JM, Lamy C, et al. Is the Montreal Cognitive Assessment superior to the Mini-Mental State Examination to detect poststroke cognitive impairment? A study with neuropsychological evaluation. Stroke. 2011;42(6):1712–6.
133. Wilder D, Cross P, Chen J, Gurland B, Lantigua RA, Teresi J, et al. Operating characteristics of brief screens for dementia in a multicultural population. Am J Geriatr Psychiatry. 1995;3(2):96–107.
134. Belle SH, Seaberg EC, Ganguli M, Ratcliff G, DeKosky S, Kuller LH. Effect of education and gender adjustment on the sensitivity and specificity of a cognitive screening battery for dementia: results from the MoVIES Project. Monongahela Valley

Independent Elders Survey. Neuroepidemiology. 1996;15(6):321–9.
135. Fratiglioni L, Jorm AF, Grut M, Viitanen M, Holmen K, Ahlbom A, et al. Predicting dementia from the Mini-Mental State Examination in an elderly population: the role of education. J Clin Epidemiol. 1993;46(3):281–7.
136. Williams MA, Sklar AH, Burright RG, Donovick PJ. Temporal effects of dialysis on cognitive functioning in patients with ESRD. Am J Kidney Dis. 2004;43(4):705–11.
137. Tornatore JB, Hill E, Laboff JA, McGann ME. Self-administered screening for mild cognitive impairment: initial validation of a computerized test battery. J Neuropsychiatry Clin Neurosci. 2005;17(1):98–105.
138. Mogi M, Horiuchi M. Clinical interaction between brain and kidney in small vessel disease. Cardiol Res Prac. 2011;2011:306189.
139. Vogels SC, Emmelot-Vonk MH, Verhaar HJ, Koek HL. The association of chronic kidney disease with brain lesions on MRI or CT: a systematic review. Maturitas. 2012;71(4):331–6.
140. Moodalbail DG, Reiser KA, Detre JA, Schultz RT, Herrington JD, Davatzikos C, et al. Systematic review of structural and functional neuroimaging findings in children and adults with CKD. Clin J Am Soc Nephrol CJASN. 2013;8(8):1429–48.
141. American Academy of Neurology guideline summary for clinicians: detection, diagnosis and management of dementia. 2013. http://tools.aan.com/professionals/practice/pdfs/dementia_guideline.pdf.
142. Albert M DC, DeKosky S, De Leon M, Foster NL, Fox N, et al. The use of MRI and PET for clinical diagnosis of dementia and investigation of cognitive impairment: a consensus report. [Last accessed on 3rd October 2016]. Available from: http://www.alz.org Neuroimaging Work Group of the Alzheimer' Association; 2004.
143. Pannu N, Wiebe N, Tonelli M, Alberta Kidney Disease Network. Prophylaxis strategies for contrast-induced nephropathy. JAMA. 2006;295(23):2765–79.
144. Ad-hoc working group of ERBP, Fliser D, Laville M, Covic A, Fouque D, Vanholder R, et al. A European Renal Best Practice (ERBP) position statement on the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines on acute kidney injury: part 1: definitions, conservative management and contrast-induced nephropathy. Nephrol Dial Transplant. 2012;27(12):4263–72.
145. Johnson KA, Fox NC, Sperling RA, Klunk WE. Brain imaging in Alzheimer disease. Cold Spring Harb Perspect Med. 2012;2(4):a006213.
146. Chen HJ, Zhang LJ, Lu GM. Multimodality MRI findings in patients with end-stage renal disease. Biomed Res Int. 2015;2015:12.
147. Le Bihan D, Mangin JF, Poupon C, Clark CA, Pappata S, Molko N, et al. Diffusion tensor imaging: concepts and applications. J Magn Reson Imaging. 2001;13(4):534–46.
148. Cowper SE, Robin HS, Steinberg SM, Su LD, Gupta S, LeBoit PE. Scleromyxoedema-like cutaneous diseases in renal-dialysis patients. Lancet. 2000;356(9234):1000–1.
