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Editors

Psychiatry and Neuroscience Update

Bridging the Divide

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This book is dedicated Prof. Dr. Enrike Gutiérrez-Argandoña who recently passed away. He was an outstanding researcher, a great friend, a truly heroic academic achiever, who was truly dedicated to his mission until his last days. He has been an example of hard work and dedication to the transcendent task of teaching and research all his life, and particularly his final days.

Foreword

The advance of neurosciences has challenged psychiatric paradigms and stressed the need for interrelationship and multidisciplinary dialogue. In this endeavour, Springer has pioneered the publishing of a monograph with papers presented at the 3rd International Congress of Neuropsychiatry held in 2000 in Kyoto. Since that time, many more clinicians and researchers are paying attention to each other's interests and findings.

The American Psychiatric Association gave birth to "translational research" sponsoring programs and projects intended to bridge the gap "from bench to bedside". The National Institutes of Health and National Institutes of Mental Health (NIMH) are funding more training programs in neurosciences and biological psychiatry across the United States and the world.

Masters and postgraduate training include subdisciplines such as molecular and cellular neurobiology, developmental neurobiology, systems neurobiology, behavioral biology, neuroimaging, genetics and epigenetics, methodology in psychiatric research, psychopharmacology, biological rythms, sleep, neurotransmitters, epidemiological and evaluative research, forensic psychiatry, biomedical ethics, and more.

The danger of becoming an expert in a small and restricted field is evident, and focusing on the intricacies of biology can obscure anthropologic richness. Human art, science, and religion need the complexity of the central nervous system, but they cannot be explained by anatomic or metabolic facts.

Terence (Publius Terentius Afer, 159 BC) wrote his famous "Homo sum, humani nihil a me alienum puto", and since then it has been applied to medicine and its related disciplines, and psychiatrists in particular should include in their studies philosophy, literature, history, and art.

The current volume is another big step in the long road in search of knowledge. Many of the contributors to this text represent a new generation of neuroscientists and it is promising that they accept the experiences of older colleagues. Springer must to be thanked for its invaluable interest and help, and we hope that translational research will be able to continue with their support.

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Humberto Luis Mesones-Arroyo, MD, DPM

Preface

The main goal of the current text is to present a wide field by developing chapters in which the actual state of research lines is presented. Psychiatry has received important contributions from the basic neurosciences, and basic neurosciences have received inspiration and objectives from the open problems of psychiatry, as professional practice, and from psychopathology as a science.

The coexistence and integration of these disciplines has not always been easy. At times they have tended to integrate and at times they have ignored one another. There have been attempts to prevail positions of one over the other that have led to difficulties. Such has been the case of reductionism, first, in which it is intended that all clinical reality can be explained by science, ignoring irreducible emerging levels to the purely chemical–physical–mathematical conception. And, in the opposite way, at the postulation of biological mechanisms for cultural events has been observed, with the consequent fall in the medicalization of everyday life. Our intention here is to bridge between the two fields, allowing a fruitful dialogue between the two groups of sciences.

The idea is to draw the borders, new trends and implications, rather than collecting classical knowledge, familiar to the audience to which this work is addressed. This presentation is not an exhaustive review of all topics in the field. We show some illustrative examples in which the research lines are trying to build a bridge between basic or theoretical facts and its correspondence with psychiatric clinical reality. Present work constitutes an attempt to advance transposing the present frontiers, leading to create a forum dedicated to the promotion of scientific and heuristical discussion. The authors, coming from different disciplines and geographic zones, are convoked to draw some possible lines for the progress of our sciences and philosophies. An open and free ambit created for the discussion of human behavior in all levels has been attempted.

This text is directed at clinicians and researchers, but also at university professors and students of medicine, biology, and psychology. It is also directed at the general physician and educator, who will find the foundations of their work here. It constitutes an actualization of some of the main topics in contemporary psychiatry and neurosciences. However, we also attempted to create a criterion for encounters between various sciences that can be paradigmatic and exemplary.

This book is divided into four main parts. The first is the epistemological basis of the study of behavior, aiming to avoid reductionisms. The purpose of the first part is to rescue the conception of "comprehensive psychology" in the sense of Jaspers. At this level we study empathy.

In Chap. 1, Mesones Arroyo includes pertinent and precise considerations. He develops a conceptual framework that allows us to study man in a comprehensive and realistic sense, essential for clinical practice. It considers somatic but also personal aspects. His contribution highlights the relevance of anthropology in the consideration of the relations between levels in the human being, and its transcendence in psychotherapy. The author has a trajectory that involves a systematic postgraduate training of 11 years in Europe, an extensive activity in public health and clinical research, a broad general education and culture and his work of about 50 years as a psychiatrist and psychotherapist. It gives his chapter the character of an integrative synthesis.

In Chap. 2, Anton-Mlinar proposes the phenomenological method as a valid way to study the essentials of human specific psychic higher levels. They include intentionality, motivation, and empathy. The role of motivation and its dynamic play promoting intentionality marks a qualitative difference with a merely causalist psychology, governed by necessity. The role of phenomenology is also remarked as a way to avoid consciousness as an elemental function with a physical localization. She sustains that consciousness is a "sphere" of convergence. The premise is rigorous and gives a solid basis for approaches to psychopathology. Some very promissory windows are here opened by Anton-Mlinar.

In Chap. 3, Sanguineti has developed a historical review of conceptions of different ways to conceptualize the relation between body and mind. It includes different positions, discussing different thought lines under the light of philosophy and recent scientific evidences. Arguments of behaviorism, functionalism, emergentism, enactivism, mind, and mental acts are discussed. The risk of reductionism is also detailed. The chapter rescues the notion of the human being as a person and constitutes the summit of a meditation of years, a tradition of thought.

In Chap. 4, Crespo uses the study of human activity, the economy, to highlight the differences between the scientifically measurable levels and human activity. He defends the possibility of facts and realities beyond a physicalist and materialist position. He postulates realities beyond matter. It should be considered as concrete evidence of previously postulated existence of levels that cannot be mechanically reduced to physicalist worldview. The chapter constitutes an example of exceeding reductionisms, written with an elegant and precise style.

In Chap. 5, Aranovich proposes psychotherapy derived from the philosophical position of Ortega y Gasset. From that point of view, some relevant philosophical concepts are proposed as analyses points and objectives of psychotherapy. It is a case of vocation, life project, and authenticity. Again, the question of a reality not directly reducible to matter emerges, and culture is conceived as an example of spirit or mental level. A complete and extensive description of the lines of Ortega with potential use solving problems of

everyday life is proposed. An important trajectory as psychiatrist and psychotherapist is conjugated with an interesting philosophical attitude, leading to an interesting proposal.

This part ends with Chap. 6 dedicated to bioethical considerations about the last edition of The Diagnostic and Statistical Manual of the American Psychiatric Association. In that chapter, Gamboa Bernal discusses many new events related to the use of this instrument. The fact that several illustrated opinions led to a cautious use of the new manual is mentioned in the chapter. The fact that the general public has reached the opportunity to express their opinion is a not usual antecedent in psychiatry. Additionally, the mode in which the manual should be used and the bioethical aspects related to it are discussed in the chapter. Some inconsistencies in psychological assumptions are criticized, giving an overview and pointing social implications of psychotherapeutic practice.

The second part is dedicated to some basic neuroscience lines with potential clinical projections. Again, the purpose is not to be exhaustive presenting all possible lines that may imply consistent extrapolations to clinical practice. The idea is to show, as paradigmatic examples, some lines in course that may clarify important mechanisms related to mental illnesses. Our knowledge about somatically based illnesses as well as psycho reactive disorders could be benefited by these research lines. Additionally, these investigation lines may be perceived as a challenge for future research projects with similar purposes.

Chapter 7 is dedicated to the functions of the renin-angiotensin system (RAS). New functions have been assigned to these structures. Since its well established role in the regulation of the blood pressure, some additional roles have been found for this system. In that chapter, the group of Paz, Marchese, Bregonzio, and Baiardi detail the new trends in the knowledge of RAS. Its involvement in processes related to psychostimulant drugs and amphetamine sensitization are interestingly described. Additionally, a role is proposed involving this system with alcohol consumption.

In Chap. 8, this group, now including Bregonzio, Marinzalda, and Baiardi realize an overview of the role of the neuropeptide angiotensin II in stress and related behaviors. Again, our knowledge about the role of RAS is expanded and enhanced in a clear manner. The role of AT1 receptors is described and clarified. The chapter constitutes a relevant actualization to our knowledge regarding stress and its mechanisms. The relevance of stress inducing some diseases is also raised.

Chapter 9 constitutes a new important contribution from the group of Marchese, Casarsa, and Bregonzio. The implication of the RAS in neurovascular cognitive functions is exposed. Consequences of inflammatory processes on microenvironment composed by small blood vessels within the brain parenchyma and its consequences, leading to vascular dysfunctions and blood brain barrier disturbances are analyzed. The relation of inflammation with brain and cognitive disorders is postulated, and the role of angiotensin receptor blockers is also suggested. In these three chapters the teams led by Baiardi and Bregonzio summarize a brilliant career of these promising and young researchers in Argentina and the United States.

Chapter 10, written by the group of Trofimiuk and Braszko, is given a support for new treatments for stress-induced cognitive impairment. The use of antidepressants and new anticonvulsants is discussed. The search for natural products is also exposed. The use of angiotensin II AT1 receptor antagonists, and H3 receptor antagonists is also proposed, opening new ways for pharmacological treatment of stress-related disorders. The convenience of a change of habits is also commented on, giving a clinical projection to the findings of this solid and prestigious research team.

Chapter 11, written by Anzulovich Miranda, is directed at the interaction of cognitive variables with circadian rhythms, describing learning and memory processes related to a circadian endogenous clock. Brain structures related to these functions are individualized, and consequences of the disorders are also considered, establishing interesting correlations between clinical and basic research. Relations between environment and organism are considered, taking into account the dialog between an organism's internal processes and environment, and the relation with cognition is established. An interesting view that integrates behavior, molecular biology, and clinical projection distinguishes the chapter, showing a remarkable ability of the author.

Cognitive function is considered from another angle in Chap. 12. The workgroup of Izquierdo, Urrechaga, Llorente, and Escanero detail an interesting body of evidence regarding the role of iron and other trace elements in the development of cognitive function. The role of toxic compounds on cognition's development is also discussed. A correlation is established between metabolic disorders and human illnesses. Iron and copper toxicity are described and related to cognitive function and brain neurodegenerative disorders. Basic research is again bound to clinical facts.

Chapter 13 is dedicated to considering new glutamate-mediated mechanisms related to benzodiazepine dependence and cocaine vulnerability. This group, integrated by Artur de la Villarmois, Gabach, and Pérez draw a prolix and interesting state of the art. Hippocampus and medial prefrontal cortex are signaled as structures clearly related to mechanisms of drug addiction and withdrawal syndromes. Some considerations about vulnerability are also suggested. The potential role of nitric oxide as a neuromodulator and its effect on psychostimulant actions is also described. Once again, an interesting and relevant bridge is constructed from basic research to clinical realities by this group.

Chapter 14, written by Nina Estrella, is dedicated to the medical effects of isoflavones. These compounds were initially used as a tool for climacteric symptoms prevention. The use of phytoestrogens and the potential role of soy isoflavones in menopausal affective symptoms is widely exposed. Some recent personal research findings are cited by Nina Estrella, and the potential role of isoflavones in depression, anxiety, and cognitive symptoms is discussed. In a broader sense, the possibility of using natural products to relieve senescence symptoms is contemplated, considering the collateral effects of conventional medicines and rationality for use. New and promissory trends are suggested by the author, Nina Estrella, Ph.D., who has been a doctoral thesis student in our team.

In Chap. 15, a general view about rationality of psychopharmacological treatment is studied. The group of Panini, Garraza, Teves, Giraudo, and Calderón proposes a reflexive consideration about rationalities and irrationalities of medicine prescription. Uses and abuses of psychopharmacological drugs are evaluated. A prudent and rational prescription is proposed, avoiding medicalization of everyday life. Pharmaceutical promotion is considered an important element in increasing prescriptions. The interpretation of some psychological processes as illnesses and its corresponding prescriptions are analyzed and some critical considerations are realized by this solid team, dedicated to rational use of medicines. They bring rationality to the use of psychoactive drugs.

The third part is dedicated to neurosciences, learning, teaching, and the role of the social environment. The development of cognition and its relation to social environment is highlighted. The part begins with an interesting conceptualization about experimental approaches and evidences in the field of neurosciences and cognition. As a continuous line in this thematic field, some clinical studies try to give support to educational interventions along with the learning process, from childhood to adulthood. Our knowledge about development and intellectual limitations may be enriched by current reviews. Additionally, it has important value in prevention of related disorders.

In Chap. 16, Díaz Véliz builds an interesting bridge between basic and clinical evidence on the issue of learning styles. Recent concepts about learning, memory, and neural plasticity are reviewed and actualized. Learning paradigms are related to styles and preferences. The change of learning and behavior as a result of experience is discussed, and experience as a fact closely related to neural plasticity. Considerations about student learning styles and studies by the author in this field complete the interaction of basic and clinical evidences. A solid scientific formation is associated with a long and rich university teaching experience that is integrated by the author in an interesting manner.

Chapter 17 is in some senses a continuation of the previous one. In it, a large group, integrated by Escanero, Soria, Guerra Silva, and our team, dedicates a study to the tool selection in studies of learning styles. Special attention is paid to some tools that we have previously used in multicentric studies on learning and teaching. Cognitive, metacognitive, and socio-affective strategies are commented on and discussed. The main objective of the current studies is to detect areas in which the scores are lower, and to propose a strategy aiming to improve them. All these efforts may improve significantly learning and teaching. The current chapter involves a trajectory of empirical research, multicenter studies, and meta-analysis.

Chapter 18, written by Ison, constitutes an interesting exposition about lines in course in her laboratory. The use of computational programs with the purpose of training and improving attentional levels in children is the central topic of the study. The role of attention in integration and processing of information is the starting point for the studies. As in the previous chapter, relevance is given to cognitive, affective and social factors involved. A psychoeducational intervention is proposed, mainly based in intervention programs designed to improve and strengthen attentional efficacy and attention

resources. A singular talent implementing intervention strategies directed to specific objectives is exhibited by Ison.

In Chap. 19, the role of social context is highlighted based on the field studies of this group. The authors, Bonantini, Cervigni, Mandolesi, Quiroga, and Gallegos review some of the findings of their previous studies with the aim of showing the relevance of the social context in mental health. The concept and correlation between vulnerability and mental health is discussed. The role of social mindset, social representations, and collective imagery is defined and integrated in an interesting and organic point of view. An important merit of this study and this workgroup is to highlight the value of relational links signaling the importance of social context in mental health.

The fourth part is dedicated to explaining human pathological behaviors. The idea is to highlight the existence of mental illness of body or somatic base. We are in the strict field of "explanatory psychology" in the sense of Jaspers. The psychopathological disorders presented are caused by somatic alterations. In this field we are talking about psychoses, in the sense of Schneider. We have somatic illnesses causing behavioral disorders that may not be "understood".

In Chap. 20, Cavicchia and Acosta describe relevant relations between basic evidences and clinical pain evidences. The chapter continues with a prolix description of nociceptors. Its importance and its role protecting the body's integrity from dangerous situations are described with a clear and didactic style. The maladaptive condition of chronic pain, as a false alarm, is also considered and explained. Some possible solutions to the problem of chronic pain are suggested, taking into account the physiological and pathological evidences about acute and chronic pain and its consequences. Cavicchia and Acosta exhibit an interesting ability linking basic evidences to clinical problems.

In Chap. 21, Bermudez and Lafuente review the neuropsycholigical disorders caused by concussion or mild traumatic brain injury. Damage mechanisms are detailed, including cellular death, cytoskeletal changes, and axonal dysfunctions. The consequences are referred, including the possibility of asymptomatic states, but usually manifested as headache, dizziness, anxiety, or insomnia. The conceptualization of a post-concussional syndrome is delineated. Predisposing factors and prognostic are also clearly exposed. A complete and clear description of the main clinical manifestations of this disorder is presented by this prestigious group.

In Chap. 22, Gouveia and Márquez de Brito describe an interesting schedule about behavioral animal models in neurosciences. The idea of animal models is discussed, taking into account possibilities, limitations, and validity. These behavioral models, closely related to experimental psychology, have been considered a very important tool to study somatic alterations linked to psychopathological behavioral disorders, but also to design and understand psychopharmacological treatments. In an interesting synthetic way, the authors give a clear explanation of this topic, with a noteworthy didactic value.

Our research contribution is detailed in Chap. 23. In the study, the team integrated by Mesones and Gargiulo talked about biological markers in

psychiatry. A historical approach is realized. First pioneer argentine studies on trace amines are reviewed, and its application to clinical practice is detailed. In a second moment of markers proposals, the theory of brain windows was an interesting step that is detailed. It implied mainly the idea of psychoneuroendocrinology as a way to study brain circuitries implied in hormone responses and, eventually, in brain responses to psychotropic drugs. Clinical neurophysiology was a next interesting attempt to evaluate brain function and responses to drugs in an objective manner. Electroencephalography, brain mapping, and event-related potentials were extensively studied in a posterior period. Its correlation with psychometric tests is commented on. An international bibliography regarding this field is reviewed. Finally, 50 years of argentine contributions to biological psychiatry and related preclinical translational research are reviewed, including our studies and publications in these fields.

In Chap. 24, written by the team integrated by Klug, Hill, and van den Buuse, the authors actualize important topics about schizophrenia. They describe the main symptoms and the accepted pathophysiology, followed by explaining the "two hits" theory of schizophrenia. A group of genetic and environmental factors appear to be necessary generating schizophrenia. The effect appears to be present if a combination or synergism of both kinds of causes is present. Following, this group describes evidences of the possible role of the brain-derived neurotrophic factor (BDNF) in schizophrenia. In a clear proposal, the main new ideas about schizophrenia pathophysiology are described by this internationally recognized team.

Chapter 25 is dedicated to therapeutic possibilities in the field of schizophrenia. In it, the team of Dubroqua, Singer, and Yee proposes a new possible strategy for schizophrenia treatment. The *N*-methyl-D-aspartate (NMDA) glutamatergic hypofunction theory is described, and the possibility that a NMDA receptor coagonist, glycine, may improve this situation without an excitotoxicity risk is postulated. Evidence is then presented proposing the use of bitopertin, a blocker of the glycine transporter 1, as a new pharmacological alternative. Preclinical evidences are summarized. The authors, members of an exceptional workgroup, show didactic abilities and a solid scientific background in a conjugated manner.

Other possibilities in schizophrenia treatment are suggested in Chap. 26. The team integrated by Ciruela, Fernández-Dueñas, Contreras, Arnau, Menchón, Vallano, and Valle León proposes a role for adenosine in the neurobiology of schizophrenia, and a possible pharmacotherapy based on it. Interactions of dopamine and glutamate are explained, mentioning the insufficiency of present treatments. For this reason, the study of alternative neurotransmitter systems is justified by the authors. Beginning with the well-known role of adenosine as neuromodulator, the authors mention that this compound appears to modulate not only dopamine, but also glutamatergic transmission. This solid team proposes, in a new approach to schizophrenia treatment, targeting adenosine receptors as additional ways to treat this illness.

In Chap. 27, an interesting window viewing brain superior functions is opened. In a brief synthesis, the team integrated by Fayed, Cifre, García

Campayo, and Viguera describes present evidences about neuroimaging in mindfulness. Brain changes during meditation are described and evaluated. The role of functional magnetic resonance during performance of cognitive tasks or at rest are analyzed in a prolix manner. The role of magnetic resonance spectroscopy of the brain is analyzed as a tool designated to allow biochemical analysis associated with neuronal integrity. Practice of mindfulness is discussed taking into consideration present evidences given by these techniques. Direct clinical schedules are presented by a solid and brilliant team, correlating subjective states to objective parameters, in a promissory way.

Fayed, Viguera, and Garcia-Campayo develop an interesting review about clinical magnetic resonance in Alzheimer's disease in Chap. 28. The use of biomarkers is postulated as very convenient in Alzheimer's disease, and some of them are mentioned. The use of magnetic resonance spectroscopy is discussed and proposed as a less-known biomarker, but it appears to be a useful one in a cross-sectional and longitudinal study. The authors show a good correlation between N-acetylaspartate levels and the progression of Alzheimer's disease. Other neuroimaging tools are also mentioned. The possibility of new objective parameters linked to prevention and prognostic are suggested by this recognized and promissory group.

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Pascual Ángel Gargiulo

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Part I

Epistemological Considerations About the Study of Normal and Abnormal Human Behaviors

1 Knowledge, by Philosophy or Science? Psychotherapy or Neuroscience?

Humberto Luis Mesones-Arroyo

The Study of Human Beings

Trying to understand human nature is a complex task in which many disciplines must cooperate. Difficulties arise from partial findings or different designations to meanings of words. Scientists, philosophers, and theologians are prone to pursue their own speculations, thus disregarding or denying the views of others.

Human beings have been defined as beings who think. Technical advances defy the conception of a sense in life that is superior to the evidence of brain at work. To be and think, or, I think therefore I am, is understood by modern science in a light conception as a product of metabolic interrelationship of neurotransmitters. To be is concealed behind scientific facts [1]. Some authors have written about how the brain is responsible for personality, stressing too much, the undoubtedly important function of the CNS for cognition, communication, and relation to the world [2]. Anthropology is not reducible to a single subject or method of study. Physical, biological, cultural, social, and historical factors show partial sides of a multifaceted reality. Time and place usually change human behavior without modification of its nature, or, precisely because of it.

Anthropology can be defined as the naturalistic description and interpretation of the diverse features of mankind. There is no single method of study, but most anthropologists seek to use direct observation of human beings in their particular society, time, and place. In the nineteenth century, biological evolution was the theory that inspired the idea that primitive social organizations would help to understand mankind because they were thought to be genuine and not yet altered by cultural interferences. That ideological bias made western ways and knowledge suspicious, and the conclusions were forced to be confirmations of the starting prejudice.

Many ethnologists and archaeologists tend to concentrate on small societies assuming that they are original examples of how the total psyche and mentality of mankind began its evolution. Culture, beliefs, ecology, and linguistics are some of the additional characteristics that need to be explored in an attempt to understand the essence of the anthropological subject.

Recent advances in genetics show findings in the complex structure of living creatures.

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Genotype and phenotype are two concepts that cannot be omitted by investigators, and many new facts are arising from Genomics. The profound significance of the work by Gregor Mendel (1822–1884) was not recognized by scientists until the beginning of twentieth century, and only in these past years of the twenty-first century have his findings evolved to a very important branch of biology. In June of 2000 the world learned of the decodification of the human genome. Worldwide collaboration has concentrated on the Genome Wide Association Studies (GWAS), a cybernetic site where investigators find the news in real time. New techniques of screening by DeoxiriboNucleic Acid (DNA) micro-arrays will hopefully allow for the prevention, diagnosis, and treatment of genetic conditions. Single nucleotide polymorphisms show predictive traits of neurological diseases. Heritability of many disorders in psychiatry has been known since the time of Hippocrates, but it is now understood that the gene and its ADN code transmits the protein anomaly, not behaviors. Genotype and phenotype influence each other. The future of genetic investigation in psychiatry should be dedicated to: (a) discovering how much heritability means in the etiology of mental disorders; (b) how this influence is exerted, and (c) how mutations can start pathology and how it can be prevented [3].

Pedro Laín Entralgo [4] has described the different levels of analysis that must be made in relation to the complex reality of mankind: science, philosophy, and theology. The first, and lowest, tries to explain how a human being is made, its anatomy, physiology, even pathology. Philosophers think about what kind of being man is, and what his place and function in the world is, his relations to his fellow men, time, and space. If the object of observation and experience is real, imagined, or ideal it can be the subject of science, but if thought reflects on essence, it will need to extend further than physics: metaphysics. And the difference will be methodical. Theology explains who man is and the sense of life, however, that all depends on belief.

Karl Popper said that the world does not confirm truths, but it refutes mistakes, and, anyway, the world exists and so does truth; the problem is that we are not sure about every detail [5]. "Being conscious of the fallibility of science is what makes the difference between a scientist and a scientificist" [6].

Psychiatrists must keep in mind the importance of this anthropological scope and accept the different methods of seeking the truth. Psychiatry, being a branch of medicine is also praxis, meaning the art of healing illness in an individual subject called a person. One of the best schools of thought up to now has been phenomenology.

Phenomenology is the study of structures of consciousness as experienced by the person point of view. Essential components of mind are intentionality and the meaning of an object or an experience. It basically defines the structure of the various types of experience ranging from perception, thought, memory, imagination, emotion, desire, volition, body awareness, social and linguistic activities; and what they mean, because meaning explains intentionality.

This particular branch of philosophy offers a theory of human subjectivity indispensable to understand, explore and treat psychiatric disorders, and there has been a "mutual enlightment" with cognitive neuroscience. By analyzing the modes in which our experience is constituted, phenomenology is capable of detecting the critical points where this comprehension is vulnerable, mistaken and open to deviations that appear as psychiatric symptoms.

Additional fertile ground is in pathology. Many discoveries occurred from observation of functional losses after injury, and paved the way for treatment of disabilities. Again, Pedro Laín Entralgo points out how much illnesses can teach about human beings. He looks back into the history of medicine and how illnesses were studied and treated in the successive ages and concludes that pathology was basic in how man was understood, and proposes to speak of Pathologic Anthropology [4]. Depression, anxiety, schizophrenia, delusions, obsessions, and neurosis are human ailments, and suffering can push men to limits against the normal and healthy wish to live, such as suicide.

Phenomenologists, for example Viktor von Gebsattel [7], characterize psychology as being necessarily personal and objective-oriented. When disorders alter normal freedom, people lose their sense of life and the disability modifies their "being in the world". Profound thinking about the meaning of a relationship with another person and the way to seek transcendence guides psychiatric art in its motivation to heal is necessary.

The reflection of philosophers and doctors about man, has necessarily confronted the mystery of body and spirit. Science and its methods cannot find an explanation for human beings. Psychiatry is the branch of medicine dedicated to the study and treatment of disorders in this crossroads. Medical anthropology received attention and awareness many years ago when Ludolf von Krehl, Viktor von Weizsäcker, Karl Jaspers, Max Scheler, and many more, had the opportunity to meet, think, and speak in the towns of Halle, Heidelberg and Freiburg. They integrated the new psychological dimension that had begun with psychoanalysis, and all the advances that technology was adding to physiology and human relationships with their fellow men. Psychosomatic medicine is one concept still in use, but it also means an unacceptable division of the integrity of human beings [8].

Anthropology is a basic step necessary to understand human characteristics for individual and social behavior. A person's well being and ailments or suffering depend on the normal or abnormal functions of organs and qualities. However, the advances in neuroscience do not explain normal behavior, only the basic predispositions to these or other reactions and pathology. Anthropology, neurobiology, social sciences, or neurosciences are partial studies of the "mystery" called man, although every one of them shows part of the truth and enables future advances.

Gnoseology Versus Epistemology

Gnoseology should be defined as the branch of philosophy that studies the nature, structure, importance, and limits of human knowledge. Two ways of understanding thought, and maybe even practice, in science are put into evidence by the following two words. Zamboni (1875–1950) suggested that gnoseology should be etymologically accepted as the science that studies knowledge in general.

According to James Ferrier (1808–1864), epistemology was more precisely focused on the acquisition of scientific information and mechanisms. We highlight the differences between both concepts and their use, because many conflicts have arisen by confusing or opposing science and philosophy [9].

Both areas of human interest and labor have the utmost importance, but their objects of investigation are different and need different methods. If we don't understand what the words mean, or at least what they mean to us and for someone else, we will encounter problems.

Not many years ago, small groups of scientific investigators were convinced that anything that could not be proved by experimental science should be discarded as nonexistent. The Wiener Kreis, founded by Moritz Schlick, published a Manifesto in 1929 with the idea of explaining the world only through scientific evidence [10]. They believed in empirism (Hume and Locke), but even scholars respected by them such as Karl Popper or the second Wittgenstein criticized their fundamentalism that left out of scope metaphysic. Popper states that he who is not interested in spirituality should be asked to leave [6].

Wittgenstein stresses the need to be conscientious of language: "there is no private language", and we should try to communicate being sure of the correct use and comprehension of words. "Meaning is related in the first place with reality, but also, and maybe more, with the meaning assigned by a group of persons" [11]. He also stresses that words change during the course of time and different societies can find difficulty in understanding each other because of language barriers.

Neuroscience is strictly empirical and its methods must be respected. However, psychiatry must be open to the many other human dimensions. "The future of neuropsychiatry is as limitless as

the human mind. Our learning about neuropsychiatric disorders will undoubtedly accelerate, and so too will our understanding of the mechanisms of the mind such as memory, learning, mood, emotions, fatigue and sleep. This era will be an exciting one for neuropsychiatry" [12].

Recent Trends in Psychotherapy

Healing by Word in Classic Antiquity is the title of a book written by P. Lain Entralgo [13]. The efforts to relieve stress or suffering have a very long and noble history in medicine. Psychotherapeutic methods have been the first choice in the cure of emotional ailments. Many of the current schools of psychotherapy can trace their techniques back in time.

Music has been a healing method used since the ancient cultures of Egypt and Greece, but we can accept that medicine men of any time and society have made patients better with rhythmic and melodic sounds, sometimes in conjunction with dance.

Moral treatment was proposed by Philippe Pinel under the assumption that insanity was the result of social and emotional stress. Organic diseases were later deemed the culprits, and psychotherapy was put aside by anatomo-clinicians until Freud began the psychoanalytic revolution, suggesting that all illnesses were a result of psychological factors.

All psychotherapies have the fundamental force of personal privileged relationship between patient and therapist in common. Some may underline spiritual beliefs; others stress directive interaction or permissive methods. Group therapy, systemic techniques, art therapy, psychodrama, and many other empirical experiences are in practice at the present time.

New psychological instruments are measuring and comparing the efficacy of the abovementioned treatments [14]. Some results favor cognitive and behavioural techniques are the more positive and short. The assessment instruments need accepted diagnostic and classification criteria, which is one of the difficulties specifically discussed here because the American Psychiatric Association has implemented Diagnostic and Statistical Manual of Mental Disorders (DSM) 5. The change from categories to dimensional measures has been immediately critiziced by former experts and by the National Institute of Mental Health (NIMH). Research Domain Criteria is the formal proposal of the NIMH, and funding for research will be assigned if investigators comply [15].

The conclusion upon completion of many reviews is that success depends on mutual comprehension and empathy. Experience on the side of the therapist and confidence from the patient allow a good prognosis. The ability to change the technique, adapting it to the personality and needs of each individual must be stressed when educating the therapist.

The World Psychiatric Association, with its Section of Education in Psychiatry, has designed two important documents that should be accepted by member national associations: The core curriculum in psychiatry for medical students and the core curriculum for the postgraduate training in psychiatry [16]. The Section of Psychotherapy requires that "for graduation, programs must certify that residents have become competent in at least five forms of psychotherapy, i.e., brief, cognitive-behavioural, combined psychotherapy and psychopharmacology, psychodynamic and supportive therapy, with competency assessed by supervisory reports, video, oral exams, case reports, direct observations or other measures" [17].

Latest Trends

There is a long tradition of objective quantification of social, economical, and health problems in the United States. Recent comparison of psychotherapy and medication for mental disorders used by the public alert health authorities and practicing professionals of a dramatic change: "…antidepressant medication is clinically effective across the full range of severity in major depressive disorders"; "…in addition, specific forms of time-limited psychotherapy are as effective as antidepressants for mild or moderate

depressions"; "…better tolerated antidepressants, increased penetration of managed care, development of rapid and efficient procedures for diagnosing in clinical practice" also explain that in 10 years (1987–1997) the use of medication increased from 37.3 to 74.5 % and the use of psychotherapy dropped from 71.1 to 60.2 % including a reduction in visits from 12.6–8.7 per user per year.

"In particular the development of *cognitive behavioral therapy* by Aaron Beck, which is a usually short-term psychotherapy focused on identifying and correcting cognitive patterns that underlie emotional and behavioural symptoms; *interpersonal psychotherapy*, a time limited therapy developed for treatment of major depression; and *dialectical behavioural therapy* , a focused therapy developed for treatment of borderline personality disorder, have been tested for efficacy in controlled trials" [14]. The problem seems to be what type of psychotherapy is most effective for what type of problem.

Diagnostic criteria have singularized clusters of symptoms as new, or newly recognized, disorders. One of them is social anxiety disorder, and can be an example of the combined treatments that offer the best efficacy in psychiatry. Psychopharmacology cannot be discarded, but it shows much better results if it is associated with psychotherapy. Four different techniques have been approved after comparative studies, and which one is best for a particular patient will depend on the abilities and experience of the interdisciplinary team. Social skills training, exposure in vivo, cognitive therapy, as well as a combination of them, have been explained by Oosterbaan and van Dyck [18].

However, a great deal more neuroscientific investigation is necessary to combine the healing force of psychopharmacology and the many new psychotherapies. For instance, music therapy has proved to influence the brain on both subcortical and neocortical levels. Brain imaging can pinpoint the areas where music is changing blood flow and electroencephalogram (EEG) waves. There is evidence of neonates hearing music from the womb that they can recognize after birth. Premature children benefit from musical stimulus that strengthen the force and speed of sucking, and probably also help in promoting growth. One special technique is called neurological music therapy and seems to train motor responses such as tapping the feet or fingers and body movements. Clinical experiences evidence useful aid with autism patients, who in many cases have a very acute musical sensibility. There is an inspiring book in the Spanish language entitled *Síndrome de Mozart?*, by Gonzalo Moure [19]. We should re-read the story of Joseph Knecht, in which he is guided to music by the Magister Musicae in the first chapter of *The Glass Bead Game* by Herman Hesse [20]. Incidentally, music is in its own category because of the importance of its history and volume of experiences.

Art therapy has many different ways of approaching and assisting with the needs and abilities of a patient. Art has been interpreted by psychoanalysis and artistic work by patients has been shown as to be a sign of disorders and rehabilitation, as in the well known case of Vincent van Gogh. Art therapy is another therapy in need of interdisciplinary investigation.

Dance therapy differs from art therapy, as it stimulates nervous coordination, self control, and expression of feelings in patients with broad types of pathologies. María Fux is a worldwide respected teacher of this technique and therapy [21]. The American Dance Therapy Association defines its goal as "the use of movement to promote the emotional, cognitive, physical and social integration of the individual".

Pets help people suffering loneliness and grief, however, dogs do not only guide the blind, and horses do not only rehabilitate spastics and vascular or traumatic brain-injured patients. Equestrian or hippo therapy has developed in Argentina and many patients benefit from it and from the different associations organized in the country. "Dogs and horses are part of an interdisciplinary team that aims to give patients with physical, social or learning disabilities a better quality of life" [22]. Interaction between patients and pets, or the need to command the horse, stimulates coordination and cognitive abilities in many ways.

Psychotherapy in Clinical Practice

Professor Jorge Insua stresses the significance that the doctor–patient relationship is always psychotherapeutic [23]. "It may be inadequate or even iatrogenic but never indifferent". If the doctor keeps this truth in mind he can diminish the danger of violating ethical limits: "If my religion makes demands on me, it probably proves beneficial to others; but if my religion attempts to control the behavior of others, it is almost certainly harmful to them" [24].

The duration of psychotherapeutic treatments can be decided according to the pathology, setting, evolution, or theoretical standpoint. But the longest are those that practice the family doctors, or rural practitioners, that become friends and lifelong confidantes.

The relationship with a patient begins before meeting, because suffering induces the move to seek help, and that is oriented by the prestige of the practitioner or the institution, and the opinion of relatives or friends. A book was written about the importance of the first five minutes of the meeting, stressing details such as opening the door by the doctor or telling the nurse to introduce the patient, alone or with companions, a handshake, a smile, the first words of welcome, etc. Transactional analysis has stressed the attitudes, words, and gestures that can make psychotherapy easier for the patient from the start [25].

A semistructured interview allows for a more personal relationship than rigid protocols. The reason for the visit can relate to childhood significant events, organic illness, and work or economic situations. The description of psychiatric symptomatology must be relaxed through questions about sports and friends. During the first meeting the practitioner should try to obtain at least a smile from the patient, which is a sign of having been understood and accepted.

Of utmost importance is finding out what is the patient's belief about the cause of his or her illness. Having performed a physiopathological diagnosis, it is also necessary to complete a phenomenological portrait of the patient's personality and his or her possibility of beginning and benefiting from psychotherapy. A person with conscience of reality and sense of duty can be a better candidate for cognitive therapy. If there are obsessive or phobic symptoms, the behavioral techniques will be more efficacious.

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Phenomenology as an Approach Method in the Neurosciences

2

Ivana Anton Mlinar

Introduction

Because of the objectively justified needs for specification and further delimitation of problems and methods for investigation, the increasing specialization of most scientific spheres has led to the differentiation and isolation of perspectives, and to losing sight in this dispersion of the whole, of the unity. However, the cognitive sciences, neurosciences, and psychiatry show a renewed philosophical interest in topics such as subjectivity, consciousness, intentionality, and the body, which is especially manifested through their dialogue with, and appropriation of, the conceptual and methodological tools of phenomenology, resulting from the realization that it is necessary for the results of their scientific investigations to be contextualized and evaluated in light of broader frameworks.

This chapter aims to present the fundamental elements of the phenomenological method, together with some defining contributions of its concept of experience which would on the one hand permit determining the legitimacy (or otherwise) of its use and interpretation by neurophenomenology and, on the other hand to warn against and overcome the neurologic determinism ruling the cognitive sciences and failing to give account of the whole and the unity of selfhood, of the person. For this purpose, we first describe the phenomenological method; second, the phenomenological comprehension of corporality from the perspective of the distinction between the lived body [*Leib*] and the (physical) body [*Körper*], as a key contribution allowing a glimpse into the personal unity; third, in order to point out the originarily motivational character of the passive stratum of the constitution of experience, we refer to a key phenomenological distinction between causality and motivation,. In this sense, the phenomenon of pain becomes paradigmatic when to showing the enigmatic unity of intentional body that resists its identification with the neurovegetative and objectifying level of the third-person perspective. These analyses of the lived body lead us to explore the phenomenological concept of empathy in order for the results obtained to be validated for the scientist or therapist to access other bodies or egos. Finally, we skim through the program of neurophenomenology, pointing out some principles and procedures that we consider misleading or being mistaken in their understanding and application of phenomenology.

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The Phenomenological Method

Phenomenology is a reflection on phenomena, on what shows itself. When we say that something shows itself, we say that it shows itself to us, to the human person. We are the ones who search for the meaning of what shows itself. In this sense, rather than "things reveal themselves", we should say that "we perceive, we are directed towards things" and we treat them consequently as phenomena, and we can come to understand their sense. Husserl has held that it is appropriate for human beings to seek sense, in such a way that it is not the fact that something exists that is of interest, but rather the sense of this fact. That is why we can "parenthesize" the existence of facts to gain access to their essence.

Why do human beings seek sense? How are human beings constituted? Husserl's novelty lies in his analysis of the subject who looks for sense, which is the second element of the phenomenological method that follows the question about the sense of phenomena. In order to arrive at the sense, an act, such as a perceptive one, needs to have taken place; we have perceived something that was before us. This mental process [*Erlebnis*] consists of the act of seeing and of the seen; it also applies to touching and the touched, to hearing and the heard, etc. The physical thing, as an existent, is outside, there, before us, but as seen, touched, heard, etc., it becomes mine, although, in turn, there exists a difference between the seen or touched thing and us, who are seeing or touching.

Husserl characterizes consciousness by intentionality, that is, by its character of always being a consciousness *of* something. This means that it is not a field that is closed onto itself, but rather goes beyond itself in order to reach objects. It turns to a something in many ways; that is why we can distinguish among several modalities of intentionality: perceiving, remembering, expecting, judging, desiring, etc.

Seeing, touching, etc, are sensations lived by us; they are mental processes [*Erlebnisse*] (i.e., they are registered by us and we are aware of them). All the acts that we register have different characteristics and qualities. Husserl pointed out that touch is the most important sense, because through it we register the confines of our own body, and thanks to it we can orient ourselves in space. The sense of touch gives us the sensation of our body and of external objects. It is through the register of the acts of the senses that we can say that we have a body and that we "have it" always with us (i.e., corporality locates us). In this respect, our lived space is the basis of all our concepts of space: it is even prior to the geometrized space of physics. Therefore, it is not correct to maintain that there is interiority and exteriority, but rather interiority and "there", the register of the acts that allows us to be aware of exteriority.

When we analyze acts we find different types: an act of annoyance because of a noise is not the same as the impulse to drink or the abstinence from drinking despite the impulse. They are corporal (thirst), psychical (annoyance), or spiritual (reflection, valuation) acts. It can be appreciated that the human structure appears as a unit of body-psyche-spirit, and they are precisely what constitute the consciousness that Husserl describes. Consciousness does not reside in any physical location, in any of these dimensions, but, on the contrary, it is through consciousness that we can notice and distinguish these dimensions. It is a "sphere" of convergence through which it is possible to give an account of these dimensions.

Phenomenology of Corporality

The (Physical) Body [*Körper*] and the Lived Body [*Leib*]

In the phenomenological analysis, which departs from the perceptive act in order to get to consciousness, Husserl is driven to the analysis of the lived body, given that things display a variable orientation in relation to the absolute here instituted by one's own lived body. It is noticeable that the latter has a unique preeminence in relation to other bodies or things, because it is the condition of possibility of the multiplicity of presentations of the other spatial objects, and

because, in addition, it is not possible to move away from it. In contrast to the mere material body, Husserl characterizes the lived body as an animated body that is both the "carrier" of an ego and a physical body. It is thus doubly defined as a "psycho-physical unity". Therefore, he distinguishes two attitudes from which it can be considered: an internal one, in which the animated aspect is predominant, and an external attitude, in which it is constituted as a physical body, comprehended as nature.

The lived body shows constituting functions that allow the very access to the world, to objects, and other subjects. The body that functions as an "organ of perception" [1] (p. 144) knows itself in its kinaesthetic freedom: it has the possibility of voluntary movement. This means that I not only experience a sensation of movement when I observe an insect, but that I can also be aware of this movement as "I move the eyes". Kinaestheses are sensations of movement relative to one's own body (i.e., and the impressions that take place when parts of the body or the organs of perception move). Every perception is consequently possible only through a "start of the ego" [*ichliches In-Gang-Bringen*] that consists of a "happening in the character of the 'I can' or 'I do'" [*GeschehenimCharakter des 'ichkann' oder 'ichtue'*] [2] (pp. 108, 164). Kinaesthesis refers to an autonomous type of happening that is possible or departing from the ego. Sensations of movement not only have a constitutive role for the appearance of the thing, but also participate in the appearance of the body itself, whereas at the same time, they are experienced as "located" in the body. Husserl took the descriptive content of this physiological concept because it is relevant for the phenomenological analysis of constitution, as it is clear that bodily sensations constitute an essential component of the experiential constitution of spatial corporality. In other words, sensations of movement accompany both the movement of the subject that perceives and the movement of the perceived object. Although unlike physical data belonging to other fields of sensation that enter into the unity of the appearance of the thing, kinaesthetic data do not belong to the "projection" of the thing. Therefore, as had already been mentioned, they constitute an autonomous type of subjective happening through which we are aware not only of the sensation but of a spontaneity of the *ego* as a principle of action.

Causality and Motivation

The descriptive features of the lived body [*Leib*] and its distinction from the mere body [*Körper*], which equates in its conscious constitution to the experience of other physical bodies or things, reveal the essential place that kinaestheses have as "mediators" and defining components of the lived body: these kinaestheses permit precisely both the constitution of bodies or things and one's own lived body, distinguishing it from the rest of the bodies as merely physical. In this sense, another differentiation emerges: that between causality and motivation. For Husserl, the notion of nature alludes to a specific spatial–temporal stratum of all experience that, therefore, founds all the superior strata of knowing and valuing. We recognize a stone or a tree as such because nature does not consist of a field of facts or appearances that we refer to as an *in-itself* without sense, but it is an intentional correlate, and we take it in its immediately constituted sense. The causality of nature then appears as "an empiric fixed regulation of coexistence and succession, always given in objective experience in the form of certainties of expectancy, as an expectant 'that must come' or 'that must be together now', i.e., after now such and such are already there from experience or have been before" [3] (p. 134). The fixed style of modifications that regulates the causality of nature is the prescientific foundation of all the inductions of the natural sciences that make both the laws of nature "valid in themselves" and their comprehension in mathematical concepts possible: it is the causality of bodies or things.

However, nature presents "a curious structure": [4] (p. 439) among things there are also bodies that include a psychical component, above all, as animating components or as a life of sensations that "makes the body a carrier of tactile

fields, of visual fields, of listening fields, etc., i.e., as a carrier of co-belonging groups of data of sensation that are immediately or mediately located on or in the physical body" [4] (p. 440), as has already been stated. Apart from this inductive-causal unity of objective nature that also includes in itself bodies purely considered as things, the experience through which we perceive a body as that of another living being, the principle of acts, gives those acts as acts of another living being, and, in that sense, psychical life is experienced as causally united with the body. Let us consider that some changes in corporality, as those in the sense organs when they are activated by pressure, touch, or eye movements, etc., involve some changes in psychic life, for example, in the life of sensations; and, conversely, we may consider that psychic changes involve physical changes (i.e., sleep-wakefulness). The body-soul unity also occurs as a natural-inductive unity [5] (p. 300); that is why the idea of a scientific psychology is justified, of a psychophysics that studies the laws of this unity [4] (p. 91), [5] (p. 105).

The fact that the unity of objective nature and the unity of body and soul are inductive-natural unities does not allow, however, to deduce that the unity of the soul is also inductive-natural. It would be an unjustifiable transposition leading to psychological naturalism (as it already occurred in the nineteenth century). For Husserl, the motivational connection is the fundamental law through which the unity of all the psychic—that assumes and includes one's own lived body—is comprehended, and not only the unity of the personal spirituality. Therefore, passive strata of the soul such as association, and the totality of the life of feelings and impulses must be explained motivationally [1] (p. 220ff), [5] (p. 107ff). This leads to the distinction between rational and irrational or active and passive motivation [5] (p. 110, 331ff). This Husserlian consideration of psychic passivity as belonging to the realm of motivation is decisive. It is precisely the existence of a passive dimension in the psychic stratum that tends to be taken as a reason to adduce that the psychic stratum belongs to the realm of natural causality, as if the passivity or independence of will that is proper to natural facts were identical to psychic passivity. The passivity of association lacking ego activity and the passivity of the physical course of nature are placed at the same level, when, actually, association is a kind of motivation, the passive type. Husserl had noticed that the active spirituality, the activity of the ego with its freedom, even in appearance could not be identified with natural passivity and was reinterpreted by naturalistic trends as an apparent image of passive sources [5] (p. 333ff). The comprehension of this point is crucial if someone is to correctly appraise the contribution of the neurosciences. Within the framework of motivation there is, for phenomenology, on the contrary, an originarily continuity between psychic passivity and activity, as can be seen in the case of kinaesthesis: to be affected presumes an act and the consciousness of the "I can". In this sense, because motivation regards the statute of all experience and consequently also that of the perception of external things, the causal connections are carried by motivational connections, which does not mean, however, that causality is to some extent an apparent structure that must be explained as motivation as suggested by Hume. On the contrary, it is transcendentally constituted as such in the subjective connections of the motivation of consciousness. Naturalistic perspectives failed because of their inability to take intentional events in their proper evidence and sense.

Pain or Afflictive Experiences

Consequently, has a human fact been accomplishedly comprehended when it has been scientifically explained? The experience of pain is one of the enigmatic touchstones for scientific analysis. Pain is the most personal and, depending on the case, bodily, of our experiences. There is no pain, as such, that is not experienced, or that is not felt. However, it is complex and difficult to show in what sense pain can be understood as a phenomenon. It is identified as experience [*Erlebnis*], as conscious experience, but it is also something that appears as object. The difficulty lies in answering to what extent pain can be considered an objective phenomenon.

In phenomenology, the term "phenomenon" may have several different meanings:

1. The concrete experience [*Erlebnis*] (having in mind, perceiving what is before us).
2. The intuited object (that appears). It is the object that is displayed and the situation, the here and now, in which it is displayed.
3. The real elements of the concrete experience, of the act of intuition; among them, sensations (visual, auditory, etc.). These are only components; the experience is not reduced to them. Conferring sense (what in Husserlian terms can be called intentional synthesis) is also a component of the experience.
4. The phenomenon understood as the manner of appearance.

There is no experience without a manner of appearance, without phenomenal multiplicities (i.e., there is no experience of an object without perspective). In turn, the appearing object is the phenomenon proper. That is, it is what shows itself to consciousness and as it is shown. The four senses in which "phenomenon" may be understood reflect the complexity with which things turn up. What appears is an object, but the act in which it appears does not depend on the object but on the subject (which is implicated in the other senses in which the phenomenon is understood). The experience [*Erlebnis*] is not anything that may be objectified inside the object, which means that the power of manifestation does not lie in the object itself. On the contrary, the originary perception of the body, except in specific situations (i.e., looking at oneself in a mirror), does not change perspective (not even in that example, because it is not the *Leib* but an external perception of the body, as *Körper*); I always have the same perspectives of my body: I cannot take a step back from it. This is the only object that resists being considered from new perspectives. In spite of that, this body is the correlate of all perspectives; it is the organ of perception, as has been said. It is the zero point of spatial orientation; from its dynamics there emerge the perspectives, it is the "here" from which all "theres" take shape. The body is the absolute dynamic "here". For this reason, all senses of the phenomenon apply to the body: it is the only phenomenon that is called phenomenon in all senses because it is or it includes all that it intuits, the intuited, the elements of the intuition and the manner of the intuition.

Beyond all possible classifications of types of pain or afflictive experiences (i.e., Buytendijk's [6] and Scheler's [7], it is clear that the description of the evidence of pain would present difficulties in principle even to phenomenological analysis, due precisely to the resistance it presents to being phenomenologically framed. However, what at first glance could represent an aporia, becomes the most solid point of the phenomenological perspective, and, more concretely, of our analysis: to show the epistemological, methodological, and conceptual value of phenomenology in order to frame the contributions of the neurosciences, cognitive sciences, and psychotherapy.

First, the phenomenological essence of pain is effectively supported by the very lived experience of pain, (i.e., by the event that I myself, in the first person, experience and have experienced pains). The painful experience is the primary irreplaceable unfolding both of the experience and of the nature of pain as Husserl [8] maintains through his "principle of all principles" in *Ideas I*: that every intuition in which something is originarily given—but also only within the limits in which it is given—is a basis of the legitimacy of knowledge. Second, this enigmatic character of the body as phenomenon, that does not allow it to be apprehended as an object or simply as *Körper*, although at the same time it can be phenomenically given in its own characteristic manner (i.e., can be intuited as such) also reveals an enigmatic unity (particularly from a purely scientific perspective) of the so-called first and third person perspectives, not in a complementary sense as the sum of data coming from two methodologies: biological-scientific and spiritualistic or introspectionistic (as in the case of neurophenomenology), but it is about the person as an intentional totality.