149. Kuo PH, Kanal E, Abu-Alfa AK, Cowper SE. Gadolinium-based MR contrast agents and nephrogenic systemic fibrosis. Radiology. 2007;242(3):647–9.
150. Rodby RA. Dialytic therapies to prevent NSF following gadolinium exposure in high-risk patients. Semin Dial. 2008;21(2):145–9.
151. Zhang LJ, Wen J, Ni L, Zhong J, Liang X, Zheng G, et al. Predominant gray matter volume loss in patients with end-stage renal disease: a voxel-based morphometry study. Metab Brain Dis. 2013;28(4):647–54.
152. Chen HJ, Zhang LJ, Lu GM. Multimodality MRI findings in patients with end-stage renal disease. Biomed Res Int. 2015;2015:697402.
153. Chai C, Zhang M, Long M, Chu Z, Wang T, Wang L, et al. Increased brain iron deposition is a risk factor for brain atrophy in patients with haemodialysis: a combined study of quantitative susceptibility mapping and whole brain volume analysis. Metab Brain Dis. 2015;30(4):1009–16.
154. Fleisher AS, Sherzai A, Taylor C, Langbaum JB, Chen K, Buxton RB. Resting-state BOLD networks versus task-associated functional MRI for distinguishing Alzheimer's disease risk groups. Neuroimage. 2009;47(4):1678–90.
155. Moretti R, Torre P, Antonello RM, Manganaro D, Vilotti C, Pizzolato G. Risk factors for vascular dementia: hypotension as a key point. Vasc Health Risk Manag. 2008;4(2):395–402.
156. Erkinjuntti T. Clinical criteria for vascular dementia: the NINDS–AIREN criteria. Dementia. 1994;5(3–4):189–92.
157. Cappa A, Calcagni ML, Villa G, Giordano A, Marra C, De Rossi G, et al. Brain perfusion abnormalities in Alzheimer's disease: comparison between patients with focal temporal lobe dysfunction and patients with diffuse cognitive impairment. J Neurol Neurosurg Psychiatry. 2001;70(1):22–7.
158. Alexopoulos P, Sorg C, Forschler A, Grimmer T, Skokou M, Wohlschlager A, et al. Perfusion abnormalities in mild cognitive impairment and mild dementia in Alzheimer's disease measured by pulsed arterial spin labeling MRI. Eur Arch Psychiatry Clin Neurosci. 2012;262(1):69–77.
159. Roquet D, Sourty M, Botzung A, Armspach JP, Blanc F. Brain perfusion in dementia with Lewy bodies and Alzheimer's disease: an arterial spin labeling MRI study on prodromal and mild dementia stages. Alzheimers Res Ther. 2016;8:29.
160. Kanai H, Hirakata H, Nakane H, Fujii K, Hirakata E, Ibayashi S, et al. Depressed cerebral oxygen metabolism in patients with chronic renal failure: a positron emission tomography study. Am J Kidney Dis. 2001;38(4 Suppl 1):S129–33.

161. Duron E, Hanon O. Antihypertensive treatments, cognitive decline, and dementia. J Alzheimers Dis. 2010;20(3):903–14.
162. Obisesan TO. Hypertension and cognitive function. Clin Geriatr Med. 2009;25(2):259–88.
163. Fried L. Albuminuria and cognitive impairment. Clin J Am Soc Nephrol. 2012;7(3):376–8.
164. Disease KCPGftMoBPiCK. Chapter 3: blood pressure management in CKD ND patients without diabetes mellitus. Kidney Int. 2012;2(5):357–62.
165. Group TSR. A randomized trial of intensive versus standard blood-pressure control. N Engl J Med. 2015;373(22):2103–16.
166. Schreiber Jr MJ. Clinical case-based approach to understanding intradialytic hypotension. Am J Kidney Dis. 2001;38(4 Suppl 4):S37–47.