Husserl does not limit intentionality only to the intentionality directed to the object or, as Dreyfus [9] maintains, he does not state that the whole mental life, even our consciousness of

practical activities and our sense of existing in a shared world, has to have the form of an objective-being-directed. On the contrary, the notion of an operational intentionality is central to Husserl's so-called genetic phenomenology. Operational intentionality [*fungierende Intentionalität*] designates the prereflexive experience which is activated without the need to explicitly adopt an epistemic or objective attitude (i.e., it constitutes the prereflexive unity of the objects, of the world, and of our life). Pain, par excellence, and in fact any human experience (even more so any pathology or afflictive experience) simply gets deleted when it is reduced to objective phenomena (as in physical–chemical descriptions), to "third person" descriptions. The same must be said of the ego that experiences: it does not equate to a unitary brain structure or to a neurological configuration. Therefore, just as a book does not contain thoughts and a machine does not understand, neither does a neuron perceive the red color, feel cold or suffer, but it is the person, the ego, who comprehends, perceives, and suffers. The notion of a lived or intentional body clearly reveals that it is impossible to separate two fields as different realities (material/bodily and psychic), sources of data pertaining to two perspectives (first and third) if we wish to describe experience in its constitutive and essential elements to understand the sense of its phenomena.

It could be argued that such an analysis finally belongs to a perspective that, although phenomenological, describes the conception, perception, and "place" of one's *own* body, but it would imply nothing for the "external" perspective of the scientist or therapist with respect to other bodies. Let us move on to the phenomenological contributions around empathy to elucidate this crucial point.

The Empathetic Act

It is clear that in our field of perception we can immediately distinguish a chair from a person; I immediately comprehend that the person is different from the chair because a different act occurs, that of empathy, together with the perceptive one through which I get both. Comprehending that the other is somebody and not something, one that is an ego like I am, presupposes that the existence of the other human being is given to me as a similarity.

Briefly, and leaving aside the developments that his theory of intersubjectivity has gone through, we can say that for Husserl, the access to others seems to have its grounds in the regular experiences of our own body and in our kinaestheses. The perception of another subject is an improper perception, understood as a kind of co-perception [*Mit-Wahrnehmung*] or co-presence [*Kopräsenz*], insofar as it presupposes an original presence, my body, as a founding moment [4] (p. 27ff) and although the other is given to me in his corporeal appearance, everything else that belongs to the meaning of "another person" and not of a mere object (as a chair) must be added in an animating interpretation [*beseelendeDeutung*], [4] (p. 50) because it is not perceived in the same way I perceive his body, that is, it is not given to me sensuously as the chair is given to me. I perceive a person, however, without any specific reflection but rather immediately. It is interesting that through his phenomenological analyses, Husserl should have noticed that the act of empathy has its origin and possibility in the givenness of one's own body as perceived and perceiving through kinaestheses. Several decades later, neurology showed that mirror neurons, which conduct motor activities, have a double function because they not only guide our movements, but are also equally activated when we observe the same action in others. For scientists, mirror neurons are not only responsible for the capacity of imitation but also for the recognition and comprehension of the meaning of "motor events" (i.e., of the acts of others), that is why they were called the "neurons of empathy".

But, what type of experience takes place and how is it possible that a lived body, a person, in contrast to a mere object as a chair, could give itself in the perception of a body? This is possible because of the similarity between my own appearance to me and the behavior or corporeal development of the other. The empathetic perception of a foreign body [*Körper*] as a lived

body [*Leib*] analogous to my own, is absolutely and essentially associated to my own corporality: the foreign body that appears refers to an internal corresponding appearance that I would have in a similar manner "if I were there"; an interiority, a source of acts is apperceived. The foreign ego, the lived body does not appear as a body that is merely governed by the laws of natural causality, but it is an intentional body that, like mine, holds that enigmatic unity that manifested itself in the constitution of my own corporality. The lived body manifests itself as active, not inert nature. It is not legitimate then to establish the causal correlation between physico-chemical facts (a neurological reaction or configuration) and acts, behaviors or human states in an obvious and immediate manner. Dealing with a human body involves apprehending an intentional body, a principle of intentions that makes that body an active source of sense, an ego. Therefore, its physical stratum of constitution is animated by the intentional unity of the self as a whole; which is why the physical stratum, as integrating self, also constitutes itself as an intentional, not *causal*, basis of the explanation of all phenomena, assimilating itself to the inert nature.

In other words, through the experience of my own body as an animated/lived body, as organ of perception which I can neither step away from nor gain perspectives of, but one that accompanies the perception of all other bodies and in that sense, as constituting the self, is governed by the causality of motivation, I can perceive another body as an animated body and not as an inert object. But the empathetic act assumes then that we have perceived a body as an intentional body, which implies that it cannot be identified with the objective nature that is simply governed by causal legality.

Neurophenomenology

One would think that the program of neurophenomenology (especially the contributions by Francisco Varela, Evan Thompson, Shaun Gallagher, and Antoine Lutz) [10–23] is intended to achieve precisely this synthesis of perspectives. Whereas "neurophilosophy" has its roots in analytic philosophy [24, 25], neurophenomenology finds its conceptual framework in phenomenological philosophy. Approximately two decades ago a new current of phenomenological philosophy emerged in Europe and North America that goes back to the source of phenomenology (i.e., to Husserl's philosophy), but is influenced by cognitive science and the analytic philosophy of the mind, and aims to contribute to these fields [11, 17] and the journal *Phenomenology and the Cognitive Sciences*. Neurophenomenology is inscribed within this current. It also grows out of the enactive approach to cognitive science which has strong ties with phenomenology [10]. The word "enactive" was coined by Varela to describe and bring together several related ideas under one umbrella term. The first idea is that organisms are autonomous agents that actively generate and maintain their identities, and thereby define their own cognitive domains. The second idea is that the nervous system is an autonomous system: it actively generates and maintains its own coherent patterns of activity, according to its operation as an organizationally closed sensor motor network of interacting neurons. The third idea is that cognitive structures emerge from recurrent sensorimotor couplings of body, nervous system, and environment [18].

The enactive approach and its neurophenomenological development converge with the growing recognition that more detailed and refined first-person descriptions are needed to characterize the *quid* of consciousness and relate it to the complexity of brain activity.

For that purpose, neurophenomenology attempts to link significant reports in the first person with appropriate neuroscientific models of neuronal performance, showing that the phenomenal properties of mental conscious states can be integrated into explanatory frames in which every property emerges in continuity with the properties accepted by the natural sciences [11]; and this objective would depend on the one hand, on the development of a rigorous methodology of observation and description of conscious experience in the first person (here lies the phenomenology of the neurophenomenology), and on the

other hand, on the development of appropriate explanatory models of the nervous system. Gathering first-person data from phenomenologically trained subjects constitutes a heuristic strategy for describing and quantifying the physiological processes that are relevant to consciousness [15] (pp. 31, 32, 45).

In that regard, the neuroscientists assume that neural models of various aspects of consciousness have been developed, and that evidence of the neural correlates of consciousness is available. However, they argue that an "explanatory gap" still remains in our understanding of how to relate neurobiological and phenomenological features of consciousness. This explanatory gap is conceptual, epistemological, and methodological [18].

Although the representatives of this school of thought acknowledge the irreducibility of conscious experience, insofar as phenomenal data cannot be reduced or derived from the third person perspective [12], such a methodological proposal would rest on the assumption that phenomenological references to the structure of experience and their equivalents in neuroscientific third person-data are related to each other through mutual restrictions: the joint consideration of the biological and phenomenological levels of the mind should cause mutual limitations and validations at the methodological level. Nonetheless, it is clear that the goal is to find the explanatory (i.e., causal) neural correlates of the phenomena of consciousness.

From a phenomenological perspective, all experience involves not simply awareness of its object (noema), but tacit awareness of itself as process (noesis). For instance, when one consciously sees an object, one is also at the same time aware—intransitively, pre-reflectively and passively—of one's seeing; when one visualizes a mental image, one is thus aware also of one's visualizing. This tacit self—awareness has often been explained as involving a form of nonobjective bodily self-awareness—a reflexive awareness of one's "lived body" [*Leib*] or embodied subjectivity that correlates with the experience of the object [26–28], as has already been mentioned. Hence, the neurophenomenological perspective maintains that any convincing theory of consciousness must account for this prereflective experience of embodied subjectivity, in addition to the object-related contents of consciousness [10, 28, 29]. In other words, it must explain "how the brain engenders the mental patterns we experience as the images of an object" (the noema in phenomenological terms), and "how, in parallel…the brain also creates a sense of self in the act of knowing…how each of us has a sense of 'me'…how we sense that the images in our minds are shaped in our particular perspective and belong to our individual organism" [30] (pp. 136–7).

Phenomenology exceeds the internalism–externalism dichotomy in the sense that the notions of "internalism" and "externalism" remain anchored in the classical inside–outside, interiority–exteriority, first–third person divisions, that have their grounds in an introspectionist comprehension. Husserl himself constantly faced psychologism and explicitly rejected the interpretations that tried to assimilate phenomenological intuition to a kind of internal experience or introspection. In *Ideas III* [31] (p. 38), it is clear that phenomenological intuition is not a form of internal experience [*innere Beobachtung*].

The phenomenological attitude with which the representatives of neurophenomenology sympathize, can be identified with the idea of *reducere*, of leading the gaze (i.e., of turning thinking away from an immersion that is lacking in reflection of the world such as it appears to us in the natural attitude). In other words, once we adopt the phenomenological attitude, we are no longer interested in *what* things are in a naïve sense, independent from the mind, but rather in *how* they are experienced in correlation with our subjectivity. The goal of phenomenological reduction is to find a way into this constituting activity of consciousness.

Husserl completed his phenomenology with the development of genetic phenomenology. Proper analyses of genetic phenomenology distinguish between active and passive geneses. Any active genesis always presupposes a passivity by which one is affected beforehand. "Passivity" is not equivalent to a state of inactivity, as has been pointed out above, but responds to an involuntary

being-influenced and -affected by habits, driving patterns, dispositions, motivations, emotions and memories. In this respect, "passivity is in itself what comes first because any activity essentially presupposes a subsoil of passivity and a preconstituted objectivity" [32] (p. 3). Perceptive apprehension and identification of a preconstituted sense of an originary passivity are already forms of activity. It is precisely this activity in passivity that mediates between the originary passivity and the activity that is called categorical proper. The tendency of the ego to establish active objectivations is only possible through this activity in the originary passivity, without which consciousness would not be what it is. Thus, past experience prefigures to some extent our manner of expecting future experience, a fact that becomes reinforced with the repetition of similar experiences. Consequently, the ego always has a horizon of acquired knowledge of familiarity that becomes a permanent component of the sense of the object as a result of a complex synthesis of association in which the similar evokes the similar. This horizon experiences continued modification because it broadens and corrects itself in the light of new experiences. One's relation to the world does not depend then exclusively on conscious and reflective acts, but is also subject to affections and habitualities of the body. In other words, the ego is affected not only in its primary passivity by sensuous data, but also in a secondary passivity by its own aggraded acts in permanent acquisitions that associatively connect with present life (the foundation, at the same time, for the possibility of habits). This explains the importance of the issue of association in genetic analysis because an intentionality of association appears, then, in the sphere of passivity before the active intentionality of the ego.

In this context, it must be noted that Husserl distances himself from the medical, physiological, and mechanical sense of the phenomenon of affection [*Reiz*], understanding the term *Reiz* in a fundamentally new manner when he establishes a motivational relationship between the ego and the intentional formations. The passive life of the ego, the affections, do not identify themselves with the natural sense of passivity, causally and mechanically determined. From this perspective, affection is no longer considered to be a blind force, but rather becomes a motivational request, a wake-up call, [33] (§§ 26–35), not a causal necessary determination.

Conclusion

The "embodied cognition" and the bodily foundation of phenomenology have awakened—as has been already explained—special interest in the neurosciences, as they find in it a series of descriptive elements and perspectives of analysis that may be suitable for the development of their research and pragmatic paradigms. Our revision of the fundamental distinctions of phenomenology has allowed us—in our view—to pinpoint, first, the mistake involved in understanding the phenomenological method as introspection. In this view, the phenomenological method would consist of the disciplined practice of a "first person" methodology that would allow one to increase and tune sensuousness towards one's own conscious mental states at diverse temporal levels through a laborious process aimed at clearing away any attentional or emotional aspects that may be applicable to conscious experience, so that through this shift, a perspective of the mind as lived, experienced mind can be achieved; so that its results can then be contrasted with those obtained by means of neurological studies. A "science of consciousness", as neurophenomenology is defined by Varela [13] (p. 330), assumes that cognition is essentially linked to the body and to the action of organisms, which also implies that *any intentional process* [*Erlebnis*] must necessarily have a manifestation or neurological correlate through which it can be identified as such. And therein lies the problem.

It must be mentioned that when these discoveries had not yet taken place in the medical field, Husserl had already warned vehemently against the process that began with "the Galilean mathematization of nature", through which nature itself is idealized under the lead of the new mathematics and becomes a mathematical multiplicity, in an infinite world—though closed in itself—of

ideal objectivities as field of work. Euclidean geometry, ancient mathematics, and even the Aristotelian syllogistic operate with a finite and closed a priori, not with the conception of an idea of infinite totality-of-being systematically dominated by rational science, which implies that our apodictic thinking simply "discovers", advancing along stages toward infinitude through concepts, principles, inferences, and demonstrations, what is already in itself and beforehand verily because everything that ideally "exists" in geometric space is already univocally decided in all its determinations [34] (§ 8). This mathematical praxis achieves what in empirical praxis is denied to us: "exactness". On the contrary, the world is given to us prescientifically in daily experience in a subjective-relative manner, without us thereby thinking that there are many worlds, but rather that we move in *the* world that contains the same things, although they appear to us in different ways. In the intuition of the surrounding world, when we direct our gaze to merely spatial–temporal shapes, we experience "objects", not ideal geometric shapes, but none other than *the* objects that we indeed experiment. Real and possible empiric shapes are only given to us—in sensuous empiric intuition—as "shapes" of a "matter (i.e., of a sensible plenitude) with what presents itself in what have been called "specific" sensuous qualities. A footnote by Husserl, in which he denounces this illegitimate identification of sensuous qualities of experienced objects with physical-mathematical ideality and sensuous data such as those of sensation, becomes enlightening and valuable at this point:

It is an ill-fated legacy of the psychological tradition dating back to Locke's times that the qualities of the truly experienced objects in the daily surrounding intuited world—colors, tactile qualities, temperatures, weights, etc., that we perceive in the objects themselves, precisely as their properties—be substituted by the "sensuous data", the data of "sensation", which are also undifferenciatedly called sensuous qualities and that are not, at least in general, distinguished from those. […]. And it is also common to substitute for what corresponds to them [immediate data] in the objects themselves with the physical-mathematical… We are talking here and everywhere, bringing faithfully to discussion the true experience of qualities, of properties of the objects truly perceived in those properties. And when we designate them as plenitudes of shapes, we also take these shapes as "qualities" of the objects themselves, and also as the sensuous, although they, as *aisthetá koiná*, are not referred to a unique organ of the senses that may correspond to them, as the *aisthetá ídia* [34] (§ 9b).

On the one hand, neurophenomenology would thereby assume that physico-chemical neurological processes, that is, what the researcher perceives in sensuous data as qualities of the objects, can be accurately described, measured, and identified with the ideal objectivity that guarantees, in turn, prediction. This method of measurement would extend to all real properties and to all causal links of the intuitive world, to all that can be experienced in particular experiences, allowing one consequently to build, explain, and predict all events belonging not only to plenitudes or sensuous qualities, but to human acts, and determining them objectively.

By means of the phenomenological analysis of corporality it was possible to suggest that the unity of the intentional body does not allow a physical localization of consciousness, neither a consideration of the body as a simple object of nature, but the unity of selfhood manifests itself in its acts, which, though of different kinds (bodily, psychical or spiritual), are proper to an intentional consciousness that is motivationally animated even in its passive strata. Pain, as an experience that reveals more clearly the coming together of the multiple and unitary manner of the body as a phenomenon (as experience, object, components of the concrete experience and manner of appearance), also showed that intentionality is already operative in prereflexive experience before objective experience, so that to assimilate every experience with "third person" objective phenomena involves their abolition.

In turn, the perception of the other as somebody in the empathetic act, which is essential to comprehend the relation of the scientist, doctor, or therapist with his or her "object" of study (a person, not a neuron or chemical substances), gives

grounds to the perception of the other as another ego, which is coupled with the givenness of an intentional body, of an intentional source of acts so as the own corporality is given. In this respect, a problematic aspect that is not taken into account at all by neurophenomenology is the "position" of the experimentalist, the scientist, as the one who is capable of connecting (and in what sense?) first- and third-person data. In relation to them, is it possible to distinguish between first- and third-person data? Are both (the neuronal and the reported data) first-person data, because they are experienced and comprehended by them, receiving a specific sense; or are both third-person data, because none of them comes from their own experience as an intentional source. It is the analysis of the position of the scientist that reveals the shortcomings of this approach: namely the attempt to dissociate first- and third-person "data".

The phenomenological perspective and method present a viable access to and treatment of corporality, of the psyche, and of the spirit, in short, of the selfhood, that do not give room to the reduction of structures, performance, and pathologies to an "objective" neurobiological explanation, but favor the comprehension and description of neuroscientific discoveries and results in the frame of an intentional unity of the person in all their strata: it is not the same, either for its comprehension or for the description of its etiology and treatment, to pose that schizophrenia is an alteration of the selfhood instead of an illness of the brain. Although the neurosciences intend to discover the correlation between actions and brain activity, they will be constantly confronted with aspects that are perhaps ontologically associated with the brain, but resist being reduced to it at the risk of falling into biological determinism or into nuances that are characteristic of ontological or epistemological dualisms, as it happens in neurophenomenology.

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Trends in Philosophy of Mind and in Philosophy of Neuroscience

3

Juan José Sanguineti

Introduction

Philosophical questions regarding the nature of the mind and its relationship with the body are usually addressed by philosophy of the mind. This area of philosophy inherits the traditional issue of the relationship between the soul and the body, interpreted in modern terms as the mind and the brain. Whereas the classical view of the problem was thoroughly ontological, going back to ancient philosophers as Plato and Aristotle, philosophy of mind, born in the twentieth century, is generally more epistemological, posing its object of inquiry within a scientific framework. Its topics are similar to those treated by the cognitive sciences such as neurobiology, computational science, and cognitive psychology.

These topics, namely perception, sensations, emotions, memory, language, thought, and free will were typically considered to be psychological. Scholars in this area of science also face questions regarding the nature of the mind and the meaning of the human person, who is seemingly made up of of mental capacities and neural processes, two dimensions related to the classical duality of the soul and body.

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The novelty of philosophy of the mind, compared with classical psychology, is that the problem is tackled in strong relationship with the natural sciences. In dealing with psychological notions such as thought, intelligence, decisions, and representations, two areas are neuroscience and computational science. Neuroscience studies and explains all that was typically reserved to psychology from an empirical perspective. Computational science seems capable of reproducing and dominating representations and thought processes. Accordingly, it seems natural that philosophy of mind will turn out to be a kind of philosophy of neuroscience and a philosophy of computation, although essentially more the former, if we take into account the recent development and prestige of neurobiology regarding human problems. This is clearly demonstrated by the proliferation of "neuro-disciplines" such as neurophilosophy, neuroethics, neurotheology, neuroeconomics, neuroaesthetics, etc.

The difficulty with the resulting discipline is that it does not share the capability of the older metaphysical psychology in acknowledging a spiritual dimension to the human person. As human thought appeared to be immaterial or non-physical, psychology classically maintained a good relationship with theology. To think of God as an immaterial Being and then to speculate on the immortality of the human soul beyond the destruction of the human body was natural for many authors. However, in contemporary

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philosophy of mind, these issues appear more problematic because ontological concepts, such as being, form, and essence, are not available in the general scientific environment, whereas scientific concepts dealing with physical, chemical, and biological concepts are familiar to scholars. Subsequent to the Kantian revolution, metaphysics was viewed with suspicion by philosophers and scientists, while the natural sciences became more consolidated and capable of vindicating the privilege of truth. Consequently, classical philosophical problems needed to be investigated in dialogue with the natural sciences, and sometimes in subordination to them. This is the current epistemological condition of philosophy of mind and philosophy of neuroscience [1].

According to this scenario, the first and perhaps most important problem of philosophy of mind and neuroscience is the alleged distinction between mental and neural acts or events, while the second problem, provided the distinction is accepted, is their causal mutual relationship. These two main philosophical questions are answered differently by a series of standard positions: dualism, neural monism, functionalism, emergentism, and non-reductive physicalism.

In this chapter I attempt to briefly present these positions, and to combine them with what can be understood as the philosophical thought of authors dealing with neuroscience rather than with a pure philosophical speculation. Many other problems, such as the relationship between neurobiology and philosophy, the extent of human freedom, the assessment of human actions in ethical issues, the particular orientation given to psychiatry, or the focus of educational efforts, depend essentially on the solution given to these fundamental questions.

Standard Positions in Philosophy of Mind

Philosophers of mind normally display an array of similar positions regarding the mind-brain duality. These different views can be found in any textbook on philosophy of mind [2–4]. My presentation attempts to go to the root of the problems and to indicate what I consider most relevant for the purpose of this book. My account is clearly favorable to some type of moderate dualism. I do not attempt to suggest being neutral on this issue.

Dualism [5]

Dualism claims that the human person is constituted through two kinds of realities: the first, material and the second, spiritual. In substantial dualism, the spiritual reality, known as the soul, spirit, or the mind, moves and guides the body, but it can also be affected by the latter. There is also the possibility of interaction between the soul and the body. Such a duality is attributed by Aristotle to animals and even to plants.

If the body and the soul are not understood as substances, they can at least be viewed in terms of properties, as is elaborated in property dualism. This type of dualism takes into consideration a group of human actions, processes, and operations as material because they are empirically verifiable, whereas others, such as thoughts, intentions, and desires are categorized as immaterial because they are experienced as *qualia*, completely deprived of material properties. With similarities to the assertion of substantial dualism, property dualism claims that spiritual operations move or guide bodily processes: a human agent as such, he or she, wants to move the hand, and thus moves it.

Dualism can be perceived either phenomenologically or through common sense. It is very intuitive to experience our thoughts, feelings, and decisions as something radically different from spatial material objects. Dualism can also be sustained through religious beliefs, and indeed, religion would be devoid of sense if all that exists were purely material. Materialism and atheism are intrinsically linked [6]. Finally, dualism can be argued and explained in philosophical terms.

In spite of the various philosophical accounts of the soul-body or mind-brain duality, dualism is a strong and persistent conviction held by many people because it corresponds to direct knowledge, or to what could be nominated as the common sense perspective. Even the most

rigorous materialists cannot avoid experiencing thoughts and consciousness. The efforts made to reduce all reality to material reality are normally very complicated. They must be argued over and over, struggling against what constantly reappears in common language and implicit belief (i.e., consider the paradox of saying: "I think that this *I* does not exist").

Naturalism or Physicalism

According to this position, nothing other than material substances or material properties exist. The apparent existence of mental acts must be reduced to something material, and this is why naturalism, or materialism, is often *reductionist* because it is committed to the effort of reducing an alleged entity to something different. Reductionism is also shared by what will later be termed "neurologist monism". The attempt to *eliminate* the notion that is reduced corresponds to the so-called *eliminativism* [7] advocated by Paul and Patricia Churchland [8, 9].

A reduction aims to be an explanation [10]. Thus, materialists typically argue that a thought is nothing but a neural circuit and the circuit explains the presentation of a thought. This epistemological procedure is taken from physics. For example, the phenomenical presentation of light can be reduced/explained through electromagnetic waves. The reduction is correct, but the phenomenal light—the light as we see it—still remains a psychological or a mental fact.

Notwithstanding the efforts to reduce the psychological process of vision to neurophysiological events, the phenomenon is not eliminated, therefore the duality between psychological and physical events persists. This is the paradox of physicalism, in its concern with reducing and eliminating the existence of mental properties.

Naturalism or physicalism [11] has its roots in the prestige of physical explanations. The reason that sustains the widespread materialistic belief is mainly epistemological. Physics deals with spatio-temporal objects, ultimately testable by empirical procedures which are based on what can be detected by external senses or by public instruments of observation. Therefore, nonobservable entities as such will never exist although they could be postulated as instrumental logical devices (i.e., physical laws or mathematical spaces) capable of explaining physical phenomena.

The physical universe of discourse—the world as is viewed by physics—is understood as closed or exclusive. Nothing can be postulated outside of this framework. God, the angels, souls, and mental entities are rejected because they do not fit into the ontological framework of natural science. Of course, it can be said that the world as it is seen through physics is an abstraction because it is the result of selecting a series of basic properties (mass, space, movement, time, force) as a methodological choice for the explanation of all phenomena. In this sense, natural science is a partial view of reality and does not comprehend reality as a whole [12].

Paradoxically, even sensations, the so-called *qualia* in philosophy of mind, are problematic for materialism. Psychological acts and states do not belong to the realm of physical and observable events [13]. Accordingly, psychic events, such as acts of vision, pain as a sensation, and so on, should be ruled out by being reduced to neurophysiological events that can be detected by our external senses or by instruments of observation.

For instance, the sheer sensation of pain cannot be externally observed. Pain, no doubt, is quite physical, but as such it does not belong to the universe of discourse of physics. If this universe is taken as exclusive, then pain becomes problematic and must be reduced, and as a psychological act it will be understood as nothing. Hard materialist philosophers view the psychological world with suspicion because they rightly feel that to accept that world could lead to the acknowledgment of something beyond matter. They opt to protect the choice of remaining within the closed natural scientific world.

Now, how can reduction be accomplished? On one hand, there is the notion that is supposed to be reduced, which in this case is the psychological reality: perception, representation, emotion, comprehension, or the self. On the other hand, some physical features (some functions of the

human body) must be chosen as the matter to which the psychological events are supposed to be reduced. Three candidates are available for this effort: external actions and reactions, neurobiological processes, and computational procedures, giving us three possible positions.

Behaviorism

Internal actions are behaviorism's preferred object of reduction. Internal operations or states, such as emotions, decisions, and perceptions, always have some relation to external actions, or at least they can be ascertained through external reports and tests, therefore, it is not difficult to attempt to assimilate the former within the latter. Accurate analyses, however, demonstrate that these two different types of acts are not equivalent.

Philosophical behaviorism [14], although not always known by this name, aims to translate internal acts into behavioral dispositions. Psychological behaviorism, however, follows the scheme of stimulus/response, and the associated notions of reinforcement and reward. In both cases, dualism should to be avoided. As is well-known, psychological behaviorism was eclipsed by cognitive psychology; but even so, this psychological school was helpful because it demonstrated the importance of paying attention to behavior when attempting to study and to follow psychological states.

Neurologist Monism

The reduction of mental acts to neurobiological processes might seem a more promising proposal. Perceptions, sensations, feelings, and conscious or unconscious cognitive states normally show a clear neural basis. The acts of vision, touching, smelling, and others associated with the senses can be described and explained in neurological terms. It can at least be claimed that mental acts always have some correlative type of neural acts or states. This type of correlation is often considered as falling under the category of *supervenience* [15]. The correlation between both dimensions must be defined in very precise terms, which is not an easy matter.

Neurologist monism (this denomination is mine) claims that a psychological act is nothing more than a neurobiological event. There is no a special term for this trend of thought in philosophy of mind, which at other times was known as the identity theory. This position seems to be similar to something that I had previously called naturalism and can be seen as the most frequent version of naturalism in our times. Although many authors take for granted that this is the correct solution to the problem, the difficulties of this position have already been highlighted above. There is no doubt that the neurological dimension of psychological acts is essential, but it can also be demonstrated that it is partial. To perceive or to be conscious, is not exactly the same as a purely neural event.

Computational Functionalism

The third attempted reduction springs from computational science and could be indicated as computational functionalism [16]. This position apparently goes beyond reductionism as understood in neurologist monism and behaviorism. It seems to recognize a certain immaterial content inside the alleged black box of the internal acts. Functionalism in philosophy of mind is, in general terms, a sophisticated position that equates mental states with causal roles or functions [17]. The supporters of this position do not accept the simple experience of feeling as a decisive feature of mental acts. Pain, for instance, should rather be defined functionally. This approach is not necessarily incompatible with the acknowledgement of *qualia* as real internal experiences. The reductionist move now appears clearly in *computational* functionalism. Mental acts could be identified with computational functions or with information processing.

The key word here is *information*, which has several meanings connected with the transmission of messages and causal effects. Generically, information implies order in nature, an order introduced within an energetic physical basis. Order is a functional notion related to a certain goal to be attained through a particular arrangement of elements in space and time. Living organisms have the capacity of controlling information, received from the environment and transmitted to the different parts of the body in

order to maintain the typical self-organization that characterizes the living system. When this information control is associated with cognitive representations (i.e., perception), then the living system, endowed with a nervous system for that purpose, is called an intentional or a cognitive organism. This is the case of animals and humans. However, it is possible to separate the flow of information from its natural basis and to treat it using an artificial device (a computer). This procedure is a calculation, namely a transformation, according to an algorithm, of some inputs into certain outcomes.

It is tempting to say, then, that psychological operations or states should be just computations. At this point inputs can be connected with outputs in a certain system through different computational procedures, as is typically done by a computer. Computational devices are able to capture informational processes occurring in the physical world, particularly in organisms and brains, and to freely manipulate them so as to simulate and emulate natural or intentional processes such as biological processes, diseases, perceptions, problem-solving, and decision-making techniques. The task of the brain would be similar to the operations of a computer.

Computational functionalism was strongly stimulated by the development of artificial intelligence and robotics. But a new sort of dualism, namely between software and hardware, has now appeared, especially because the software, corresponding to the mind, is multiply realizable in different physical media (e.g., a brain, a standard computer, a quantum computer, etc.). Therefore, the computational mind appears to be independent from its bodily realization.

The problem with functionalism is that it concedes too little importance to the neurobiological basis of psychological states. It is easy to produce pieces of information manifoldly, just as a book can be printed or registered in any kind of computer. But this is not the case in a real cognitive operation or in an emotional state. Two people can share the same thought, such as $2+2=4$, but nevertheless, each one of them has a personal thought.

This is the reason why neurobiologists normally pay little attention to computational functionalists. Engineers and computer scientists, instead, are more attracted by the functionalist proposal. The computational theory of mind favors the idea of a possible introduction of consciousness into a computational machine. But what kind of consciousness would a robotic consciousness be? It would be an imitation, not a real psychological state. Functionalism takes one aspect of mental states, the informational one, and ignores those states of lively action performed by real people. In this sense, functionalism is a new form of reductionism.

Emergentism and Antireductionism

The recognition of irreducibly higher levels in a stratified natural universe—life over non-life, sensitive consciousness over life, and human reason over sensitive consciousness—in the twentieth century produced the anti-reductionistic position called emergentism. This position emphasizes the existence of new kinds of properties emerging from the construction of lower layers, provided that they are organized in a certain way [18]. Namely, mental states naturally emerge from a precise organization of neural integrated circuits.

There can be strong or weak versions of emergentism. The strong versions are not far from a moderate dualism, such as that found by Popper [19]. The weak versions (Bunge) [20] interpret the emergent properties as new global structures constituted by the assembly of many parts. The whole is more than the sum of the parts, as a house is more than a pile of bricks. John Searle [21] follows an intermediate position.

One of the problems faced by emergentism is the difficulty of providing an account of the causality between mental and neural events. Clearly the neural basis enables the subsistence of psychological states, and when this basis fails to function, impairment and disorders follow (bottom-up causation). However, it seems that insights and decisions, for instance, the ideas flowing through my mind that cause me to write these lines, also spontaneously select many brain activations in very specific areas (downward causation). Musical performance and linguistic

abilities are responsible for specific brain activations and shape cerebral patterns in several ways. Emergentists usually stress downward causation while attempting to avoid interactionism in a dualistic sense. They criticize all types of reductionism, whether neurologist or functionalist.

> Connections that give rise to correct sounds or utterances are reinforced during the learning process, while those that produce the wrong results are not; and the difference is determined by the semantic rules that govern the systems. In this sense, certain connections within the brain, as well as with nerves and muscles, are selected and shaped through a process of downward causation: from the contents and meanings of the musical and linguistic signs, according to the semantic rules of musical notation and ordinary language, to neural and neurophysiological connections. To this extent, content and meaning, which, as externalism has it, go beyond the individual's brain and bear an objectivity of their own, are causally responsible for the actual shaping of the neural connections and networks required for a competent musical or linguistic performance [22] p. 197–198.

Neurophilosophical Proposals

In addition to the official philosophers of mind, neuroscientists who are sensitive to humanistic topics frequently present opinions on epistemological and anthropological items regarding the problem of mind and brain, the nature of knowledge, human identity and free will, and other themes that justly correspond to the philosophical domain, even in ethics and religion. Their observations on these topics are sometimes episodic or very brief and are frequently found in popular books. However, in other cases, they can be more systematic, ambitiously delineating a complete view of man. Their reflections can be located on the frontier between science and philosophy. They convey an amount of useful information regarding neuroscientific achievements and usually enter the philosophical field without the sophistication of professional philosophers. Hence, they risk being naïve in subtle matters or unduly mixing what can be scientifically demonstrated with what needs careful philosophical argumentation. In spite of these difficulties, the contribution of these authors to the philosophy of neuroscience is undeniable and can be considered complementary to the philosophers' efforts in the corresponding areas.

It is not easy to identify clear-cut positions among the authors involved in these neurophilosophical writings. Some of them more directly engage the current problems in philosophy of science and propose a solution. The solution can be dualistic, as in the case of Eccles, which is currently rather exceptional, or it can be materialistic. A number of them share a less than well-defined naturalistic background. While they usually reject a drastic dualism, being open to some form of imprecise non-reductionism, they contemporaneously include neurophysiological items that can enrich the anthropological view. Two other related areas of research are neuroethics and the so-called neurotheology. The former studies not only the problem of the legitimacy of deep neural interventionism human beings, with their consequences for personality and society, particularly in the areas of health, education, marketing, and culture, but also the biological foundations of ethical inclinations and actions. Neurotheology is concerned with the correlations between religious experiences and brain activations. Depending on their philosophical position, namely either materialistic or perhaps open to the spiritual dimension of man, researchers involved in these areas sometimes draw contrasting conclusions regarding the distinction and causality between mind and brain.

Without any attempt at classification, this section sketches out in broad lines those authors and insights that can be viewed as paradigmatic of these positions and as an expression of the major concerns and attitudes on the topics considered in this chapter.

Before continuing, certain ideological movements should be mentioned, such as antipsychiatrism and transhumanism, which have had an impact on many questions debated by neuroscientists and philosophers of neuroscience. The *antipsychiatric* movement negatively evaluates standard psychiatric practices, partially in reaction to certain abuses, but also as a result of its anthropological vision. One aspect of this negative

evaluation can be illustrated in the criticism of the very concept of psychiatric disorders (definition, classification, and treatment), something that is linked to the patients' relationship with society. A balanced account of these topics must be considered in an overall philosophy of psychiatry [23]. *Transhumanism* claims that neural and genetic human enhancement of our abilities and potentialities are to be promoted even to the point of changing our species in the future into another, better post-human transspecies [24].

These movements, such as those dealing with fundamental ethical questions, involve an evaluation of the risks and benefits of medical and psychiatric interventions in the brain, both by means of pharmaceuticals and through computational interfaces. Positions regarding this problem range from optimistic views, which soar to unlimited heights or to the transhumanism view, to cautious and sometimes pessimistic caveats.

The assessment of what contemporary neuroscience enables us to do in human and social affairs creates many challenges in social policies, education, medicine, and ultimately depends on some basic views held by philosophical anthropology. Ethical codes and prudential practices are not enough unless we go to the ontological and anthropological root. In this sense, philosophy of mind and of neuroscience could be considered a crucial part of anthropology. The basic positions on this theme shall be presented in this chapter, especially those of particular selected authors.

John Eccles

John Eccles (1903–1997) was awarded a Nobel Prize in 1963 for his work on synaptic transmission. He was an enthusiast of Charles Sherrington (1857–1952), another Nobel Prize winner for research on neuron functions. Eccles [25] held a clear dualistic position that he shared with Sherrington [26] and in some aspects with his colleague Robert Sperry [27] (1913–1994), whose philosophical position is rather emergentist (Sperry won the Nobel Prize in 1981 for his studies on split-brain). The distinction between dualism and strong emergentism is not always clear cut. In practice, Sperry diverges from Eccles only because the former does not believe in the survival of the immaterial mind after death. Popper's philosophy, wholeheartedly followed by his friend Eccles, is likewise akin to Sperry on this point.

According to Eccles [25, 28], there is no way of explaining the unity of human self-consciousness and its active role in guiding the conscious experience without the presence of an immaterial entity, called the self-conscious mind. This immaterial entity is capable of interacting with cerebral networks and, more specifically, with the dominant linguistic hemisphere (normally the left) at the cortical level. The mind is the source of the continuous selective integration of various activated neural centers that are continuously being spatio-temporally reorganized during the state of wakefulness. The self-conscious mind, which is the root of personal identity, plays a central role in the conceptual interpretation of the information it receives from cerebral patterns as well as a role in guiding attention in order to focus perception, to awaken memories, and to promote active voluntary movements of the body by acting upon several cerebral open modules. Eccles [25] estimated that this conjecture should not be rejected as anti-scientific, provided one accepts that it is normal in neurobiology to take into account psychological immaterial concepts such as intelligence, comprehension, and the unity of the self.

Michael Gazzaniga

Michael Gazzaniga (1939–) worked under the guidance of Sperry in split-brain research. He stressed the importance of the dominant left hemisphere in the process of verbally and consciously integrating representations arriving from the various cerebral modules. Additionally, he studied to what extent patients with perceptual disorders resulting from cerebral damages tend to confabulate rational explanations of incoming data in a coherent way. This task is attributed to a specific area of the left hemisphere, where he places a so-called *interpreter*. Corresponding to

the linguistic consciousness emerging in the left hemisphere, the function of this hemisphere is extended to the creation of all human beliefs and to the manipulation of the different "selves"—sometimes in conflict—that pertain to other regions of the brain so as to maintain the appearance of one self with its own story and identity. This action requires a special skill to alter memories so as to adjust them to the dominant self. In this sense, in Gazzaniga's view, what happens as a pathological confabulation in the case of impaired perceptions, as when someone does not perceive a leg as his own, rather attributing it to someone else, is transformed into a universal procedure for the production of ideas and beliefs, even in the ethical and religious domains [29–31]. This position, although emergentist, is actually not far from materialism position because the interpreter is simply produced by the left hemisphere.

> The interpreter constantly establishes a running narrative of our actions, emotions, thoughts, and dreams. It is the glue that unifies our story and creates our sense of being a whole, rational agent. It brings to our bag of individual instincts the illusion that we are something other than what we are. It builds our theories about our own life, and these narratives of our past behavior pervade our awareness [32].

There is something unintelligible in the notion of the interpreter by Gazzaniga. It is a creator of everything that one thinks in relation to himself and to the world, a fictional person who does not truly correspond with oneself. To say that this person is generated by the left hemisphere is an obscure statement. We can understand that brain injuries produce pathological perceptions, but it is vacuous to say that the brain generates ethics, philosophy, religion, etc. Paradoxically, dualism is resuscitated, but in a strange way: the dualism of the interpreter and the brain.

Jean-Pierre Changeux

Jean-Pierre Changeux (1936–) is a well-known French neurobiologist who studied those allosteric mechanisms involved in the function of nicotinic receptors and related to cognitive functions. On the basis of those studies, he formulated a theory of epigenesis by synapse selection. He also proposed a theory on the global neuronal workspace, which was associated with consciousness. A great humanist, Changeux published several books on the neuronal basis of cognitive and affective consciousness as well as on neuroaesthetics [33]. He was interested in the problem of the biological foundation of ethics. His concern for certain topics such as religion, ethics, cognition, truth, beauty, and the general good, brought him near the field of philosophy.

Changeux adheres to an "enlightened" materialism, claiming that the neural structure of man [34] is sufficient to explain consciousness, ideas, love, and ethical problems such as the need for tolerance and reciprocal respect, human rights and obligations, and even the existence of a universal natural ethics based on fraternity, freedom, and peace. The idea that there is something beyond the human body, spirit, or mind, must be abandoned as useless and anti-scientific.

It is difficult to understand how Changeux could believe that he was able to draw his ethical convictions from neurobiology. In a book published in conjunction with the philosopher Paul Ricoeur entitled *What Makes Us Think* [35], the latter tries unsuccessfully to convince Changeux that the neurobiological perspective is partial and insufficient in providing a real foundation for ethics, unless one previously has ethical convictions. There is a fundamental methodological obscurity when too many human achievements are expected to simply be the result of the structural and functional dynamics of our nervous system. This point will be highlighted when discussing the following two authors.

Maxwell Bennett and Peter Hacker

In 2003, Maxwell Bennett (neurobiologist, 1939–) and Peter Hacker (philosopher, 1939–) published a book on the philosophical foundations of neuroscience [36], a publication which was quickly followed by another in 2008 on the history of cognitive neuroscience [37]. They asserted that too frequently many neuroscientists uncritically attribute to the brain, or to parts of the brain, psychological acts that should properly be assigned to the person. To say that the neurons

perceive, that a neuronal circuit decides, that our hippocampus remembers, or that some cerebral region interprets is not false, but rather nonsense. This type of discourse is a mereological fallacy, referring to the parts what should be ascribed to the person, which is not a mere sum of the parts. These authors aim at bringing some conceptual clearness to cognitive neuroscience in order to avoid reductionism and materialism based on an abuse of language.

> We do not know what it means to say that the brain thinks, fears, or is ashamed ([37], p. 255).

These authors accept that we understand, love, or perceive, thanks to the brain, but not that the brain itself is the subject of those acts. Neuroscientists can discover that some neuronal events are related to psychological acts, but nothing more. Bennett and Hacker are not dualistic because they also deny that psychological processes should be referred to consciousness or to the mind.

> To ascribe a mind to a creature is to say that it is a creature with a distinctive range of capacities: in particular, capacities for concept-exercising thought, self-consciousness, memory, and will ([37], p. 13).

These are the capacities that confer the status of person, but the authors do not develop a theory of the human person, nor do they explain in what sense the brain is causally responsible for our psychological acts. Their position seems to reach toward Aristotelian-based views.

> It would be better to say, with Aristotle, that human beings are ensouled creatures (empsuchos)—animals endowed with such capacities as confer upon them, in the form of life that is natural to them, the status of persons ([37], p. 262).

Neuroscientists Contributing to Improve the Anthropological View of Man

Neurobiologists, including those already mentioned, sincerely believe that they contribute to a better knowledge of man. This section refers to certain neurobiologists whose discoveries and theoretical reflections, independent of a more or less clear account of the specific topic of philosophy of mind, make specific contributions that could quite well be integrated into a philosophical vision of the human person. These discoveries and reflections necessitate further discussions and adjustments. The complete list could be very long, however, because of space limitation we will discuss a selected few.

Antonio Damasio

Antonio Damasio (1944–) investigated the role of emotions in knowledge, specifically in the basic psychosomatic levels of human consciousness. His work delineated a new picture of primary and secondary emotions, moods or background affective states, and feelings. He proposed useful distinctions between an unconscious protoself, linked to the overall state of the organism, a higher core consciousness, and the self, based on an extended or autobiographical consciousness [38]. The interplay between consciousness, body, and feelings with relation to the environment is far from the older simplistic views that go back to the rationalistic account of man and to Descartes. This interplay is a dynamism geared to the consolidation of homeostatic states tending to "the good life" of the human person. Damasio's preference in philosophy of mind tends to reach toward Spinoza rather than Descartes: the mind is like the "idea of the body" [39].

Gerald Edelman

Gerald Edelman (1929–2014), winner of the 1972 Nobel Prize for his studies on the immune system, developed a theory of human (and animal) cognition and its neural substrate, based on an idea that can be labeled as neural Darwinism. This hypothesis posits a spontaneous neuronal process of selection between populations of neurons, in contraposition to the idea of information processing by instructions [40]. Leaving aside technical details, the hypothesis is convergent with research on epigenesis through synapse competition with Changeux.

Another major thesis by Edelman is the role of mapping in brain activity, which at some level

of the brain is a way of creating representations of the objects perceived, similar to the mapping of the human body contained in the somatosensory area of the cortex. There is a continuous re-entry or informational exchange between brain maps receiving various kinds of information from different areas (this point is convergent with Damasio's views on the same topic). There are also brain maps of maps, a process which explains the formation of concepts, memories, and learning beginning with the aforementioned Darwinist principle of selective competition between brain synaptic patterns [41].

Christof Koch and Giulio Tononi

Christof Koch and Giulio Tononi investigated the neural correlates of consciousness (NCC). Tononi holds that the basic substratum for being conscious (i.e., awake) is a wide group of selected neurons firing together according to adequately synchronized oscillations associated with the talamo-cortical system [42, 43]. The NCC is not a special area, but a talamo-cortical network distributed throughout the brain. This theory introduces a measure of integrated information called ϕ, quantifying the reduction of uncertainty (i.e., the information) that is generated when a system enters a particular state through interactions among its parts, above and beyond the information that is generated independently within the parts themselves (hence integrated information) [44].

This measure quantifies the neural requirement to be conscious in a context of biological complexity and provides the possibility of explaining unconscious cognitive states, such as those linked to nearly automatic behavior, while opening the way to deeper cognitive unconscious states corresponding to creative pre-representational states.

Josepf LeDoux and Jaak Panksepp

Cognitive consciousness must be completed with affective consciousness. This new understanding of consciousness became object of research for Joseph LeDoux (1949–). He considered neural dynamisms connected with basic emotions to be linked to animal survival, nutritional, and sexual behavior [45]. His research was complementary to that cultivated by Damasio. Jaak Panksepp (1943–), who coined the expression *affective neuroscience*, presents an ambitious scenario in animal behavior, integrating complex emotional systems such as seeking, pleasure, pain, panic, rage, anger, and anxiety [46]. These systems, based on instincts, are unconscious, but have conscious manifestations. They are realized through neural circuits related to meaningful stimuli that have an impact on perceptual systems and preside over behavioral responses such as defense, attack, and the search for food. The whole of these systems constitutes the affective dimension of consciousness, which is rooted in subcortical brain levels with projections into the cortex. Panksepp advocates the existence of an unconscious affective self, involving subcortical and cortical regions, both in animals and in humans.

Benjamin Libet

The thought-provoking experiments performed by Benjamin Libet (1916–2007) had a special impact on the neurophilosophical discussions on consciousness. A person is asked to freely move his or her finger or hand at any moment within a range of time, and to disclose, by pressing a button, the exact moment in which he or she was aware of the decision to perform the motor action. Electronic devices detect the correlated neural activations and the movements of the muscles. The result was the delineation of a specific neural firing called *readiness potential*, which briefly preceded (by nearly 1 s, but in later experiments even longer) the awareness of the decision [47].

A quick reductionist explanation of this experiment, such as "the brain decides for us", can arguably be avoided when the conditions and limitations of this experiment are analyzed. Simultaneously, insights on the role of previous unconscious preparations for decisions can be drawn. The experiment also provides a better understanding of the different levels of voluntary

actions, some of which can be clearly planned, while others can be accomplished in a semi-automatic way. These neuropsychological findings leave the nature and the relationship between consciousness and will open to debate.

Mirror neurons

Important for its potential implications in the philosophy of man, another discovery in neurobiology refers to the *mirror neurons*. This topic has become very popular, finding a place in books and magazines and providing an occasion for speculation and debates in many fields. The fact that the observation of meaningful or teleological actions accomplished by other subjects provokes the firing of sensory–motor-specific neurons in the observer seems to indicate that the observer mimics the actions of others, at least in imagination. In this sense, there is a kind of natural participation in what people see or perceive in others' actions and passions, such as pain. This form of knowledge is relevant for anthropological topics, such as empathy, which is theorized by phenomenologists, and also for a better comprehension of the process of learning by imitation [48, 49].

Some authors consider this discovery a breakthrough in the history of neuroscience [50]. Obviously, mirror neurons cannot be made responsible for the entire framework of personal interrelations. However, if integrated with many other aspects of brain dynamics, they do play a role in an account of the human and animal mind and consciousness in which relations, even from the prenatal period, must be put at the center of the human person and in his or her development in cognitive and affective states. Accordingly, family, friendship, and society appear to be essential in evolving human life. This idea is far from that of the traditional approach which tended to view mind and brain as isolated items.

From the previous convergent neurobiological accounts regarding consciousness, some comprehensive conclusions can be drawn. These provide a whole vision of the human person, under the various labels of the emotional brain, the social brain, the empathic brain, etc. In addition to the aspects relative to the higher levels of the embodied mind (as it is often called), rational as well as emotional dimensions can be added regarding ethical and religious behavior inasmuch as they can be followed in their cerebral expressions.

Neuroethics and Neurotheology

Neuroethics, both theoretical and empirical, the latter using functional magnetic resonance imaging, considers, among other things, the interactions between cortical and subcortical networks in the brain, attempting to assess their weight in moral reasoning, judgments, desires, and impulses related to ethical behavior [51–54]. Another field of interest in neuroethics is the cerebral corresponding patterns, occasionally investigated through connectionist models in which stable states of mind are related to virtues and mindfulness [55, 56].

Something similar can be performed regarding religious experiences. This field of study is usually called *neurotheology*, however, a more precise name would be neurobiology of religious and spiritual experiences [57]. Different neurotheological perspectives range from reductionistic views, which propose that these experiences (i.e., mysticism, meditation, and religious beliefs) are merely a product of particular brain states and activations, to an account of different psychological states (cognitive, emotional, and behavioral) based on special brain circuits and associated with religious acts that are considered to be an authentic anthropological dimension related to God.

This field is broad and complex. Religious acts, like any other human performance, certainly have a neural substrate, although it may not necessarily be specific. These acts, that can be very different and can be realized in various ways, can exercise a downward causation on brain circuits (motivational, cognitive, emotional). Inversely, particular neurophysiological states modulating moods, motivations, etc., can develop an upward causation that affects the way specific religious acts are experienced by individuals or groups.

The relationship between theology and neuroscience is in some ways similar to the relationship between theology and psychology of religion. However, the claim that neuroscience explains religion would be analogous to claiming that neuroscience explains mathematics, literature, philosophy, and so on. Neuroscience is an important auxiliary discipline for the understanding of many aspects of human behavior, but it is not the key to a complete comprehension of man.

Enactivism

Enactivism, among whose proponents are Francisco Varela, Evan Thompson, Eleanor Rosch, and Alva Noë, can be considered a psychological and anthropological approach competing with the classical philosophies of mind. The latter had become overwhelmingly dominated by an isolationistic view of mind and brain [58]. Such a perspective, typical of dualism, materialism, and functionalism, stands in stark contrast to enactivism which emphasizes the active relationship of bodily agents, or humans, to the environment and to the world.

Enactivism rejects the representationist view of a brain primarily concerned with its own states and subjective representations. The brain is important, but it cannot be seen as a center endowed by cognitive powers that simply creates the world. The brain is a part of an organism, viewed as a complex system, and the mind is not only a function of the brain, but is an aspect of the entire body (*embodied mind*). The unity of the embodied mind and brain is "extended" to the world through action (*enactive mind*), while at the same time the world appears to the agent as functionally patterned in accordance with his or her needs, capacities, and experiences [59]. Reducing the human being to the brain is parallel to the Cartesian reduction of man to consciousness.