167. Sawada T, Yamada H, Dahlof B, Matsubara H, Group KHS. Effects of valsartan on morbidity and mortality in uncontrolled hypertensive patients with high cardiovascular risks: KYOTO HEART Study. Eur Heart J. 2009;30(20):2461–9.
168. Fuentes B, Fernandez-Dominguez J, Ortega-Casarrubios MA, SanJose B, Martinez-Sanchez P, Diez-Tejedor E. Treatment with angiotensin receptor blockers before stroke could exert a favourable effect in acute cerebral infarction. J Hypertens. 2010;28(3):575–81.
169. Fournier A, Oprisiu-Fournier R, Serot J-M, Godefroy O, Achard J-M, Faure S, et al. Prevention of dementia by antihypertensive drugs: how AT1-receptor-blockers and dihydropyridines better prevent dementia in hypertensive patients than thiazides and ACE-inhibitors. Expert Rev Neurother. 2009;9(9):1413–31.
170. Lovell MA, Abner E, Kryscio R, Xu L, Fister SX, Lynn BC. Calcium channel blockers, progression to dementia, and effects on amyloid beta peptide production. Oxid Med Cell Longev. 2015;2015:9.
171. Turnbull F, Woodward M, Neal B, Barzi F, Ninomiya T, Chalmers J, et al. Do men and women respond differently to blood pressure-lowering treatment? Results of prospectively designed overviews of randomized trials. Eur Heart J. 2008;29(21):2669–80.
172. KDIGO. Clinical practice guideline for lipid management in chronic kidney disease. Kidney Int. 2013;3(3).
173. Khella S, Bleicher MB. Stroke and its prevention in chronic kidney disease. Clin J Am Soc Nephrol. 2007;2(6):1343–51.
174. Wattanakit K, Cushman M, Stehman-Breen C, Heckbert SR, Folsom AR. Chronic kidney disease increases risk for venous thromboembolism. J Am Soc Nephrol. 2008;19(1):135–40.
175. Reinecke H, Brand E, Mesters R, Schäbitz W-R, Fisher M, Pavenstädt H, et al. Dilemmas in the management of atrial fibrillation in chronic kidney disease. J Am Soc Nephrol. 2009;20(4):705–11.
176. Saracyn M, Brodowska-Kania D, Niemczyk S. Oral anticoagulant therapy in patients receiving haemodialysis: is it time to abandon it? Scientific World Journal. 2013;2013:170576.
177. Ezekowitz MD, Bridgers SL, James KE, Carliner NH, Colling CL, Gornick CC, et al. Warfarin in the prevention of stroke associated with nonrheumatic atrial fibrillation. Veterans affairs stroke prevention in nonrheumatic atrial fibrillation investigators. N Engl J Med. 1992;327(20):1406–12.
178. Shah M, Avgil Tsadok M, Jackevicius CA, Essebag V, Eisenberg MJ, Rahme E, et al. Warfarin use and the risk for stroke and bleeding in patients with atrial fibrillation undergoing dialysis. Circulation. 2014;129(11):1196–203.
179. Carrero J, Evans M, Szummer K, et al. WArfarin, kidney dysfunction, and outcomes following acute myocardial infarction in patients with atrial fibrillation. JAMA. 2014;311(9):919–28.
180. Wiesholzer M, Harm F, Tomasec G, Barbieri G, Putz D, Balcke P. Incidence of stroke among chronic hemodialysis patients with nonrheumatic atrial fibrillation. Am J Nephrol. 2001;21(1):35–9.
181. Clase CM, Holden RM, Sood MM, Rigatto C, Moist LM, Thomson BKA, et al. Should patients with advanced chronic kidney disease and atrial fibrillation receive chronic anticoagulation? Nephrol Dial Transplant. 2012;27(10):3719–24.