Thomas Fuchs

Thomas Fuchs (1978–), a German philosopher and psychiatrist endorsing enactivist claims, presents a philosophy of neuroscience in which the brain is understood as a relational organ rather than a mere information processor [60]. It is an organ of the person, and not the seat of consciousness or of a mind. Its function is to integrate experiences so as to regulate and modulate the entire organism, to transform information, and to enable communication with others while simultaneously mediating the various cycles of organism–environment interactions. Biological plasticity enables the brain to be in a continuous process of transformation, flowing from the interactions of the person with the physical environment, culture, ideas, and other persons. The brain is an organ of possibilities that are accomplished through neural processes and whose agent is the human person. The plastic condition of the brain enables the individual to grow as a person through experience and to create his or her own personal history. The person—not the brain—thinks, lives, and interacts with the world, thanks to brain activity. Fuchs strongly criticizes Thomas Metzinger's thesis according to which the self would be a purely phenomenic construction of the brain [61].

Fuchs follows a phenomenological approach in a broad sense of the word, coherent with the enactivist premises in psychopathology. He argues that mental disorders must be seen in all of their dimensions, as they disrupt the unity of the person and his or her relationships with the environment. Psychiatric therapies should be both biological and psychological [62]. Contrary to the methodology of reductionism, the therapists in this field should bear in mind the circular complex causality within a systemic biological framework. Any single interaction within the system has holistic effects at all levels.

According to Fuchs, interventions and psychiatric treatments are efficacious whenever they follow the systemic and ecological concept of mind and brain [63]. This idea implies a comprehension of the interconnection among psychological, social, and pharmacological approaches. Psychological therapies influence the structure and functions of the brain by altering synaptic plasticity and gene expression, following a top-down causality. Conversely, neuropsychological

and biochemical dysfunctions influence moods, emotions, and ways of perception, exercising a bottom-up causality.

This is a circular complex causal process displayed between the brain, the organism, mental or psychological states, and interactions with the environment, with the brain acting as a mediating entity [64]. There is no separation, but rather a reciprocal transformation or translation between psychological and biological processes. For instance, depression is perceived by Fuchs as a psychophysiological desynchronization [65]. A loss that the individual is unable to cope with is translated by the brain into a neurobiochemical pattern affected by the uncoupling of rhythmic physiological processes, which in turn increases depressed mood. Every level triggers and influences the other. Analogous views can be supported regarding schizophrenia and many other mental disorders [66]. The classic positions on philosophy of mind such as dualism and functionalism examined in the first part of this chapter, are unable to obtain these insights into psychopathology.

Conclusion

Among the many conclusions that can be drawn from the delineation of the different positions enumerated in this chapter, two considerations can be proposed. The first is epistemological. Neurobiology, as far as it is concerned with human capacities such as language, reasoning, understanding, and free decisions and actions, is not purely biological. It is a hybrid science that presupposes and employs anthropological and ethical knowledge [67]. This epistemological feature is unique in neuroscience. It entails a complementary interaction between the anthropological (as well as psychological) and the biological perspectives. As a purely biological science, neuroscience involves a partial and not a total explanation.

The second consideration is ontological and is the basis of the former. The human person, and in a different way, animals as well, is a multi-layered complex and systemic unity. Each level, the vegetative, the sensitive to various degrees, and the rational, possesses its own autonomy while at the same time it influences the others, not extrinsically, but essentially, according to the various modes of integration. An important way of integrating could be understood following the hylomorphic Aristotelian model (given in a broad sense) that explains how higher levels are capable of giving a new sense to lower and more material levels while at the same time depending on the material conditions of the former [68]. Such is analogous to the game of chess, wherein intelligent moves and the rules of the game provide a new dynamism to the physical chess pieces, which otherwise merely obey gravitational and other physical laws. Accordingly, there are many senses of being causally influent in several reciprocal directions.

The limits of dualism, materialism, and functionalism are overcome by this systemic view [69, 70]. The latter part of this chapter was dedicated to enactivism and to Fuchs' view because it is an approach that seems more complete and promising than the others. The Aristotelian framework together with an account of the complex unity of the human person add a more comprehensive ontological view to this perspective.

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Neurosciences, Neuroeconomics, and Metaphysics

4

Ricardo F. Crespo

Science and Metaphysics

The idea that theoretical or even metaphysical notions influence the meaning and perception of scientific evidence has by now garnered widespread acceptance. The road to this conviction has taken many steps. French scientist Pierre Duhem is considered one of the first thinkers to note the theoretical commitments of empirical scientific investigation—the later so-called theory-ladenness. For him, the result of a physics experiment is the fruit of an observation interpreted by virtue of the theories held by the observer. When using their instruments, physicists, chemists, and physiologists "implicitly admit the accuracy of the theories justifying the use of these pieces of apparatus as well as of the theories giving meaning to the abstract ideas of temperature, pressure, …" ([1], pp. 259–60).

In *The Logic of Scientific Discovery* (1934), Karl Popper wrote that "for even singular statements are always *interpretations of the 'facts' in the light of theories*" ([2], p. 423, italics in the original). Popper notes that any descriptive statement contains universals, which are hypotheses or conjectures; given that, for him, "universals cannot be correlated with any specific sense—experience" ([2], p. 95)—because "they transcend experience" ([2], p. 424)—these propositions cannot be verified. A scientific community convention is then required to establish an empirical basis ([2], Chap. 5).

In 1951, Quine challenged the analytic–synthetic distinction, arguing that empirical propositions cannot be isolated from their associated theories. As a result, there is "a blurring of the supposed boundary between speculative metaphysics and natural science" ([3], p. 20). Hans-Georg Gadamer, affiliated with a different tradition (hermeneutics), refers to the "horizon", "the range of vision that includes everything that can be seen from a particular vantage point" ([4], p. 302).

In 1958, Hanson coined the expression theory-ladenness in his well-known statement: "seeing is a 'theory-laden' undertaking" ([5], p. 19). Much has been said about the meaning and scope of his notion and the concept of incommensurability set forth by Thomas S. Kuhn and Paul K. Feyerabend. Because both thinkers underwent an intellectual evolution over the years, a moderate interpretation of their theses may be considered. While relevant differences separate the ideas of all the authors mentioned above, e.g., (Heildelberger

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[6]), a certain influence of theory in observations and experiments remains undisputed and clear. This influence may be conceptual or semantic—the meaning of observational terms is (partially) determined by theory—or "perceptual", by the cognitive theory biases of the observers.

Empiricist Bas van Fraasen ([7], p. 81) also supports theory-ladenness. More recently, Jim Bogen explained that "by Bayes' theorem, the conditional probability of the claim of interest will depend in part on that claim's prior probability. (…) One's use of evidence to evaluate a theory depends in part on one's theoretical commitments" ([8], p. 11). Additionally, Julian Reiss complained that evidence theories fail to take into account that evidence about "a hypothesis is dependent on how the world works and our knowledge there of" ([9], p. 302). In summary, as James Ladyman pointed out, "the degree of confirmation of a scientific theory is heavily theory-dependent, in the sense that background theories inform judgments about the extent to which different theories are supported by the available evidence" ([10], p. 214).

Finally, moving beyond this thread of thought, Craig Dilworth [11] underscored that modern science applies specific, fundamental metaphysical principles (uniformity of nature, substance and causality), which outline a physicalist, deterministic (though not rigid) view of reality that is not accepted without reservations by all scientific disciplines, depending on their subject. These principles determine what is ontologically necessary or possible within every discipline, provide the structure of scientific rationality, set guidelines for developing science, and define the basic concepts. They are not necessarily true, but it is assumed that they were.

Aggazi ([12], p. 19; quoted by 1, p. 71) states:

> Science […] cannot be pursued without one's using certain criteria of intelligibility which are prior to the specific tasks it involves. In fact, every advancement of some science which has been presented as a 'liberation from metaphysics' has actually been tantamount to discarding a *particular* metaphysical framework and accepting (often unconsciously) a different one […] Therefore it is much more reasonable to be aware of the metaphysics one has, rather than have a metaphysics without knowing it.

Dilworth shows how these metaphysical criteria or principles have shaped the methodology of the empirical aspects of science. Regarding him, we should mention 'principle-laden' (cf. [11], p. 94) concepts, rather than theory-laden notions. "Neither these principles," Dilworth argued, "nor the physicalistic interpretation they have been given by modern science are inviolable, however, and to a large extent both have been adopted" ([11], p. 193). In his paper on "the metaphysics of neuroeconomics", Michiru Nagatsu admited that "metaphysics is an indispensable part of scientific practice that provides scientists with worldviews and directions in research" ([13], p. 198).

In short, metaphysics—understood as a worldview—is always present in science, and the current metaphysics of science is materialistic. As Schouten and Looren de Jong simply put it, "science and philosophy have turned materialist: all that exists exists in space and time and must be considered fundamentally physical" ([14], p. 1). Let us see how this applies to neurosciences, economics, and neuroeconomics.

The Metaphysics of Neurosciences

Dilworth ([11], p. 265) notes:

> The fundamental problem for modern science with regard to the spirit is evident already in early Greek atomism, with its lacking categories for the self and psychic states. This problem remains in modern science, both as a paradox with respect to the nature of its own activities, as well as a major lacuna with respect to what it is capable of explaining. […] [T]he spiritual element generally acknowledged to exist in human activities cries for explanation. Science, limited as it is to physicalistic categories, cannot handle either of these issues.

Indeed, this limitation introduces a tension in the philosophy of neurosciences. Though a complete materialistic reductionism largely prevails (e.g., Patricia and Paul Churchland), not all authors share this belief. Morality, responsibility, complex or high reasoning, conscience, affective relations are evident realities pointing to something beyond matter. Finally, many reductionists cannot accept that all can be explained by biological interactions.

David Chalmers [15] spoke about an explanatory gap, or "the hard problem of consciousness", while Bickle et al. ([16], p. 11) wondered, "Why should that particular brain experience give rise to conscious experience?" The introspective aspect of individual sensory experiences also raises doubts.

Bennett and Hacker ([17], Part I, Chap. 3) argued that neuroscientists are plagued by a "mereological fallacy", attributing psychological acts pertaining to the whole human being to a part of him—the brain or the mind. That idea is drawn from Aristotle, who wrote in *De Anima*: "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 that the soul is the instrument whereby man does these things" (*On the Soul* 408b 12–15, [18]). Anthony Kenny [19] referred to that mistake as "the homunculus fallacy", while Wittgenstein noted that "only of a living human being can one say it has sensations; it sees, is blind; hears, is deaf; is conscious or unconscious" (*Philosophical Investigations*, & 281, [20], p. 97c). The human being is an organic whole not reducible to the sum of its parts. Voices have also risen against materialist reductionism in psychiatry and psychology. More generally, William Wimsatt (e.g., [21]) spoke about "functional localization fallacies"—that is, attributing a property of the whole to one of its parts.

However, this is not the dominant view, which is mainly reductionist. The topic of reductionism is thoroughly complex and, although tensions in this field still remain, the balance is inclined toward an epistemological and ontological reduction of the mind to the neural, according to the underlying physicalist worldview. Yet the situation is not hopeless: in the introduction to their book on reductionism, after saying that the most reductionist position on the book is John Bickle's, Schouten and Looren de Jong ([14], p. 21) concluded:

> Most other authors, however, will acknowledge that to a more or lesser degree higher-level explanations are indispensable, but not autonomous; and that psychology and neuroscience are and should be connected and perhaps integrated, but not unified along physicalist lines.

While avoiding a dualistic view of the human being, Aristotle's hylemorphic conception of the soul as the form of the body allows for two compatible non-reductionist explanations (*On the Soul* 403a 39–403b 2) [18]:

> 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.

Nonetheless, to make these explanations compatible, physicalism must be replaced by another metaphysical naturalist—yet not a materialist view.

The Metaphysics of Economics

There is a parallel problem in the application of modern science's physicalist, metaphysical commitment to social science. Dilworth ([11], p. 130) explains,

> Some of the basic problems regarding their applicability in the social sciences are those of synthesizing uniformity and free will, the vagueness apparently inherent in the notion of a social substance, and the dominant position occupied in social thought by the notion of final causes.

These characteristics also introduce a tension to social sciences, including economics, as Dilworth once again points out, specifically about economics and freedom ([11], p. 135):

> [T]here is a particular tension in the economist's conception of human nature. On the one hand the notion of free will is integral to it, since without free will the rationality principle would make no sense. On the other hand, however, no economic actor has the freedom not to follow the rationality principle, which itself determines how he or she is to act.

Throughout the nineteenth and early twentieth centuries, economics was closely related to psychology. Freedom was present, but not always the star. The roots of the law of decreasing marginal utility lie in psychology, and the view of utility from authors such as Gossen, Jevons,

Menger, Walras, and Marshall are also associated with psychology. For Keynes, psychological factors strongly influence behavior. However, it should be noted that the ordinal utility theory, which started with Pareto, Hicks and Allen, and Slutsky, began to belittle psychology, planting the seeds of a "logical", non-psychological, theory of rational choice, the core of a formal science of economics.

Let us take a glance through history. Economics started to become a formal science in the nineteenth century. Nassau Senior was the first economist to strongly argue against consideration of ends and the normative character of economics, maintaining the distinction between positive or neutral analysis and providing recommendations for economic policy in his *Outline of Political Economy* (1836). In 1860 he delivered the presidential address to Section F ("Economic Science and Statistics") of the *British Association for the Advancement of Science* ([22], pp. 19–24). As Terence Hutchinson ([23], p. 9) remarked, "Section F had to assert its scientific respectability and its worthiness to be included alongside the established subjects of natural science". According to Hutchison ([23], p. 13), Senior sketched a narrow and limited vision of economic science. In other words, under the pressure of natural sciences requirements, economic science was forced to modify its subject of study in order to conform to this particular conception of science.

Thus, we arrive at the definition formulated by Robbins ([24], p. 15) –influenced by Menger, Weber and Mises: "Economics is the science which studies human behavior as a relationship between ends and scarce means which have various applications." That is to say, economics is the science of a specific vision of choice. In this way, economic science is turned into a formal science. It is formal because its subject of study is not a field related to material human needs, nor to production and distribution. It becomes a choice, any choice, to the extent that it requires adaptation of means to certain ends: it is an approach to human action. In fact, it was initially correlated with economic matter, viewed as efficient distribution of resources, but it quickly applied its logic to the analysis of other human realities.

The key to fitting human action in a specific framework is to consider ends or preferences as given. Stable, exogenous preferences (the ends, as considered by economics) prepare the field for the development of a certain scientific subject. Menger entitled Appendix VI of his *Investigations into the Method of the Social Sciences with Special Reference to Economics* "The Starting Point and Goal of all Human Activity are Strictly Determined". In that work ([25], p. 217) he sustained that "[e]conomy is really nothing else than the way which we travel from the previously indicated starting point of human activity to the previously indicated goal." Strictly speaking, it is a technical path that enables formulation of exact laws whose formal nature does not differ from that of the laws of all other exact sciences and of the exact natural sciences, particularly (cf. [25], pp. 217–219). Therefore, economic science considers ends as given. As Robbins ([24], p. 29) maintains, "economics is not concerned at all with any ends, *as such*. It is concerned with ends in so far as they affect the disposition of means. It takes the ends as given in scales of relative valuation". Freedom is thus put into brackets.

Economics then attempts to be a formal logic without psychological, sociological, and moral elements, a "rational choice theory". Though Robbins ([24], p. 83ss) tried to move psychology aside, he recognized that it was "half of the equation". The very word "utility" carries a psychological resonance. Samuelson ([26], p. 62; [27], pp. 243–253) subsequently developed his theory of revealed preference, "dropping the last vestiges of the utility analysis": we come to know preferences by looking at their external manifestations, quantities and prices. However, the word preference itself refers to psychology. Finally, John von Neumann and Oskar Morgenstern [28], as well as Leonard Savage [29] have come up with a completely formal theory of rational choice: the expected utility theory. An axiomatic theory, it states that if people are rational—in the specific sense they have been defined as such—they will behave as if they were maximizing utility. The order of "well-behaved" (consistent) preferences and probabilities are given and the solution is exact. However, the theory embodies very strict

assumptions that make it even narrower than Robbins' theory, because it assumes an over-simplification of the problem of uncertainty.

Hence, there are two wings eluding the topic of freedom and introspection: the positivist of Samuelson's revealed preference theory and the axiomatic of von Neumann, Morgenstern, and Savage. Nuno Martins ([30], p. 252), explains:

> There are two dominant approaches in contemporary rational choice theory, which in turn underpin mainstream microeconomic theory. In the first of these approaches, we start by defining a set of axioms, from which a preference ordering is obtained. This preference ordering reflects self-interest, and can be represented by a utility function. It is also assumed that actual behavior, and 'rational' choices, will be driven by such preference, which is the 'rational' preference.
>
> In the second approach, instead of starting from a set of axioms from which a preference ordering that explains choice is obtained, we start from observed choices, and infer an underlying ordering preference that is consistent with those choices. This is the approach that establishes Paul Samuelson's (1947, [31]) theory of revealed preference because, through this approach, an underlying ordering preference is inferred from observed behavior, and rational behavior is defined as any type of behavior that is consistent with the revealed ordering preference.
>
> Sen (2002, [32]) notes that even though these approaches have opposite starting points, both are committed to the postulates that there exists a single and complete ordering preference that characterizes rational behavior, and that actual behavior mimics rational behavior so defined.

Assuming there is a complete set of preferences, represented by a utility function and deriving in a maximizing behavior, it becomes unnecessary to examine all possible behavior motivations, which can be reduced into a single one: maximizing utility. This set of preferences suffices to predict resulting behaviors. However, this is not true in real life. Sen criticizes both approaches, as Martins ([30], p. 252) notes in this passage:

> Sen (1982, 2002, [32, 33]) criticizes mainstream rational choice theory, and the mainstream microeconomic theory grounded on the latter, for failing to recognize that human behavior cannot be described in terms of a single complete preference ordering only. Sen (2002, [32]) notes that human behavior may be driven by motivations other than self-interest, such as social commitment, moral imperatives and conventional rule-following, and argues that not all of these motivations can be described by the same preference ordering. Furthermore, Sen argues that preference orderings need not even be completely specified. Limited information, value conflicts, or the need to act before the judgmental process has been made, may lead to incomplete preference orderings.

The simplification of the rational choice theory adopted by current, standard economics captures preference content and motivation in a proverbial black box. However, experiments on behavioral economics have rejected the rationality axioms of the rational choice theory as unrealistic, as they are based on the assumption of a hyper-rationalistic individual that does not exist. Thus, it is necessary to open this black box to examine preference causes, processes and contents.

Behavioral economics is an attempt to improve the explicative and predictive strength of economics by incorporating psychological elements. This approach draws from earlier North American institutionalists who gave a relevant role to psychological considerations. Its rebirth can be attributed to the joint work of psychologists Daniel Kahneman and Amos Tversky, particularly since the 1970s. Other academics from this branch of economics later included Richard Thaler, Matthew Rabin, George Loewenstein, and Colin Camerer, to name a few. Its epistemological or methodological characteristics include its evident interdisciplinarity; its acceptance of non-observable entities, such as beliefs or emotions, as valid sources of scientific knowledge, and its consequently close ties to cognitive sciences and their developments which have recently given way to a new branch called "neuroeconomics". Also noteworthy are the attempts of behavioral economics to conduct experiments to objectify evidence and draw conclusions.

Kahneman won the Nobel Prize in 2002, with Vernon Smith received it along with him for a methodological innovation: "having integrated insights from psychological research into economic science, especially concerning human judgment and decision-making under uncertainty." The corresponding committee stated:

> Traditionally, much of economic research has relied on the assumption of a "homo œconomicus"

> motivated by self-interest and capable of rational decision-making. Economics has also been widely considered a non-experimental science, relying on observation of real-world economies rather than controlled laboratory experiments. Nowadays, however, a growing body of research is devoted to modifying and testing basic economic assumptions; moreover, economic research relies increasingly on data collected in the lab rather than in the field. This research has its roots in two distinct, but currently converging, areas: the analysis of human judgment and decision-making by cognitive psychologists, and the empirical testing of predictions from economic theory by experimental economists. This year's laureates are the pioneers in these two research areas. (http://nobelprize.org/nobel_prizes/economics/laureates/2002/press.html)

Controlled experiments are mediators between hypotheses, theories, and models, on one hand and reality on the other. They are used more and more in economics, as they make way for the analysis of phenomena that had not been previously considered. For example, they allow for the development of theories on the contributing factors in such human realities as happiness, altruism, and reciprocity. The purpose of behavioral economics is to open the black box of human preferences, values, and decisions. Neuroeconomics can be viewed as a development within behavioral economics that looks for the content of this black box inside the brain.

The Metaphysics of Neuroeconomics

The purpose of this section is to establish whether the above-mentioned tension—i.e., for or against physicalism—is also present in the field of neuroeconomics. Neuroeconomics is not a well-defined and homogeneous research field: Roberto Fumagalli ([34], pp. 121–22) listed five different definitions for it, while Caterina Marchionni and Jack Vromen [35, 36] narrowed them down to two, noting ([35], p. 104; [36], p. 2; see also Harrison and Ross [37], and Glimcher et al. [38], p. 7):

> One might easily get the impression that neuroeconomics involves a one-way transfer of data and insights from neuroscience to economics and that if neuroeconomics is to make a lasting contribution, it should be to the field of economics. But this is not at all obvious. As most of the essays collected in this Special Issue recognize, there are at least two rather different strands within neuroeconomics: *Behavioural Economics in the Scanner* (BES) and *Neurocellular Economics* (NE). Whereas BES takes existing neuroscience to task to better understand economic behaviour, NE takes existing ("standard") economic theory to task to better understand neural activity in the brain. Whereas BES argues for radical, if not revolutionary changes in economic theory, NE argues for radical, if not revolutionary changes in neuroscience.
>
> As several authors in this special issue observe, some leading proponents of neuroeconomics expect that neuroscientists have more to gain from introducing standard economic theory in the study of neural activity than economists can gain by trying to accommodate neuroscientific data and insights in the study of traditional economic phenomena.

In addition, Colin Camerer et al. ([39], p. 10) make a distinction between neuroeconomics as an "incremental" approach (adding variables to standard economics) and a "radical" approach that might deeply change economics. The former type of neuroeconomics tries to analyze economics using neuroscience experiments because, as mentioned, the rationality of both the rational choice theory and the expected utility theory—the basis for standard economics—has been challenged and often refuted by behavioral economics. The second type of neuroeconomics, advocated by Don Ross [40, 41], assumes that the brain's internal logic is the neoclassical economic logic. Although Ross makes a good case for such a bold thesis, I will not delve into that topic in this chapter.

The first type of neuroeconomics, as Harrison and Ross ([37], p. 187) describe it,

> consists of repeating protocols that putatively demonstrate human 'irrationality' under neuroimaging, and trying to show how 'anomalies' in rational choice have origins and explanations in framing effects that results from the computational processing architecture of the brain.

As neuroeconomists, they suggest replacing the traditional economic notion of utility—the already mentioned mathematical representation of consistent preferences refuted by actual experiments of behavioral theory—with neural utility—a true, objective utility, measured by the activation

of particular areas of the brain. In other words, the intention to overcome the oversimplified analysis of standard economics with a refined search of the causes and content of preferences, far from doing away with the materialistic realm of utilities, deepens the materialist approach. It skips the realm of free conscious decisions—rational or not—to move straight into their supposedly materialistic causes. Neuroeconomics opens the black box only to find neural mechanisms. As Graziano ([42], p. 32) explains, in neuroeconomics "[t]he utility of a choice is not determined by formal preference relationships, but rather it is the result of a complex [neural] mechanism." He adds ([42], p. 40), "the *Homo Economicus* is replaced by the *Homo neurobiologicus*, whose behavior derives from a neurobiological development able to generate sentiments, beliefs, actions, and the capacity to make decisions."

Many economists frown upon the science of neuroeconomics. Its most severe critics, Gul and Pesendorfer [43], point to the definition of economics: while neuroeconomics pays attention to the choice process involved in opening the brain's black box, economics deals with rational choices under specific conditions. The relevant data for this new field are revealed by observable external information, not by information about the brain's internal interactions. According to those authors, neuroeconomics deals with other areas of concern and has nothing to do with economics. And it's back to metaphysics once again, as Nagatsu concludes ([13], p. 203), "In this paper, I have attempted to identify a genuine, metaphysical disagreement between the advocates and critics of neuroeconomics [see also Levine [44] for a similar argument]. The disagreement arises when scientists take different stances on the same object of investigation." Additionally, Fumagalli [45] raises doubts about the accuracy of neural measurements. This is a generalized criticism of neuroeconomics.

Robert McMaster ([46], p. 119) also found divergent ontological positions about the structure of the brain in a number of neuroeconomic research studies. However, regardless of their different ontological views, they are all materialist notions of the brain. The debates between economists and neuroeconomists, as well as in the neuroeconomic field are largely methodological. And when they move beyond this narrow dimension onto a metaphysical ground, they do not move past the physicalist arena.

Thus, the science of neuroeconomics seems plagued by paradox. Its goal is to open the black box of preferences—a worthy endeavor as part of efforts to learn more about choice causes and processes as well as the ends of economic agents. The goal of neuroeconomics is to replace economics' typical "as if" or *ceteris paribus* assumptions with realistic "as is" observations. However, this intention leads neuroeconomics to eliminate all human intent and freedom: there is nothing but neural interactions. Knowing them, we will be able to predict human responses in different economic (in a broad sense) scenarios, because "neural activity causally determines economic choices" (Fehr and Ragel [47], p. 3).

Nonetheless, there are voices that have risen in opposition. For example, basing his claims on specific neuroeconomic experiments, Antonietti [48] concluded that neuro-mental correspondences only feature a heuristic purpose, at most providing for conjectures, "which must be verified by psychologists in the context of mental (and not neural) phenomena and which must be explained in psychological (and not neurobiological) terms" ([48], p. 217). Kuorikoski and Ylikoski [49] also point to the priority of psychology, relying on a theory of explanation—"neuroeconomic data are explanatory and relevant only when they inform a causal and explanatory account of the psychology of human decision making" ([49], p. 227). Similarly, Roberta Muramatsu ([50], p. 283) notes, "[a] careful look at some experiments lead us to suggest that they identify some interesting statistical associations (correlations) between variables (parameters) but there is no room for an indisputable move to a 'causation talk'." Harrison [51] also assigns a priority role to psychology.

An additional key issue remains unresolved. Economics does not only have a descriptive, predictive, or explicative role: it also serves a normative purpose—what should an economic agent or an economic policy look for? Typically, the

answer is maximizing individual or social utility, but, what is the definition of a true utility, a hedonic utility? Or should "eudaimonic" aspects be included? The normative role in economics is increasingly taken into account and refined. Amartya Sen's capability approach focuses on the capabilities, opportunities, or freedoms that people should have, but what are these capabilities? Happiness economics also pursues a descriptive and normative goal. Which concept of happiness should we adopt? Crespo and Mesurado [52] argue for a "flourishing" notion of happiness, which is a very refined construct that includes the social impact of individual actions. What are the neural counterparts of the components found in flourishing? Additionally, as Martins notes, "The relevant conception of reality in neural analysis is that of an open system" ([30], p. 255). Not everything is deterministic—not even inside the brain.

Wouldn't it then be more sensible to note a correlation between neural activities and psychological movements while prioritizing psychology? This sounds like a more prudent approach, but the narrow straitjacket of today's prevailing physicalist worldview prevents it.

Conclusion

Modern philosophy has leaned toward reductionism, reducing spirit to matter, human rationality to instrumental rationality, causes to the efficient cause, freedom to determinism. This set of reductions has shaped a physicalist, materialistic metaphysics that pervades modern science and curtails its explanatory capability. The tensions of neurosciences and social sciences point to the limited scope of this metaphysics. Neuroeconomics focuses on these tensions but tries to solve them in materialist fashion, which is is an example of the need for broader metaphysical view in science. The materialist approach of neurosciences and neuroeconomics reveals that both are current products. Barely 50 years ago, German pathologist Franz Büchner ([53], p. 205), faced with the reality of the close link between mind and body stated:

> The human body is expression of the human soul and represents not only the manifestations of our conscience but also the whole no conscious sphere. Thus, we face the question whether the soul is not the dominant principle of human existence, if it is not essentially the creator of our body and if the latter is not a creature of our soul. In another stage of these reflections we arrived to think that what is truly real in our human being is our soul and that our body behaves only as symbol of our soul.

Looking at the same reality, this interpretation opposes that of neuroscientists and neuroeconomists. We perceive a relation, but causality is a metaphysical reality and is not captured by our senses, but by rather our minds: it is a metaphysical knowledge. True metaphysics is required to secure proper knowledge. Hopefully, the future will bring new and better explanations and practices, with non-materialist naturalism as a better option to make sure it does. At the end of the day, not everything is material in nature. For example, though supported by matter, structures, forms, actions, and thoughts are not material things, but they are natural. A metaphysically sensible approach would be integrative rather than reductive, underscoring psychology and the soul (see Craver and Alexandrova [54]).

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Towards a Psychotherapy with a Philosophical Basis

5

Ricardo Aranovich

Introduction

In somatic medicine, biological knowledge is the base of the therapeutic actions, and when questions arise, experimental works which are focused on better therapeutic behaviors are used. Psychotherapy, a therapeutic intended activity, lacks an equivalent basis. Being a land belonging to what Dilthey denominates Sciences of Spirit (as opposed to those of nature), the experimental method is of dubious application. While there are a growing number of jobs that deal with the results of psychotherapy, assimilation of the validity of these results to those obtained by experimental methods in biology is problematic. On the one hand, the diagnosis and evolution is often established by scales in which the results require an interpretive step that makes them lack the same value an objective fact has (even though it is pretended that they DO have the same value). Furthermore, if you try to compare treatment groups with "placebo" groups, such is the number of variables exposed to interpretive bias (diagnosis, features of each case, training and personality of the therapist, technique, rapport) that the results (even though they may statistically favor any technique in relation to others) do not yield, in general, certain conclusions. Another limitation of this type of testing is that the data are retrospective and, as such, do not rule out other intercurrent factors in the course of treatment that influence its evolution. This is not intended to question the various psychotherapeutic methods or the type of research in which the limitations are based on the object of study, but shade their conclusions, and therefore reaffirm the need for a basis for the psychotherapeutic task because it is not found in the scientific field, but in other fields that have always been taking care of reflecting on the human being: the philosophy and philosophical anthropology. It is in this quest that we find the work of José Ortega y Gasset, which we want to share as a basis and resource for therapeutic action.

Regarding implicit philosophical thinking, Ortega says:

> Strictly speaking, the truth is that the ground on which man always stands is neither Earth nor anything else, but a philosophy. Man lives *from* and *a* philosophy. This philosophy can be scholarly or popular, from others or from ourselves, old or new, brilliant or stupid; but the fact is that our being always ensures its living plants in one. Most men do not notice because that philosophy that they live is not shown as a result of intellectual effort (therefore, that themselves or others have done) but it seems "The Truth"; i.e. "reality itself." [1]

The philosophy which we might call "implicit," as opposed to that which will later be

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proposed as "explicit," in the case of psychotherapy consists of the conceptual knowledge with which the human being is focused and the manifestations of his conduct. Currently, what will happen more often is that Darwin (and in other cases Pavlov), with their common positivist affinity, will underlie in what "most men do not notice."

It is necessary, if any clarity is intended in any topic related to humans, to start from an anthropological hypothesis that provides an answer to the inevitable question: What *is* man? So far it has been the positivist thought and biologist who have taken care of that answer, with good results, precisely, in the aspects that depend on biology, but that approach involves a human nature similar to that of our brother mammals, with whom we have much in common, no doubt, but leaves out the most important thing that is to be determined: what is specifically *human* in *man*.

> …Man has no nature. Man is not his body, which is one thing; nor is he his soul, psyche, consciousness, or spirit, which is also a thing. Man is not a thing, but a drama—his life, a pure and universal event that happens to everyone and in which everyone is not, but an event as well [2].
>
> And because of this it is not the pure reason, eleatic and naturalist, who will ever understand man. So far, man has been a stranger. [3]

A workaround for the lack of that foundation is to try to obtain it from "experience." From a certain amount of experience which is considered favorable, these are generalized and a true anthropology which argues that difficulties in human life originate in this or that conflict that takes universal dimensions is built. Be it (the conflict) Oedipus, the need for power, early objective relationship, projective identification of the primal trauma, predator behavior, filicide, the search for meaning or simply an inadequate conditioning and such occur and proliferate "schools" that populate the auditorium of the specialty.

Again, we insist that no value is removed for any of these buildings or is their effectiveness questioned. With a covering philosophical foundation, each of these approaches may become cosmopolitan and live with each other, because when they each cease being the "ultimate truth" about the human being they would be illustrative examples of possible conflicts.

The importance of Ortega to the topic at hand is:

> Since 1914 (see my *Meditations on Quixote*, in Collected Works, Vol. I) it is the intuition of the phenomenon known as "human life" that is the basis of all my thoughts. [4]

Therefore, it is a philosophy *of* man. Not only *on* humans but, for it, it is located *from* humans.

> …Whatever we think about our life and its ingredients is something we do by being in our life; it, then, with all its ingredients, *is* already before we get down to thinking about it and them, respectively. [5]
>
> There is the "fact" previous to all facts, in which all others float and that all emanate from: human life as it is lived by each person [2].

Radical Reality

This condition of "previous event" to all has led Ortega to regard life as "radical reality":

> To live is the way to be radical: all the ways of being which I find in my life, inside it, as it´s details and referring to it… The most abstruse mathematical equation, the most solemn and abstract concept of philosophy, the universe itself, God himself: these are all things I find in my life, things that I live. [6]

"Radical Reality" implies that it is also a fundamental fact. It is the data that do not require any other concept to be "explained." This is possible because life is evidential:

> All living is to live ourselves, to feel alive, know to exist, where knowing does not involve any intellectual knowledge or special wisdom, but it is that surprising *presence* that life is for everyone; without knowing that, without realizing that *that* toothache would not hurt us. [7]

So all "intellectual knowledge" or "special wisdom" is posterior and secondary to the prior act of living. To develop any knowledge or wisdom about life, it is necessary to *be* alive, living, so life is prior to any explanation of the attempt to determine it. Life is, at once, what requires nothing and can't be explained, but also the data which

one should take as a starting point if any reflection on what is human is sought. As human beings, our human life is an astonishing and incomprehensible phenomenon. It is legitimate and inevitable to make theories, but these cannot reverse the shocking prior act: the life of everyone. To escape this contact with life itself, to visit the territory of abstractions and intellectualizations was the cause of the modern man to lose touch with himself and to get lost in forms of an increasingly superficial and artificial life, inauthentic.

Fundamental Data

Any consideration needs to rise from important data that does not depend on another and that constitutes…

> …An order or area of reality that, because of being radical, does not leave any other under him, rather, because of being the basis, all others are forced to appear over it.
>
> This radical reality in strict contemplation on which we need to base and ultimately ensure all our knowledge of anything *is* our life, human life. [8]

To the ancient Greeks, those irreducible or fundamental data were provided by the objective, physical world: "things." But objects deteriorate and change, we do not know if they exist when we do not see them, so at the middle of the seventeenth century, Rene Descartes proposed *thought* as fundamental and irreducible data. The thought is immediate, what people think, it is what certainly exists and furthermore, certifies the existence of the human being: "cogito ergo sum." Life moves away from itself and moves toward the thought, but:

> The basic fact of the universe is not, simply, that either thought exists or I exist—but if there is thought there are, *ipso facto*, I, who thinks and the world in which I think, and they exist with each other, without possible separation…I am for the world and the world is for me. If there are no things to be seen, thought, or imagined, I would not see, think, or imagine. Therefore, I would not *be.* [9]

The basic fact of the universe and as such basic, undeniable, and obvious and that does not require demonstration, is the fact of everyone's life. And that, the life of each one, is what everyone *is*.

> The being of man is what he calls his life. We are our life. Now, the life of each of us consists on that it is found having to exist in a circumstance, environment, world, or whatever you want to call it. [10]

I and Circumstance

Life, then, is a relationship between the "I" who lives and the "circumstances" in which he lives. There is no I if it isn't in a circumstance or a circumstance that isn't one for I. It may exist in an infinite set of objects, but these would not be integrated in that particular perspective that organizes them in world or circumstance for who lives between them until an *I* appears to face them.

> This circumstance or world in which, like it or not, we have to live, cannot be chosen by ourselves. Without our prior consent, and without knowing how, we were fired at it, dumped toward it, shipwrecked on it, and forced to sustain it, to live. We are left no choice but to always do something, to "go take a swim". [11]

One of the conditions of human life is to always face a situation and have to do something about it. Because unlike our brothers on the zoological scale, humans lack the instinctive knowledge that provides responses to the environment:

> Our life, therefore, is given to us—we have not given it to ourselves—but it is not given to us already made. It is not a thing whose being is fixed once and for all, but it is a task, something that needs to be done, a sum, a drama. [12]

The circumstance will submit favorable and unfavorable aspects to *us*, and the *I (us) must* have a plan to stick to those alternatives and avoid being destroyed by an unfavorable circumstance. Because the circumstance is, in addition to the present, the inscrutable future and that future's uncertainty is the origin of the anxiety of human existence that at times acquires an epidemic character.

Now materialize some elements of Ortega's thoughts that approach the application to

psychotherapy. When Ortega argues that life is the radical reality and the fundamental fact, he rescues the human being from both the old realism (for which the human being is another object among objects, an animal with some virtue or capacity added, such as being rational), and the modern idealism that considers that *being* is made up of *thinking*. Ortega says that man's *being* IS his life, and that it precedes any attempt of identifying it, or of including it in any theory "about" the human being that limits it and considers it a *thing,* an *object*. With that Ortega rescues man from nature without needing to enroll him in any supernatural context. The human being *is*…his life. And this allows him to…be whatever he wants to be. Nothing is determined. This way of seeing is different from others that preset their needs, ambitions, and goals, which make it difficult in achieving them, the cause of their suffering and "disease."

> "The most uneven form of being have passed through man; then, to the despair of intellectuals, man *is* to pass, to pass through thing after thing, to be stoic, to be a Christian, be rational, be positivist, be what he will now be…Man goes through all these ways of being; Pilgrim of *being,* he *is* and then he *is not*, i.e., he *lives* them." [3]

What is the value of this in the therapeutic context? To appreciate this, we must include it in the picture of what can be considered as "bad weather" these days. Consultation motives have changed much since the "five lectures on hysteria" at least in the regions of the world which consider it useful to employ the professional assistance that receives the curious name of psychotherapy. At that time the difficulties appeared to be a result of a repressive and Victorian culture. Freud would have ended up creating much more negative reactions than the ones he had initially intended, and consequences far worse for their author. Actually this aspect of psychoanalysis was an attack against a terminal state of culture. However, today it is not sexual repression or the Victorian culture which can be blamed for the discomfort that increasingly spreads in the "civilized" world, as we shall see, currently, what is repressed is the contact with oneself, the authenticity, to *be* what everyone one *is*. People live shallowly and, consequently, aimlessly. Meanwhile, the World Health Organization (WHO) foresees that by 2020 depression will be the second largest public health issue, and that by 2030 it will be ranked first, in addition to the huge growth of anxiety disorders and unspecified forms of unease that haven´t found their place in the many diagnosis manual (such asDSM IV, CIE 10). It seems that the undoubted and stunning advances in science are not accompanied by an equivalent improvement in the way people feel in life.

Cultural Crisis

> …The culture is the interpretation that man makes of his life, the number of solutions, more or less satisfactory, which he invents to obviate his vital problems and needs; being understood under these words is the same as for those of material order or of a spiritual kind. [13]

When these solutions are no longer satisfactory, what is perceived as "critical problems and needs" deepens, and unrest, uncertainty, and anxiety grow. Successful solutions are those in which the human being lives his life and feels acceptably safe either in the "material or spiritual order." Faced with these difficulties, this situation takes a turn towards the medical area, it suffers a detour to the fields of medicine, which is not assumed as cultural failure and, incidentally, medicinal drugs are sold. Therefore, whoever is not happy with the "way things are" is sick.

> …In short, what is called "crisis" is but the transition man goes through from living pinned to some *things* and leaning on them to live on and supported by other *things.* [14]

Ortega theorized three crises in the West: the first was caused by the fall of the Roman Empire, after which humanity decided to settle on Christianity. The second was the Renaissance, in which the Christian faith is replaced by faith in science, clarifying that it is not because of being science, but because it is another kind of faith: faith can only be replaced by another faith. And the third, in which we are currently engaged, is that we have lost faith in science as a solution to the problems of human life and we have not yet found a replacement.

> As always occurs when a new faith emerges, an amount of time to establish its promise was given to it faith. But as the time passed it was seen that while science was prodigiously solving problems regarding things about man, it kept proving itself less and less able to say anything clear about the deepest of human problems. [15]

The inability of culture to fulfill its role of providing security to those who integrate it leads to a reaction that, in turn, worsens the situation. Insecurity and instability, along with the resulting distrust in the links and institutions increases expectancy and sharpens the need to "find" a way out. However, this is searched for precisely where it cannot be found: in the "outside" because as the situation is a crisis, there is nothing in the social context in which the person can steady him or herself. So, a true vicious circle appears. In it the greater insecurity and instability cause an equally high expectation of something to emerge. This something would calm this insecurity and instability, and causes the individual to turn more and more "outward," making him lose touch with himself. Personal and social lives become shallower and this causes most of the difficulties that sometimes end in the patient consulting a professional.

> …The origin of the crisis is precisely for the man to feel lost, because he has lost touch with himself. [16]

Withdrawal or Inward-Looking and Alteration

This trend toward increasingly shallower lifestyles had already been announced by Ortega more than 80 years ago in many of his works, emphasizing that humans should get back in touch with themselves, pouring inward on a process he called "withdrawal" or "inward-looking." The opposite, to be turned outward, he called "alteration" and manifests as an inexplicable feeling of emptiness, lack of meaning even in the midst of situations apparently favorable. Intimacy in situations of coexistence becomes problematic, because for a deep connection with another person to take place, a deep connection with our inner self is needed by everyone. The marriage and family life is invaded by the "outside" and this implies the need to always be doing something. Predominance of the action for the action itself: confusion. Failure in relationships because this leads to situations of loneliness, which becomes intolerable because there is no ability to find any possibility of inner calm. Solitude (even though one is physically accompanied) sharpens fears and anxiety and gives way to depression, in which despair manifests.

> We do not know what happens to us, and this is precisely what happens to us, not knowing what happens to us… [17]

While Ortega shows us the issue with astonishing precision, he also gives us the resources to find the solution. In all his work, there is a consistent appeal to authenticity, to the inner quest to find what the true desire is, and find in it the source of energy for action.

> There is no other effective way of being that is effective other than to look inwards, into ourselves, this being, before doing anything, before giving your opinion on anything, to stop for a moment and instead of doing anything or to think the first thing that comes to mind, to strongly agree with ourselves, this is, enter ourselves, stay alone, and decide what action or opinion among the endless possibilities is truly ours. [18]

Life Project and Vocation

In critical situations there are no collective solutions. If there were any it would not be considered a crisis. The solution must be sought by everyone inside themselves, in contact with their true desire and to flourish in a life project born of vocation. Ortega argues that in each of us there is a bottom or true self that seeks to manifest in the form of a life project that, as we saw, is the way the "I" likes facing the circumstances. Here we find a clear example of the distorting effect of crisis: the current way of life ignores both the vocation and the authenticity and favors economic performance so that the activity becomes hollow and forced, and for relief, distraction and entertainment are used, which accentuates the remoteness of oneself. Ortega invites us to a life

according to a vocation that exists in each of us, and also a beating life project that is ours alone, untradeable, from whose realization derives the only chance of fulfillment in life.

> Almost everyone is altered, and in alteration man loses his most essential attribute; the possibility to meditate, to withdraw within himself, to agree with himself in what he believes in; what he really cares about and what he really hates. Alteration blinds him, numbs him, compels him to act mechanically, in a frantic sleepwalking. [19]

By withdrawing, the individual may "be collected within himself" to go dusting off his real life project, his vocation.

> To know how to be alone…and turn inward is one of the most difficult tasks. The passions, appetites, and interests usually shout louder than the vocation and darken his voice. [20]

Let us see, then, in which way Ortega's intuitions lead to a psychotherapeutic action that corresponds to the real need of the patient in the current situation.

The critical situation, such as cosmic rays, goes through it all. It passes through everything. It does not stop at the office door, it goes with the patient and not only that, it also goes to the therapist. Not taking it into consideration means ignoring a subtended reality that affects the development of treatment and the relationship with the patient. Otherwise a fiction that puts the whole problem in the intricacies of the mental patient on the assumption that the world is waiting with open arms once its atavistic conflicts are solved would be developing.

On the contrary, considering the crisis lets us address the situation in the simultaneity of all difficulties. Be it for reasons of history or because it fails to "get the hang" of your current life or, more often, by combining both instances, the task is the same: to make the patient arrange himself from the "inside" and face life with the knowledge thus acquired, by using introspection. Such is the case for example, of the patient having marital difficulties and has already spent countless hours of "therapy" complaining about her husband, whom she held responsible for all their woes. The solution (for her and her husband) is that she finds within herself any resource that allows her to take control of her own well being, a life project that frees the husband of being the sole person responsible for his spouse's well being. To achieve this she must discover a vocation that gives meaning to her own life, an activity that takes the place of the activity of housewife and mother, who once had been a person with a vocation that absorbed her life, but was, with the time and the growth of her children, disposed of. Something similar can happen to the businessman for whom the years of enthusiasm for success that was achieved have passed, and have been replaced by incessant fatigue caused by complications of the situation. He no longer "loves" what he does. Finding the vocation does not mean to "kick the board" but to leave the state of forced labor, because vocational action reconciles with life and provides energy. The same occurs for the young man who does not find any career interesting, or retiree who has lost his place in the world. In most cases there is a frustration because when discomfort is felt, one can only hope for circumstances to change in the right way because of the subject's claim. Withdrawing allows momentarily abstracting from the circumstance and creates a space for dialogue with himself and by which balance and inner peace that were impossible to experience in the midst of the furious fighting hostile circumstances are restored. Discovering that inner space is in itself a therapeutic experience. The disturbance caused by the crisis leaves no choice, "either I make things as I intend them to be, or I will have no opportunity to feel self-achieved." However if the patient discovers that without none of the things he thought his well being depended on is needed for him to be well by changing his way of being, and that it depends on him and not on changing the world, a space to relate with ourselves and harmonize opens up. Of what is this harmony made up of? It consists of the ending of inner struggles, as opposed to the outer struggles. Expectations, slogans, self-reproach, ambitions, failures, humiliations, mandates, competition, guilt, happiness, and sadness occupy that space. But life had left no time to order it; everything is there as if it were an abandoned attic. It is only possible to run forward with the hope that something might happen

to bring order to life. But that order is not out there, it is in agreeing with YOU, forgiving mistakes, accepting failures, to stop claiming greater or different things from those achieved. But it is not just payback; there are new things to discover and do that were crushed under the rubble. This process is of such dynamism that it is beyond any attempt to trap it between the lines, but understand that the goal is to achieve the greatest harmony with ourselves, and it marks a direction to the task, both for the patient and the therapist.

> By living I have been released to the circumstance, the chaotic swarm of stinging things in which I get lost… I get lost in things because I lose myself. The solution, salvation, is finding ourselves, coincide with us once again, be very clear about what is my sincere attitude to everything…The substantial problem, the original one, and in that one sense, unique, is fitting in myself, agreeing with myself, to find myself. [21]

Ideas and Beliefs

Ideas and beliefs are an issue of major importance in psychotherapy and also, incidentally, for any activity aimed at humans, besides living. Ortega distinguishes mental contents in "ideas" and "beliefs." The ideas are the result of the thinking activity of each, they are our own, and are therefore also known as "occurrences." Because they are ideas that occur to us, they are burdened with a load of doubt that the possibility of error accompanies in any trial. Instead, beliefs are free of that burden because they are not created by each person: they have always been on us and consist of the particular type of ideas that represent "how things are" or reality itself.

> The "ideas" persuade us, convince us, are "obvious" or are "proven"; but they are all that because they never cease to be mere ideas that are not our reality itself, unlike what we believe in. [22]

These are the elements we were given to carry out our life, and without them there would only be pure distress. Beliefs are absorbed without noticing it, they are absorbed just by living. The culture itself is a major supplier of beliefs.

From birth we run a constant effort of acting as receptors, a process of absorption: in family life, school, reading and socializing, which decants to us those collective beliefs [23].

Any action taken is taken because of a belief. If a trip is undertaken it is because "it is believed" that it will come to term, if a date is going on, for whatever reason, it is because you expect (believe) the other person will be present. You attend to work because it is assumed and expected (belief) that there will be financial compensation. These are all examples of things that are believed will happen, and therefore generate much discomfort when they do not. Ortega gives a great example: when someone is about to go out, for whatever reason, they think about the temperature, the clothes that they will wear, they will look at the time, but he does not ask himself whether the street will be there or not, which would be crucial, because he goes toward her. He "expects" the street will be there, as well as a huge amount of things and facts which he also expects and has in his life. It is the case for friends to be there, the affection of his family, the loyalty of your spouse, the support of children, the concurrence of the emergency medical service, effectiveness of vaccines, economic stability, legal framework, the daily sunrise, in short: everything that the social and natural environment puts at our service which we use to live and that we incorporated as reality itself, as "the way things are."

> We do nothing with beliefs themselves, instead, we are just *in them*…you are in the belief, and you have the occurrence (idea) and you hold it. But belief is what has us and sustains us [24]

We live sustained by beliefs, sustained and mobilized. Suppose a scientist, day after day, locks himself in his lab looking for a result. He will have reasonable doubts about whether he *will* do it, but not about whether he *can* do it. Doubt is the idea; the certainty of possibility is the belief. Without that certainty he would not be able to take a single step toward his lab, what would be the point? What drives the human being? To do in the

reality, no matter how redundant it may seem, what he "believes" that reality is. He will not try to pass through a wall or walk through the air without any device or beat the world's Boxing Champion without the necessary expertise. When something like that happens we find the miracle, either the old one that we can read about in the Scriptures or the current ones that we see in the movies. But in ordinary situations things considered possible are undertaken. On two fronts: the aforementioned reality and what each of us "makes" about himself. No one tries anything they are not sure they can achieve. You cannot be absolutely sure you *will* achieve it, but you cannot be completely sure you will *not* achieve it.

Is the importance of beliefs appreciated in psychotherapy? (And in life!) Both, what we each *believe* "things" to be as what he *believes* about himself, in constant interaction, will determine his action in life. And what each believes about themselves is the introjection of the lived and the way it has been interpreted.

> The most effective thing about our behavior lies in the latent implications of our intellectual activity, in everything that we have, and, since we have it and count on it, we do not think about it. [25]

> Additionally, much of our beliefs pass unnoticed to us. [22]

Consequently, motivations for action are submerged and hidden in the level of beliefs. If they were not so, they would not be beliefs, they would just be ideas, and ideas do not move us because they lack the strength of evidence that beliefs have. At this point we have proof of the systematic thought of Ortega: different themes are interconnected and it would be ideal to expose everything at once, which, for now, goes beyond the author's skills. We must return to the life project, because what motivates us is in the plane of beliefs. But beliefs obtain their power from their existence being secret, if they came to the light they would become ideas. This is an important therapeutic resource to neutralize inconvenient beliefs. So the life project is not a plan whose stages are previously known.

> The project that I am, I find myself being it even before I wonder what project am I. Furthermore, none have managed to ever think the whole project which they are... Ordinarily, it is the course of life which shows us what project we are, who we are. [26]

The life project is in the same space as beliefs but it is not a belief. Sometimes they are helpful (like when they give security), and sometimes make things difficult (like when you face a vocation you are uncertain about from the economic point of view), generating fears. The life project is born from the need to face the circumstances, with all their uncertainties and risks. Human beings have the permanent need to know what to expect from circumstances. What concerns us, which is a constant problem, is not the past but the future.