182. January CT, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JJC, et al. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. J Am Coll Cardiol. 2014;64(21):e1–e76.
183. Herzog CA, Asinger RW, Berger AK, Charytan DM, Diez J, Hart RG, et al. Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int. 2011;80(6):572–86.
184. Chan KE, Giugliano RP, Patel MR, Abramson S, Jardine M, Zhao S, et al. Nonvitamin K anticoagulant agents in patients with advanced chronic kidney disease or on dialysis with AF. J Am Coll Cardiol. 2016;67(24):2888–99.
185. Masaki KH, Losonczy KG, Izmirlian G, Foley DJ, Ross GW, Petrovitch H, et al. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. Neurology. 2000;54(6):1265–72.
186. Morris M, Evans DA, Bienias JL, Tangney CC, Wilson RS. Vitamin e and cognitive decline in older persons. Arch Neurol. 2002;59(7):1125–32.
187. Cote S, Carmichael PH, Verreault R, Lindsay J, Lefebvre J, Laurin D. Nonsteroidal anti-inflammatory drug use and the risk of cognitive impairment and Alzheimer's disease. Alzheimer's Dement J Alzheimer's Assoc. 2012;8(3):219–26.
188. Yip AG, Green RC, Huyck M, Cupples LA, Farrer LA. Nonsteroidal anti-inflammatory drug use and Alzheimer's disease risk: the MIRAGE Study. BMC Geriatr. 2005;5(1):1–6.

189. McMahon JA, Green TJ, Skeaff CM, Knight RG, Mann JI, Williams SM. A controlled trial of homocysteine lowering and cognitive performance. N Engl J Med. 2006;354(26):2764–72.
190. Durga J, van Boxtel MPJ, Schouten EG, Kok FJ, Jolles J, Katan MB, et al. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. Lancet. 2007;369(9557):208–16.
191. Brady CB, Gaziano JM, Cxypoliski RA, Guarino PD, Kaufman JS, Warren SR, et al. Homocysteine lowering and cognition in CKD: the Veterans Affairs homocysteine study. Am J Kidney Dis. 2009;54(3):440–9.
192. Troen AM, Scott TM, D'Anci KE, Moorthy D, Dobson B, Rogers G, et al. Cognitive dysfunction and depression in adult kidney transplant recipients: baseline findings from the FAVORIT Ancillary Cognitive Trial (FACT). J Ren Nutr. 2012;22(2):268–76.e1–3.
193. Kurella Tamura M, Vittinghoff E, Yang J, Go AS, Seliger SL, Kusek JW, et al. Anemia and risk for cognitive decline in chronic kidney disease. BMC Nephrol. 2016;17:13.
194. Marsh JT, Brown WS, Wolcott D, Carr CR, Harper R, Schweitzer SV, et al. rHuEPO treatment improves brain and cognitive function of anemic dialysis patients. Kidney Int. 1991;39(1):155–63.
195. KDOQI. KDOQI clinical practice guideline and clinical practice recommendations for anemia in chronic kidney disease: 2007 update of hemoglobin target. Am J Kidney Dis. 2007;50(3):471–530.
196. Locatelli F, Barany P, Covic A, De Francisco A, Del Vecchio L, Goldsmith D, et al. Kidney disease: improving global outcomes guidelines on anaemia management in chronic kidney disease: a European Renal Best Practice position statement. Nephrol Dial Transplant. 2013;28(6):1346–59.
197. Shaffi K, Tighiouart H, Scott T, Lou K, Drew D, Weiner D, et al. Low 25-hydroxyvitamin D levels and cognitive impairment in hemodialysis patients. Clin J Am Soc Nephrol. 2013;8(6):979–86.
198. Liu GL, Pi HC, Hao L, Li DD, Wu YG, Dong J. Vitamin D status is an independent risk factor for global cognitive impairment in peritoneal dialysis patients. PLoS One. 2015;10(12):e0143782.