> Precisely because life is always rooted in disorientation, perplexity, and not knowing what to do, it is always an effort to find direction, to know what things are, and the man between them. Because he has to deal with them, he needs to know where they stand and what to expect from them. [27]

But to have enough energy and face the future, the project must be authentic and must respond to a vocation. Today this vocation is a neglected topic; however, he who finds his vocation and goes ahead with it is in a better condition to carry out his life.

"Belief holds us and sustains us," Ortega once said. Consequently, it can be assumed that behind a consult with a therapist lays a difficulty or conflict in terms of beliefs that makes us not comply with it, its support function. Beliefs make up a system, but not a logical system. Beliefs support each other and the fall of one of these beliefs drags others down like dominoes placed in a row. Thus, for example, someone is scammed, meaning, he "had" his partner's honesty, to the economic loss he adds loss in confidence that he can recover from that loss, which he supposes will distance him from his social relationships with which he counted, and had once expected would help him carry on with his activity. If his love life was not consolidated he would fear a breakup. He will also have fears about his health and his ability to receive any treatment, etc. Although the example is somewhat "light" and life can get worse, the object is to illustrate the ripple effect produced by the fall of a belief. Sometimes this is expressed with a fairly accurate phrase: "The world will collapse." All faiths fell together and the subject lost everything that could provide

security in life, plain and simple. As beliefs come from culture, this collapse of the belief system throws out the culture and exposes him to the primitive terror humans felt when they lacked the resources of civilization. This or similar situations can lead to what is called post-traumatic stress, in which it is hard to reset the belief system that sustains the needed confidence, in both the medium and the subject itself, to resettle in life.

But beyond these extreme situations, evolving in life is to go changing the belief system. Life itself is responsible for forcing us to that process. Within the limits of any generalization, we can say that every crisis in life is determined by the fall of a belief, something that we had "counted on." Be this belief that loved ones will live forever, that the groom or bride will hold their oath of eternal love, that "my son will never lie to me," that "my parents will never divorce," that "the company needs me," that "I am as healthy as my mother," that "when I finish school I will find work." Sometimes beliefs crumble because of social facts, such as in 2001 in Argentina, in which people, until that time, *believed* that their money was safe in banks. It could be the case also for insecurity for criminal acts, or a change that requires adaptation, it being of the marital kind or of economic "status," neighborhood, country, or taking a parent to intern in a nursing home. Examples are necessary but are always rough and rudimentary to the boundless range of human possibilities. The language has, in this regard, a confirmatory value. Faced with a disturbing event, it is often said, "I *knew* (thought) that this could happen but I never *thought* (belief) that it could happen to me." It is the final language of facts that modifies beliefs.

The plan of action for therapy is, therefore, that of beliefs. Conflict means conflict between beliefs. The action rises from beliefs. Disorientation shows the lack of strong beliefs. Culture is the provider of beliefs. When not in crisis, these beliefs are shared, reinforcing the coexistence and are genuine and mobilizing in the whole society. These are the highlights of humanity: civilizations are born, countries are born, and collective enthusiasm is displayed by faith appearing art, science, politics, democracy. Confidence grows in all and between all, and also in humanity itself. In a culture in crisis no beliefs are shared. What is worse, those received as a result of "constant effort of reception, absorption" are contradictory and chaotic: Should I follow my vocation or continue the profitable family business? Should I get married and have children or should I follow a career that makes me an independent woman? Should I divorce and live my life, which is one, or should I dedicate my life to my family? Should I take this opportunity and solve my life´s problems, or should I stay in this painful mediocrity? (Some time ago a bank accountant fled with several million dollars and caused admiration: "How well he did it!" was what everyone said). It should be repeated that the examples provided are always rudimentary. To present others that are more subtle you would have to rely, perhaps, on clinical cases, which would lengthen this work improperly.

Beliefs turn the gears of action. If they are conflicting the action gets stuck, locks. Resolving this conflict is the goal of therapy (and beyond). To resolve it, reverie is used to find a coincidence with ourselves, meaning to find an overlap between beliefs. Therefore, the inner world is rearranged and the subject can return to action. The therapist (the friend, the priest, the confidante) provides that inner dialogue which consists of confrontation between contradictory beliefs in pursuit of a new harmony. However, conflicts are not resolved in the plane in which they occur. If they were already settled there would not be such a conflict. The solution requires the intervention of another level operating as mediator in order for it to absorb the contradictions of the conflict and to generate a conciliatory synthesis. "The only way to escape a maze is to move upwards, not forward" This higher level is the spiritual level.

> If we compare hunger or sexual pleasure with the thought in which Einstein formulated his abstract theory or the heroic decision a man makes when he succumbs to duty, we will find such distance and difference, that we seem forced to divide our intimacy in different worlds or orbs. [28]

Ortega discusses these orbes or worlds in his essay: "Vitality, Soul and Spirit" (El Espectador V, OC, T II).

Vitality, Soul, and Spirit

What is most evident when we look at a human being is that it has a physical body. But this body may be at rest or in activity, and the activity may manifest differently. We can see someone walking lightly, as if the body does not gravitate, while another seems to find their body heavy and drags it wearily. There are some who feel that they are overflowing with energy and some who seem to live only with the necessary amount of energy, and this not only something that is different in each person, but also something that changes from one moment to another in the same subject. Ortega called this phenomenon "Vitality."

> There is, indeed, a part of us that is infused or rooted in the body and it is like a physical soul… this physical soul acts as the seat or foundation to the rest of us. [28]

The most immediate thing we could think about this phenomenon, given the inevitable "scientific" propensity that still dominates our way of thinking, is that it depends on biological (health/disease, vitamins, antidepressants), genetic (born well) or momentary (lack of sleep or fatigue) aspects. But it then happens that our oppressed subject inserts his hand into his pocket and takes out a curious rectangular object, about ten inches by five, and inexplicably it to his ear and begins to talk without having any partner in sight. And to our surprise, after a few minutes we find that his status has changed. He reintroduces the small pocket device into his pocket and resumes his march faster and stronger, his face lit up with a smile. How much would we all like to have one of those revitalizing gizmos! It turns out our subject has received very good news and that is what has been changed. But on other occasions we have seen that in similar conduct, through the gizmo, the subject has been beaten and powerless. What has happened in either case? The vitality has been changed by the intervention of another factor for which we find no better way of naming than "emotion." And the enclosure in which emotions are is what Ortega called "soul." And, as we have seen, the border between the vitality and the soul is ever-shifting and undetermined.

(continued)

> It is false; it is unacceptable to pretend sectioning humans in body and soul. Not because they are not different, but because there is no way to determine where the body ends and begins the soul. [28]

In the soul reside emotions, and these have certain characteristics. One is that they extend in time: you can be sad, happy, or angry and that feeling has some duration. The other is that various and even contradictory emotions may coexist: love and hate, fear and desire, rejection and attraction. If, as we have seen, conflicts tend to hinder the action, this feature of emotions is not what most favors these conflicts to be resolved. It is even more unfavorable if we consider that beliefs are a result of the amalgamation of ideas and emotions.

Consequently, an order-bringing item is needed, an element that integrates, that absorbs the diverging and sometimes even opposing emotional tendencies that nest in the soul for life to maintain a certain course. Because circumstances will always offer some nice options, also called "entertainment," or, when they are most dangerous, "temptations" that redirect the project as their voices are often more attractive than the harsh call of vocation. That guiding element is the "spirit."

> The spirit has no feelings: it thinks and it wants. The soul is what desires, loves, hates, rejoices and becomes sad, dreams, and imagines. Both powers collide perpetually within us, being of remarkable notice that the spirit is primarily concerned to stop our soul's automatism. [29]

Unlike emotions, which extend in time, spiritual phenomena are instantaneous.

> The spiritual or mental phenomenon does not last: psychic phenomena take time. Understanding that 2+2=4 is done in an instant…For "to think" we mean the succession of many acts of thinking, each of which is a mental lightning. Similarly, you want or do not want in a moment. Volition, which perhaps takes time to form, is a ray of intimate activity which fulminates it's decision. Instead, all that belongs to the fauna of the soul lasts and stretches over time. [30]

We have the vitality that blends with the emotional or psychic world which requires the spirit's intervention to prevent a dispersion that would probably lead to subjective and objective chaos.

It is interesting to investigate the repertoire of efficient actions that the spirit possesses over the soul, and, on the other hand, to notice its limits. The spirit or "I", cannot, for example, create a feeling or directly annihilate one. Instead, it can, once a desire or emotion has arisen at some point of the soul, close the rest of the soul and keep this desire from overflowing it, from filling its entire volume [31].

Although the term "spirit" is filled with supernatural overtones, again we find that Ortega can remove humans away from nature without enrolling it in any supernatural or religious context, but, at the same time, without amputating any features that make the absolute originality of human beings. The most notable one is the ability to access universal instances and participate in modes of existence that transcend the individual-subjective.

> Whoever thinks a truth realizes that every spirit must think it as he did. Instead, my sadness is mine alone, no one can feel *with* me and *like* me [32].

The functions of the spirit are thinking and wanting. When we understand something or when we come to a decision that emanates from a well-defined will, the spirit is at work.

> What does seem clear however, is that when we think or want, we give up our individuality and start participating in a universal world, where all other spirits lead and participate as ours does. Even though it is the most personal thing that there is in us–if person is understood as the source of all actions–the spirit, strictly speaking, does not live of itself but of the Truth, Norma, etc., in an objective manner on which it rests, from which it receives its particular frame. In other words; the spirit does not rest in itself, but it has its roots and foundation in that universal and transsubjective world. A spirit that worked in and of itself, in its way, taste and genius, would not be a spirit, but a soul. [32]

What is the importance of spirit to the topic at hand: psychotherapy? Now comes the time to reap what was sown above. We saw that for Ortega, "The substantial problem, originally, and in that one sense, is for me to fit in myself, agree with myself, to find myself." That fitting, that coincidence, is the result of the integrative action of the spirit over all psychic life.

> I consider the spirit is the set of intimate acts of which each feel as real author and protagonist. [30]

We also said that: "today what is repressed is contact with ourselves, the authenticity, to be the one everyone is. People live shallowly and, therefore, aimlessly" and that given the critical situation in which culture is, the solution "…Is sought, precisely, where it cannot be found, in the 'outside' since as the situation is a crisis, there is nothing in the social context in which to assert." Therefore, what is left is to withdraw inwards, inside ourselves, and self-absorption is the state in which the spirit can act:

> Without a strategic withdrawal within ourselves, without any warning thought, human life is impossible. Remember all that man owes to certain large withdrawals! It is no coincidence that all the great religious founders preceded first of their apostolates famous withdrawals. Buddha retreated to the mountain; Muhammad retired to his tent, and even within his tent, he withdraws by covering his head; above all, Jesus leaves into the wilderness for 40 days. [33]

We have seen that society is undergoing a cultural crisis that permeates everything, even the therapeutic field. The result of this cultural crisis is for life to become shallower, with an increasing loss of touch with ourselves as a social phenomenon, meaning it affects those that seek consults and those who do not. Consequently, and beyond the specifics of each individual case, a psychotherapeutic action which tries to be effective must take into account this situation and be alert to the real help and "cure," which comes through authenticity, contact with ourselves that allows withdrawal and allows, resorting to the spirit, to truly exercise thought.

> …Man needs to think if he wants to live, whether he likes it or not. If you think incorrectly, that is, without intimate truth, you live badly, in pure anguish, trouble, and distress. If you think correctly you will fit in yourself, and *that* is the definition of happiness. [34]

Happiness

In such a context, the stories legitimize their presence not because they are good but because of what the message is that they are intended to convey. This is no exception. As an example, we may imagine that a patient attends a consultation with a therapist and says, "Doctor, I feel well, you could almost say I'm happy, but all my friends happen to visit you and I also want you to know my side of the story." Except in terms of fiction, it is not expected that someone who is happy will request a consultation, at least not for himself. Consequently, psychotherapy must sooner or later deal with the problem of happiness. It is actually our well-known cultural crisis that has worsened the problem and has displaced the instances which traditionally would have taken care of answering this question (priests, philosophers, "teacher") and has made the whole psychiatry-psychology responsible for, nothing more and nothing less, than human happiness. A discipline which originally dealt with patients who were "sick" has greatly expanded its reach, but still does so by using an instrumental designed for pathological situations. In fact it is still called psycho*therapy*. This has generated a response in a movement called "Positive Psychology," that aims to generate a body of knowledge relevant to those who wish to improve their lives without being "cured."

As was noted above, Ortega says that life has been given to us, but it has not been given to us *already made:* making our life is our task. The task is to deal with the circumstances and to address this task we must assert ourselves in a life that draws its strength and power from being the realization of a vocation. However, one of the worst consequences of life becoming shallower is that together with the lack of contact with ourselves, people have lost touch with the vocation. Self-absorption is required to retrieve it, and great determination and clarity to follow when trying to retake it.

It happens that man feels happy when he can apply the different vectors of his intentionality to a target and, additionally, get a positive outcome from those procedures, like when vitality, soul, and spirit are integrated into an action. It is of note however, that this integration is not static. It is like the bike: if you stop moving, you fall. It is pure dynamism and is the objective which, by concentrating the various rays of intent, holds the tension of the whole. But this convergence requires the essential action of the spirit, which, being in another plane, can achieve the synthesis between both emotions as well as beliefs, emotions that are amalgamated with received ideas. The same happens when said in an informal language intended to suggest a state rather than to make a polished conceptual exposure.

Ortega refers to that state when he says:

> …Our life is never to simply be, to just lie. To live is always to live, by something or for something: it is a transitive verb. Hence there cannot be a human life without a vital interest, which holds, makes up and organizes that life. At the moment all vital interest comes loose completely and effectively, life would cease to be. [35]

And that vital interest is a vocation taking place. When you are not in a life in which this happens, suffering occurs:

> Obviously, it is our life—project, which, in the case of suffering, does not match our actual life; man dilutes, splits in two, the man he had to be and the man he is. The dislocation is manifested as pain, anxiety, anger, moodiness, and emptiness; the coincidence, however, produces the prodigious phenomenon of happiness. [36]

Positive Psychology has moved in this direction with the Flow Theory, in which an ideal state is described as one in which the actor of a task is fully involved in the activity he performs and that in which personal skills are at the height of the present difficulties. This was said by Ortega in "Theory of Happiness" (OC, T II, pg 222) in 1916. He had already said:

> Not like possessed or obtained do they [things] contribute to make us happy, but as reasons for our activity, as a matter on which this is shooting and from mere possibility becomes exercise. When we ask existence to be clear on their meaning, we do nothing but demand for it to show us anything capable of absorbing our activity. If we noticed that something in the world was enough to replenish the volume of our vital energy, we would feel happy and the universe would seem justified… When have you heard of someone completely absorbed by an occupation feeling unhappy? [37]

It is worthy to note that the Theory of Flow refers to what happens to the subject with himself, while Ortega states that "To live is always to live by something or for something," meaning

this ideal state of being absorbed by an occupation requires giving something to something that transcends the subject: a mission.

> That is why every human life has a mission. Mission is this: the consciousness that every man has of his most authentic self which is calling him to become who he really is [38].

This must not be interpreted as a sacrifice or giving up on something. On the contrary, Ortega says that living this way is what leads to a happy and fulfilling life. That unhappiness is the result of not delivering, lacking a way, lacking a meaning, a result of a cultural situation that leads to an increasingly shallow/superficial and inauthentic life and contact with ourselves. In other words, who keeps his life will lose it, and who gives his life away, shall win it.

> Human life, because of its very nature, must be set to something, to a glorious or humble enterprise, a famous or trivial destination. This is a rare but inexorable condition, written in our existence. On the one hand, life is something that everyone does by itself and for itself. On the other hand, if this life of mine, that only I care about, is not given by me to something, it will lack and will be without "form." These years witnessed the gigantic show of countless lives, marching, lost in the maze of themselves because they lack something to deliver themselves to. [39]

Corollary

The psychotherapeutic activity should have a philosophical foundation that gives some clarity on what the man and his life are or should be.

The human being is not a thing, he is his life, which is a pure event produced by the encounter between an *I* and its circumstance (which consists of the body and mind he was given and with which he must fulfill his life project). But this project cannot be just any project; it must be the realization of a vocation. Vocation means life as a mission, and requires the integrative action of the spirit, which, through contact with him, retains the authenticity and saves us from the confusion that the circumstance's alternatives may give in a culture in crisis.

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DSM-5: A Bioethical Overview

6

Gilberto A. Gamboa-Bernal

Introduction

Psychiatry, since its origin, has been marked by controversy. Its initial developments soon adjusted to the scientific method and even today, certain approaches to patients and to treatments are questioned as being far from proven methods and with more empirical than scientific characteristics. In this field, like no other, it is still believed that magic and science go hand in hand; that the boundaries between both are so blurred that they end up being confused.

Nevertheless, it is also true that another phenomenon occurs with psychiatry, perhaps derived from the above: at certain times and places it has become one of the products within the orbit of fashion: to go to a psychiatrist or being in therapy of this specialty is perceived as a sign of distinction, elegance, or style; and therapists become the subject of conversations in social clubs, spas, beauty salons, etc.

However, this phenomenon, which could well be described as "postmodern fickleness", does not erase the long history of sadness and humiliation to which patients have been subjected for decades while psychiatry achieved a minimal status and scientific development. Fortunately not all has been negative in the journey of psychiatry, and much less in recent decades when the development of modern psychotropic drugs has changed the way that psychiatric patients are perceived, sensibly improving the prognosis of many pathologies which hovered over them until the middle of the last century almost without leaving hope for improvement and, much less, healing.

Historical Approach

Since the eighteenth and nineteenth centuries, thanks to advances in statistics, doctors or psychiatrists began to think of disease classification, but from the perspective of mortality: the first attempts were directed to determine the causes of death and crystallized in the International List of Causes of Death.

It is striking that the forums dealing with these topics revolved around the International Statistical Institute and that it was only in the first decades of the twentieth century, within the League of Nations Health Organization, that they expressed an interest in classifying diseases. They did not start from zero: years before, they had identified groups of diseases (Hippocratic classification in classical Greece [fifth century BC] with variants: Galeno [130–200], Philipe

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Barrough, in The Method of Physics of 1590; François Bossier de Lacroix o Sauvages [1706–1777], published the Nosologia Methodica [sic]; Linneo [1707–1778], wrote the Genera Morborum; William Cullen [1710–1790], in 1785, published the Synopsis Nosologiae Methodicae), in accordance with a number of criteria, but never reached the level of global consensus.

In addition, this is how the International List of Diseases was born, the forerunner of the International Classification of Diseases (ICD), with a criterion set more on morbidity than on mortality. Since the First International Conference held in Paris in 1900, where a ranking parallel to the Detailed International List of Causes of Death was presented, numerous conferences have reviewed it. It was not until 1944 that the Manual for Coding Causes of Illnesses according to a diagnosis code for tabulating morbidity statistics was published in the United States. This document was the basis for the approval of the Manual of International Classification of Diseases, Injuries and Causes of Death in 1948. The participation of the World Health Organization (WHO) was a very relevant fact within the scope of the First World Health Assembly.

From the sixth revision of the international lists (1948), the WHO went on to take the reins assuming the responsibility of making updates every 10 years. This review is particularly important to the topic at hand because it is here that mental pathologies are included for the first time.

However, the inclusion was apparently not quite so fortunate for both the American Psychiatric Association (APA) and the New York Academy of Medicine. They were unhappy with previous classification attempts and because of that, they decided to work together to develop an acceptable nomenclature for the United States. Even when some previous attempts were made in Europe, these are the most closely related antecedents of the DSM of Mental Disorders, which was released in 1952 [1]. From this first version of the DSM unstable equilibria attempted in its design.

A remote antecedent is constituted by the classification made by the American Psychological-Medical Association in 1869. Subsequently, in 1883, Emil Kaeppelin (1856–1926) published in Leipzig his Compendium der Psychiatrie, and in 1899 the Manual of Psychiatry, which gave him the title of "father of the classification of mental disorders".

DSM-I: leading the editorial team were organicists and psychodynamic psychiatrists; a place was given to the United States Armed Forces and the National Institute of Mental Health (NIMH) specialists; a non-theoretical classification was sought based on the consensus of experts. The welcome to this classification was not as expected and its use did not have the invoked extension. Criticism was immediately heard from both the field of Medicine, as well as from Psychology: the use of diagnoses as labels, the lack of identity in the concepts, the absence of criteria for classification, and the reliability of categorical clinical trial judgment, etc. was questioned.

The subsequent versions of the DSM were marked by a number of various characteristics.

DSM-II: more predominance of the psychodynamic over the organicist vision and the application of the medical model making symptomatic clusters.

DSM-III: an attempt to approach the revised versions of the ICD, to be open to many more institutions and experts from other countries, to incorporate a multiaxial system of European origin, to use diagnostic criteria supported by an empirical basis (by which the psychoanalytic approach lost ground); diagnostic comorbidity was admitted. More mental disorders were included. Of the 106 mental disorders included in the DSM-I there are now 265 in the DSM-III (Cfr. Table 5). This is a brilliant document, although its use unveiled its problems—its inability to anticipate neuroscience or genetic advances.

DSM-IIIR: the reorganization of certain categories and the inclusion of a new axis on the degree of adaptation of patients allowed DSM to be generalized and be the object of greater acceptance.

DSM-IV: a simplification of the criteria and more clarity of language was attempted, empirical data of recent discoveries and many other new mental disorders are included.

Table 6.1 Disease classification systems

Year	International classification of diseases (Europe—WHO)	Diagnostic and statistical manual of mental disorders (USA—APA)
1900	ICD-1[a]	
1910	ICD-2[a]	
1920	ICD-3[a]	
1029	ICD-4[a]	
1938	ICD-5[a]	
1948	ICD-6	
1952		DSM-I
1955	ICD-7	
1967	ICD-8	
1968		DSM-II
1975	ICD-9	
1978	ICD-9—CM	
1980		DSM-III
1987		DSM-IIIR
1992	ICD-10	
1994		DSM-IV
1996	ICD-10 (children)	
1998		DSM-IV-R
2000		
2013		DSM-5

[a]Classification of diseases with organic base only
WHO World Health Organization, *APA* American Psychiatric Association, *ICD* International Classification of Diseases
Adapted from Del Barrio V. Roots and evolution of the DSM. Hist Psychol. 2009;30(2–3):81–7 [1]. With permission from the University of Valencia

However, the greatest achievement may have been the work methodology, the systematic and transparent processes by which it was developed: this was an example of collaborative, open, and participative work. In the opinion of some, this was a "systole" in the rating system, with epistemic blinkers to progress and the impossibility to investigate in comorbidities [2].

DSM-IVR: clarifications were made on prevalences, subgroups were added in many categories, and newest data and information derived from recent research were taken into consideration, the number of mental disorders rose to 297.

Table 6.1 illustrates the publication dates of the disease classification systems.

DSM-5

The presentation of the new version of the DSM-5 during the annual meeting of the American Psychiatric Association, in San Francisco, California, in May of 2013 was preceded by a strong controversy, where not only the scientific community intervened but also the printed and digital media. Through these media, the general public also witnessed an unprecedented situation in the development of psychiatry: just as participation of the scientific community had been paradigmatic in the DSM-IV, for the DSM-5 an attempt was made for the diffusion of the broadest spectrum, taking advantage of the resources offered by the globalization in information technology subjects. Not only common people were being informed, but they also had the opportunity to express their points of view through social media, web sites of specialized publications and of digital newspapers or magazines.

Despite the aforementioned, not all information was disclosed and many points remained in the shadows that put into question the way in which the process of creating the manual was carried out. In addition to the positive aspects of the fifth version of the DSM, diverse opinions regarding the limitations of the instrument, roughly illustrated, were being made public.

In order to understand these reactions and weigh them, it is necessary to describe some characteristics of the works that preceded the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders*, considered by some as an example that represents the latest in scientific thinking, both in content and organization criterion, as well as in the structure of the mental disorders.

The process of reviewing and updating the manual began 1 year after the appearance of the DSM-IVR. From 1999 to 2007, the necessary material was collected and the research was designed that would support the new version. Thirteen teams were formed with a total of more than 400 psychiatrists. Twenty diagnostic groups and six study groups in which 1,200 psychiatrists from six subspecialties and

4,000 doctors from six medical specialties took part. The total cost of the project rose to $25 million dollars.

A pilot test was conducted in 2010 and the proposals of the teams were reviewed.

In the following year proposals related to the criteria, the proposed diagnostic spectra, and the dimensional measures were revised. In 2012, the final draft of the text was prepared and following several revisions, was approved in May 2013 [3].

Objectives

One of the intentions of the project was without a doubt to confirm the scientific nature of psychiatry. The deployment of evidence-based medicine in the last two decades of the last century, hypertrophied up to a point, and the desire to provide empirical evidence available to Psychiatry of the XXI century, were powerful engines to warrant revision of the DSM.

The appearance of the biomarkers was promising for the task of deconstructing the classification of mental disorders. The moment had arrived to open up to and to start taking into account the scientific advances of other specialties: genetics, neuropharmacology, neuroimaging, neuropsychology, epidemiology, epistemology, postmortem research, psychopathology, cognitive psychology, etc.

Among the objectives of the review, reconsidering the relationship between the DSM classifications and research was also included. If psychiatry was to be provided with a firmer scientific basis, then it was essential to rely more on research.

The Neo-Kraepelinian model, basic for the previous DSMs, needed to be reconsidered or at least explore some basic changes [4]. This involved approaching a current medical model from a categorical perspective to a more dimensional point of view, also seeking greater specificity. Taking into consideration the preparation of the ICD-11, a homogenization was attempted with the ICD-10.

Tools

One of the tools used was the study of diagnostic categories over the dimensional diagnosis, supported by a multiaxial system, to the dimensional diagnosis: it went from only using two possible values (present or absent) to using three or more ordinal values.