199. Jovanovich AJ, Chonchol M, Brady CB, Kaufman JD, Kendrick J, Cheung AK, et al. 25-vitamin D, 1,25-vitamin D, parathyroid hormone, fibroblast growth factor-23 and cognitive function in men with advanced CKD: a veteran population. Clin Nephrol. 2014;82(5):S1–4.
200. Health NIo. Vitamin D supplementation on physical and cognitive function — Pilot Study.
201. Harris S, Lamping D, Brown E, Constantinovici N. Clinical outcomes and quality of life in elderly patients on peritoneal dialysis versus hemodialysis. Perit Dial Int. 2002;22(4):463–70.
202. Mizumasa T, Hirakata H, Yoshimitsu T, Hirakata E, Kubo M, Kashiwagi M, et al. Dialysis-related hypotension as a cause of progressive frontal lobe atrophy in chronic hemodialysis patients: a 3-year prospective study. Nephron Clin Pract. 2004;97(1):c23–30.
203. Kurella Tamura M, Unruh ML, Nissenson AR, Larive B, Eggers PW, Gassman J, et al. Effect of more frequent hemodialysis on cognitive function in the frequent hemodialysis network trials. Am J Kidney Dis. 2013;61(2):228–37.
204. Murray AM, Pederson SL, Tupper DE, Hochhalter AK, Miller WA, Li Q, et al. Acute variation in cognitive function in hemodialysis patients: a cohort study with repeated measures. Am J Kidney Dis. 2007;50(2):270–8.
205. Jassal SV, Devins GM, Chan CT, Bozanovic R, Rourke S. Improvements in cognition in patients converting from thrice weekly hemodialysis to nocturnal hemodialysis: a longitudinal pilot study. Kidney Int. 2006;70(5):956–62.
206. Wolfgram DF, Szabo A, Murray AM, Whittle J. Risk of dementia in peritoneal dialysis patients compared with hemodialysis patients. Perit Dial Int J Int Soc Perit Dial. 2015;35(2):189–98.
207. United States Renal Data System; USRDS. 2005 Annual data report: atlas of end-stage renal disease in the United States. Bethesda: MNIoH, National Institute of Diabetes and Digestive and Kidney Diseases; 2005.
208. Lin Y-T, Wu P-H, Kuo M-C, Chen C-S, Chiu Y-W, Yang Y-H, et al. Comparison of dementia risk between end stage renal disease patients with hemodialysis and peritoneal dialysis — a population based study. Sci Rep. 2015;5:8224.
209. Radic J, Ljutic D, Radic M, Kovacic V, Sain M, Curkovic KD. The possible impact of dialysis modality on cognitive function in chronic dialysis patients. Neth J Med. 2010;68(4):153–7.
210. Harciarek M, Biedunkiewicz B, Lichodziejewska-Niemierko M, Debska-Slizien A, Rutkowski B. Cognitive performance before and after kidney transplantation: a prospective controlled study of adequately dialyzed patients with end-stage renal disease. J Int Neuropsychol Soc. 2009;15(5):684–94.
211. Kaya Y, Ozturkeri OA, Benli US, Colak T. Evaluation of the cognitive functions in patients with chronic renal failure before and after renal transplantation. Acta Neurol Belg. 2013;113(2):147–55.
212. Griva K, Thompson D, Jayasena D, Davenport A, Harrison M, Newman SP. Cognitive functioning pre- to post-kidney transplantation — A prospective study. Nephrol Dial Transplant. 2006;21(11):3275–82.
213. Gelb S, Shapiro RJ, Hill A, Thornton WL. Cognitive outcome following kidney transplantation. Nephrol Dial Transplant. 2008;23(3):1032–8.
214. Van Sandwijk MS, Ten Berge IJ, Majoie CB, Caan MW, De Sonneville LM, Van Gool WA, et al. Cognitive changes in chronic kidney disease and after transplantation. Transplantation. 2016;100(4):734–42.

Index

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7

R