In this new approach on how to make the diagnoses, it was necessary to take into account an assessment of disorders throughout life; the reality of the transculturality; the links between psychiatry and general medicine; the variations in the processes of psychophysical deterioration and the growing incidence of disabilities; and the use of instruments for the diagnostic evaluation.

Since the previous versions of the DSM, the gender perspective has been increasingly present. One cannot deny that the role of women as psychiatric patients has had certain special connotations, not only as a historical subject but also as an analytical category, which has made it possible that gender perspective be present in psychiatry [5, 6]. The ideological tendency is much more marked in the DSM-5, as will be discussed later.

The disorders contained in the DSM-5 are classified in the following dimensions: substance use disorders, mood disorders, psychotic disorders, anxiety disorders, childhood disorders, and personality disorders.

Developments

Several new elements that can be seen in the DSM-5:

The multiaxial system disappears and is replaced by the dimensional system.

Greater attention to the role of cultural differences in the appearance and development of the disorders, using a phenomenological approach that is underlying the whole design of the manual, taking distances from the exclusion of phenomenology, is present in both the DSM-III and the DSM-IV [7].

A different model was used in the field studies to find a greater diagnostic reliability and an

Table 6.2 Structure of DSM-5 [8]

Neurodevelopmental disorders	Spectrum of schizophrenia and other psychotic disorders	Bipolar disorders and related disorders
Depressive disorders	Anxiety disorders	Obsessive–compulsive disorders and related disorders
Trauma or stress-related disorders	Dissociative disorders	Somatic symptoms and related disorders
Eating disorders	Elimination disorders	Sleep/Wake cycle disorders
Sexual dysfunctions	Gender dysphoria	Disruptive disorders, impulse control and behavior
Substance use and addictive disorders	Neurocognitive disorders	Personality disorders
Paraphilic disorders	Other disorders	

increased acceptability of the manual. In order to minimize the bias resulting from the evaluation of several observers, it was decided to find a coefficient of interreliability using the kappa coefficient and give these studies a greater credibility backed up by the concordance of the evaluators of mental disorders.

Another contribution, in terms of methodology, was the meta-structure approach through clusters: the analysis of different mental commitments in various pathologies, such as reviewing the neurocognitive commitment both in patients with bipolar disorder as well as in individuals suffering from schizophrenia. The clusters used were neurocognitive, neurodevelopment, psychosis, emotional and externalization.

Some of the major changes contained in the DSM-5 are in the field of disorders related to moods, neurodevelopment, child psychiatry, personality disorders, neurocognitive disorders, sexual disorders or affective disorders, and the use of cultural formulation interviews.

The organizational structure of the new manual is similar to DSM-IV and is summarized in Table 6.2. [8].

Some topics of the DSM-5 have been revised we will discuss their advantages and disadvantages from a bioethical perspective.

Points in Favor of the DSM-5

It would seem that the "rivalry" initiated with the ICD-6 is close to ending. Part of the effort in drafting the DSM-5 was the purpose of finding lines of greater convergence with the future ICD-11 [9], through a rating that holds a certain parallelism and allows an eventual mid-term unification, which goes far beyond the numerical codes. However, not all experts are optimistic about this [10].

The approach has been sought for DSM-5, where research—and the results—are taken into a greater scope, makes it presumably that in this field it has a wider use. This situation could create the low use of the DSM in clinical practice. The correlation between the use of DSM and ICD will be discussed in the Disadvantages section.

The technological advances, which have largely contributed to the emergence, development, and acceleration of globalization, and the effect these developments have had on individuals, society, and culture in general, have also been taken into account in the DSM-5 [11]. The pace of life, which has increased as the twenty-first century advances, is also etiological of pathologies. New disorders caused by these two fronts are opening a space in the new nosological classifications, although this situation has not been without difficulties [12].

Earlier versions of the DSM in Spanish were translations made in Europe, which had more than a few drawbacks derived from the semantic adaptations and the translation of the codes, and the comparison with those of the ICD, in particular the DSM-IV and the ICD-10 [13]. This time, the DSM-5 in Spanish depends directly on the APA, which hired professional translators for both the breviary of the diagnostic criteria of the DSM-5 as well as for the whole manual.

The trend in the versions of the DSM has been to organize groups of criteria which better include the psychopathological characteristics of individuals suffering a mental illness. Some diagnostic criteria contents in the DSM-IVR were, however, too large or were difficult to recall; for example, those of the major depressive disorders [14]. Efforts were made in this sense for the DSM-5, although with only relative success.

It is very positive that the updating of mental disorders has had an inclusive and participatory design [15] as an extension of the impulse on previous work in the DSM precursors. In this sense, the DSM-5 is no worse than the DSM-IV [16].

The presence of field tests, developed in a clinical and academic environment, led by a good number of randomly selected professionals, is one of the predictable guarantees of the manual. In addition, the 11 medical centers chosen to make the protocols in the United States and Canada [17] give reassurance about the results the enormity of this work.

However, there is a concern that cannot be proven: the result of the work of these teams who conducted the field trials depended not so much on their efforts and their personal and professional qualities as the design of the protocols and the previous work on the proposed diagnostic criteria that were proposed: If there were levity in these circumstances, the risks of false positives and negatives would be multiplied (mainly the false positives) and the field trials would shed more distortions than certainties.

Unfortunately, there are indications in this sense: the dates of submission of the findings were postponed more than once, the availability of electronic data is limited, an environment of limited information was experienced, as well as secrecy, concealment, and dissimulation which do not allow sharing the enthusiasm with which the DSM-5 had been received. Some of the issues that are at the basis of this skepticism are described below.

Visit http://www.DSM5.org/Research/Pages/DSM-5FieldTrials.aspx; when trying to access "To read the studies in AJP in Advance, click on their titles: DSM-5 Field Trials in the United States and Canada, Part I: Study Design, Sampling Strategy, Implementation; DSM-5 Field Trials in the United States and Canada, Part II: Test-Retest Reliability of Selected Categorical Diagnoses and Analytic Approaches; DSM-5 Field Trials in the United States and Canada, Part III: Development and Reliability Testing of a Cross-Cutting Symptom Assessment for DSM-5", the result is: http://www.ajp.psychiatryonline.org/error404.aspx?aspxerrorpath=/Errors/404.aspx.

Conflictive Issues of the DSM-5

One phenomenon that has attracted the most attention is the presence of Allen Frances at the head of the diehard critics of the DSM-5. Phrases like "Many of the changes included in the DSM-5 are clearly unreliable and scientifically flawed"; "Some of the decisions included in the DSM-5 are not only not supported by scientific evidence but even defy common sense", in addition to the presentation of a list of the ten errors which—in his opinion—the new manual has [18]. See Table 6.3.

Pressure from the media was the reason that some of the changes proposed by the teams and some new "pathologies" were not taken into account in the final draft. There is no certainty as to the scientific support that the DSM-5 experts had, not even to include or discard them [18]. See Table 6.4.

It is not possible to lightly dismiss the assertions made by Dr. Frances: it was he who led the team that worked on the DSM-IV, and became

Table 6.3 Top ten errors of the DSM-5 [18]

Disruptive mood dysregulation disorder with child's disruptive behavior
Bereavement due to emotional loss as a cause of a major depressive disorder
Minor neurocognitive disorders
Attention deficit disorder in adults
Overeating disorders
Change in the definition of autism
Onset of substance abuse
Addictive behavior
Generalized anxiety disorder
Post-traumatic stress disorder

Table 6.4 Changes and diagnostic not taken into account in the DSM-5 [18]

Risk of psychosis
Mix of anxiety and depression
Addiction to the Internet and to sex
Rape as a mental disorder
Hebephilia
Uncomfortable personality
Significantly lower thresholds for many existing disorders

Table 6.5 Evolution of the number of disorders contained in the different DSM [26]

DSM	Year	Number of mental disorders
I	1952	106
II	1968	182
III	1980	265
IV	1994	297
V	2013	312

part of the design teams of previous DSM who has a profound knowledge of the subject [19].

Meanwhile, Robert Spitzer, who led the teams that structured the DSM-III and introduced the field studies and tools aiming to ensure the reliability of the level of agreement on the diagnostic criteria (kappa coefficient), also expressed his concern. Many of the indexes obtained in the field work were insufficient, and others yielded divergent results [20]. However, the DSM-5 group has denied these facts [21], but also recognized limitations in the studies, which in later versions need to be corrected [22].

Frances and Spitzer sent a joint letter [23] to the APA on December 10, 2010 in which they expressed their perplexities on several points: that confidentiality agreements continued (the participants of the working teams could not reveal anything relating to their work in the DSM-5); the composition of the panel appointed to evaluate the quality of advanced work (except for two members, the others were not unbiased because they worked with the DSM-5), the suitability to be a part of the scientific group to review the work (in spite of being prestigious professionals, they had no competences in primary care, public health, health economics, or forensic medicine); the method was not reliable (among other things because a standard operating procedure did not exist neither to review the literature nor for the fieldwork, which implied a non-significant disorder); the time when the team was formed (it was a year late, which prevented a calm and careful work).

Gary Greenberg [24] stated that thanks to the DSM, in psychiatry there is an overdiagnosis and a considerable increase in the prescription of psychotropic drugs. This has been achieved by the multiplication of alleged pathologies for which the pharmaceutical industry supplies medicines. "It is not about real diseases such as measles or hepatitis, but useful constructs that reflect the way people commonly suffer" [25].

Data on the increase of the disorders included in the various versions of the DSM corroborate this multiplication of nosological entities, hardly supported by clinical practice [26]. See Table 6.5.

The phenomenon of the increase of mental disorders lead to another problem: the relationship with the pharmaceutical industry, previously mentioned, which generates conflicts of interest.

Other experts on the subject have not kept silent and the academy and the media have expressed their concerns and criticisms [27]. Some of the topics covered are the perception that the new update is too ambitious to attempt; that they want to change the paradigm of both the concept of patients with mental disorders as well as of the same concept of what is considered to be healthy. This leads to redefining what a mental disorder is [28].

The issue related to the economy of media deserves special mention. The DSM publication system is very eye-catching [29]. See Table 6.6. Only the DSM-IV has reported to the APA the non-inconsiderable amount of US$100 million. The APA website shows the links that lead to the DSM-5 collection. There are 13 titles that are being offered for online purchase and it is not unreasonable to think that some of them may be rated as a bestseller, with the consequent increase in sales. The price of the DSM-5 is US$149, more than double the price of the DSM-IV; the other titles have an average value of US$80.

Table 6.6 DSM-5 Library [29]

1	A research agenda for DSM-5
2	DSM select at PsychiatryOnline.org for individuals
3	Public health aspects of diagnosis and classification of mental and behavioral disorders. Refining the research agenda for DSM-5 and ICD-11
4	DSM-5™ guidebook. The Essential Companion to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
5	DSM-5™ handbook of differential diagnosis
6	Guideline of diagnostic criteria of DSM-5™. Spanish edition of the desk reference to the diagnostic criteria from DSM-5
7	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)
8	DSM-5™ clinical cases
9	Desk reference to the diagnostic criteria from DSM-5™
10	The pocket guide to the DSM-5™ diagnostic exam
11	DSM-5™ repositionable page markers
12	Study guide to DSM-5™
13	DSM-5™ self-exam questions

We should mention here a paradoxical effect that has been seen in the previous DSM, especially IV: the increase in sales is not followed by a profuse use in the clinic, but presents just the opposite effect. In a recent study [10] that compared the use of the ICD-10 with the DSM-IV, the preferences of the professionals were in favor of the ICD: "Approximately 5,000 psychiatrists took part, which came from 44 countries worldwide. At a global level, 70 % of the psychiatrists who participated affirmed that the ICD-10 was the diagnosis system they used most, and only 23 % declared they used the DSM-IV more. In Europe, these data were even more pronounced: more than 80 % of some 2,700 European psychiatrists who participated in the survey used ICD-10 more often and only 13 % frequently used the DSM-IV" [30].

Another hot topic lies in the field of relations with the pharmaceutical companies industry. It is true that the authors of the DSM-5 have publicly stated their relations with this industry, but it is striking that they have not done so in the context of the conflicts of interest, but as another characteristic of the work developed by them. However, the issue is not to be dismissed so lightly. The details of those relationships have generated in the public opinion a veil of suspicion and reaction or rejection, as it is very difficult not to distrust some alliances where ethics is compromised [31].

It is very understandable and with the previous editions of the DSM it has happened that, given the novelty of the new classification, diagnoses takes off and with them treatments. One of the main problems is that they start calling mental disorders the everyday situations that have come to be regarded as variations in the normal, everyday functioning. And at present more focus is being placed on the brain than on the mind [32], because of the biological prejudice, consumption of psychoactive drugs is increasing exponentially.

It is foreseeable that comorbidities will be increased: more than one diagnosis will be ascribed to a patient without necessarily presenting various diseases. This happens because the new classifications are not sufficient to bring together in only one diagnosis where all the symptoms that are presented in a given clinical manifestation. These large artifacts will cause diagnostic errors and frustration as a logical consequence of the poor results of therapeutic measures. An example of this situation occurs with comorbidities in the autism spectrum [33].

This manual is not only used by psychiatric professionals, and this, which is apparently positive, can cause problems. Many other instances will benefit from the DSM-5, but those benefits may be only relative because not all of them will be prepared to assimilate the contents and succeed in using them.

Only a few examples suffice to illustrate the point. In primary care, general practitioners and nurses may venture diagnostic impressions and make or omit inappropriate or unnecessary referrals. In psychology, the guidance provided in the DSM-5 will also help in the field of diagnosis, but can provide an excess of information which does not necessarily produce a benefit for patients.

On the other hand, journalists, social workers, as well as lawyers will seek support in the new classification for the exercise of their professions, with unexpected consequences. It is also foreseeable that insurance companies will find an important source of information to itemize or back up decisions, for or against patients.

The Philosophical and Ideological Problems

An idea expressed in different ways, facilitated by advances in neuroscience, is taking shape in the mental health environment: find an empirical basis for the new system. It is a fact that the knowledge of the pathogenesis of mental illness has not changed much and that is why the seduction to change is large.

Neither has psychiatry been immune to the influence of the gender theories that have infiltrated vast terrains trying to change society and culture. This is clearly seen in the evolution which, from the DSM-IIIR, have been having the paraphilias [34], sexual dysfunctions, and the way in which this ideology has positioned itself in the different versions of the DSM [35, 36].

It is somewhat striking that factors relating to gender, race, and culture have been given thorough consideration in the DSM-5. A specific working group has been created for them, as well as gender and cultural features, which attempted to provide empirical evidence for some diagnostic categories to be sensitive to these factors. In the background of these changes is one common denominator: the little knowledge we have about who is the person that is suffering a mental disorder.

Assuming that the psychiatric patient is just a mechanism which, due to lack or excess of certain neurotransmitters, has some more or less important imbalances in the operation of his psychic life and can only be restored with the administration of some psychoactive drugs (the mechanism goes back to running without problems), is a form of reductionism that results from not knowing who the psychiatric patient really is.

The trend, even unconscious, of "labeling" patients by the type of disease they suffer will always be a burden and a stigma hard to erase. The very same complexity of the psychiatric pathology causes that the forecasts made will never be specific. This is why the psychiatrist and the health care personnel must be exquisitely prudent and primarily careful as to how the diagnostic impressions as well as the prognosis are communicated to the patient and their families.

That fact that modern psychopharmacology has solved many problems and completely solves some of the cases is unobjectionable. However, its efficacy in other cases is not absolute, even in patients in whom their symptomatology finally goes into remission. This is why following the medical model for treating patients should not prevent the therapist from using other safe and proven alternatives that have nothing to do with the biological treatments.

Nevertheless, the approach to the medical model also intended to be used with the DSM-5 led to a new pragmatic, provisional classification based on observations which use phenomenological criteria and abandon the criteria of Robins and Guze, completed two decades later by Kendel.

The effects of this way of approaching the patient with a mental disorder have led to conceptions such as the one expressed by Joel Paris [37]:

> We psychiatrists could help more people if we spent less time writing prescriptions and more time dedicated to listening and talking to our patients. However, this does not mean that we should return to the psychoanalytic divan. This means, at least in part, that psychiatrists have to be concerned with the lives of our patients and understand how events influence symptoms. We also need to recognize what we know and what we do not know, so we can treat our patients intelligently and effectively.

It is also necessary to take into account that there is a gradual separation between the NIMH of the U.S. and the APA on occasion of the DSM-5, despite the open letter addressed to the visible heads of both institutions [38] by the National Institutes of Health (NIH), where they make sure that the APA as well as the NIMH have shared interests in the line of making sure that patients

and health representatives have the best tools to diagnose and treat mental diseases.

However, it is known that Tom Insel, the Director of NIMH, questions the validity of the DSM-5: although the manual is presented as the "Bible" of psychiatry, for him it is nothing but a simple "dictionary", a collection of labels and their definitions.

In his blog [39], the director of the NIMH poses the need for a new nosological system supported on the data provided by leading edge research in genetics, neuroimaging, and cognitive sciences. He also points out the weaknesses of the DSM-5 diagnoses, that they are based only on a consensus about clusters of clinical symptoms and not on any objective or para-clinical information.

With a somewhat biologistic vision ("Mental disorders are biological disorders involving brain circuits that affect specific domains of cognition, emotion or behavior"), Insen reports that the NIMH has launched a project to transform the psychiatric diagnosis on the basis that all mental illnesses depend on a biological impairment and that "research domain criteria" are necessary to be (*the Research Domain Criteria, RDoC*) supported by clear and contrastable empirical data.

The reported detachment has made the NIMH, at the request of the NIH, make a decision to refocus and fund research apart from the categories proposed by the DSM-5.

Conclusions

The approaches presented suggest taking a closer look at how the DSM-5 should be applied because authors recommend restraint and care in its use. Nevertheless, it should be taken into account that what is new is not always good because it is new. For subsequent versions it is necessary to improve the drafting, to proofread each one of the translations and, above all, make a point to perfect the terminology so that it does not end up being aggressive for patients and their families.

The approach fostered by the DSM-5 for the mental disorders, up to a certain point "contrived" because it is more focused on nosologic rather than on the person who suffers from them. This fact, combined with the clinical practice in health systems that have problems, necessarily tends to forget the patient and to focus on their disease.

At the root of this situation is the lack of an adequate anthropology in the general approach, both for the problems as well as the solutions. This leads to misunderstandings that are expressed in the mechanistic approach to the patients; in the "medicalization" of psychiatry, in its commodification; in the reductionism in which clinical practice slides when it is not understood that patients are people; in the categorical fallacy according to which "what is in the DSM-5 is a disease"; to consider as a disease which is a normal event and current; in the evaporation of the difference between what is considered normal and abnormal; between health and disease; the growing number of people who regularly use psychotropic drugs, etc.

With the advent of psychotropic drugs, many practices in psychiatry quickly became things of the past. When properly prescribed and if the diagnosis has been accurate, these drugs can reduce anxiety, agitation, depression, insomnia, hallucinations, delusions, suicidal or homicidal ideations, etc. They can stimulate inner calmness and sleep, a better mood, and a desire in the patient to communicate and find a way out from ostracism and be prepared to face the reality that is everyday life.

However, an arsenal of drugs is not the solution for all mental disorders. There is a need to not fall into the trap of the biologistic fallacy and a priori to rule out other resources, which, as with psychotherapy, have proved their efficacy both when used together with psychotropic drugs, and when used exclusively, provided that the diagnoses that support their use are appropriate.

With the advancement of science–and in psychiatry there has been a lot, though perhaps is more what we ignored than what we know–a parallel phenomenon has been taking place: the growing gap between health care personnel and the patient: the doctor–patient relationship has been rarefying. Psychiatry also suffers from this harsh reality that needs to be remedied. But, more properly speaking, those who suffer are the psychiatric patients and their families.

Progress has been made in understanding how human beings functions, his thoughts, his feelings, his sensorial perceptions and his behavior. Also much is already known about the techniques to intervene in times of crisis; about the diagnostic approaches which, with instruments such as the DSM-5, should be increasingly refined, in order to establish treatment schemes consistent with the reality of the mental disorder suffered by the patient.

But are patients being treated better presently? Does the status of psychiatric patient make them lose the reality of being a person? Is there hope for the mentally disabled or handicapped? Or, do they have to conform to carrying that stigma and being treated as disposable individuals? The procedures used to manage patients suffering from a drug addiction and who are subjected to a series of indignities and humiliations, are they pertinent because they are supposedly therapeutic? Are certain attitudes and actions acceptable from therapists who, sheltered by fragmentary and incomplete theories, recommend and even help to apply practices and customs head-on contrary to a well-lived sexuality?

These and many other questions demonstrate the need to reject a professional practice, which is at a close risk of dehumanizing both patients and psychiatrists. Instruments such as the DSM-5 should assist in this task of recovering the Hippocratic sense of practicing medicine in general and particularly, psychiatry.

Mental illness means no partiality of individuals, age, or professions. It can be suffered within the most diverse circumstances and be found in any social group. It does not affect a few and it is possible to be found in anyone. It is certain that there are special conditions that favor it and very diverse factors which facilitate it: genetic, biological, family, social, environmental, psychological, cultural, economic, etc. These factors influence each other in all individuals, families, and cultures.

That is why psychiatry needs a reorientation, including taking distance from the "marketing" of the pharmaceutical companies and the insurance companies and approach serious research and a real contrast of the validity and reliability of the mental disorders classification.

We must not forget that the psychiatric patient is a person and demands respect for his dignity, the same humane treatment and the same attention and help as a healthy person and even more as a result of their very helplessness and weakness.

We must also not lose sight of the people who are classified as normal or healthy within a society and could be considered abnormal, sick, or deviant within another human group. There is no single definition of a "normal person" which can be applied to all individuals, at all times, in all situations.

It may be a mistake to equate the "normal" person with an ideal or an idealization. People who are under the definition of "healthy" also have flaws and make mistakes; sometimes they can have "crazy thoughts", internal conflicts, and are not absolutely balanced, reliable or responsible, at all times.

It is possible that the idea of a completely normal person may not be based on reality. The concept of a healthy or normal person covers a wide area of performance and human adaptations. The healthier a person is, it will be more possible for this person to become a part of and react appropriately to unexpected or changing situations, or to events causing tension within their surroundings. One must fight for normality, even though it may never be fully reached. This always requires constant work with the goal to maintain and strengthen the mental health in individuals and in the various human groups.

In this sense, it is important to mention the family as that small community of individuals that can and should become not only the first educator, but also the first preserver of mental health, because it strives to promote and maintain an optimal performance and affective support.

The more or less normal individuals are not developed by accident; they were raised within family organizations, which made such development possible. The psychiatrist needs to keep this in mind: the psychiatric intervention cannot be limited to an individual management of the patient, but must seek to improve their family and social

environment, for such management to provide a good, stable, and durable outcome.

Some recommendations can be derived from the above approaches, aimed at using the DSM-5 to continue the bioethical principle of precaution [40]. It is a reality that this manual is being used and its strengths should be taken advantage of. At the same time, it is necessary to be aware of its limitations for a proper clinical practice and to conduct a respectful research with humans.

These recommendations can be proposed following some reference points for action, derived from bioethics centered on the human person and from those operating habits pointing toward doing good, offered by natural ethics which are crucial for a thorough professional practice: prudence, justice, fortitude, and temperance.

For proper use of a disease classification system it is necessary, as stated above, to start from an adequate philosophical anthropology that gives real reason as to who are the people they have as patients. It is very important to understand that, while it is true that life is a fundamental value, life is not an absolute value and the value of life of each individual of the human species cannot be separated from its transcendent and relational value.

These fundamentals will lead us first to think of the well being of the individual person and his or her family, social, and cultural context rather than trying to "pigeonhole" them into a diagnostic category and establish a prognosis and a treatment. This order facilitates that prognosis as well as management are not depersonalized and that they be settled within a real scenario, including the environment of the person suffering a given disorder.

It is also important to keep in mind an integral concept of the person within the field of research. In such sense, the duty of the Research Ethics Committees is to carefully and professionally study those protocols on new drugs, marked by the "needs" of the market, the extension of patents, the combination of two or more active ingredients, the multiplication of indications, etc., because their duty is to protect the persons included in clinical trials whose relevance can be questioned.

Taking into account the relation of priority and complementarity between person, society, and the environment, the application of the DSM-5—or later versions of the same—will, among other things, lead professionals using the manual to be especially careful and not accept undue or disproportionate incentives offered by the pharmaceutical industry to promote the prescribing of their products. Providing all pertinent and sufficient information on both the diagnoses and treatments to patients, their relatives, or legal guardians should also be ensured so that the process of informed consent is truly valid.

Psychiatrists are recommended to take care not to medicate their patients on their first appointment, when this is not essential and even less when the diagnostic impressions are not clear or when those disorders have been recently included in the DSM. It is also recommended that they be extremely cautious when handling patients with a mild or discontinuous symptomatology: in these situations, it may be preferable to use resources other than the pharmacological resources.

By having a clear concept of human love as an exclusive, complete, and permanent endowment, the application of the DSM-5 will be particularly useful in handling patients with difficulties within this important dimension of the human person. Products offered by the market as "treatment" for sexual dysfunctions, as well as therapies compromising the exercise of sexuality and a properly framed affective experience are to be avoided.

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Part II

From Basic Neurosciences to Human Brain

7 Brain Renin-Angiotensin System: A Novel Therapeutic Target for Psychostimulant and Alcohol Related Disorders?

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Introduction

Ang II is a neuropeptide with multiple actions on the brain. The distribution of its AT1 receptor in the central nervous system (CNS) coincides with several cerebral regions known to regulate cardiovascular and body fluid homeostasis [1, 2]. It is now known that a brain RAS exists [3], with actions largely complementary to those of the systemic peptide [4, 5].

Ang II does not cross the blood–brain barrier, but, through generation at the periphery, can stimulate the brain RAS at specific brain sites such as the circumventricular organs (specific sites in the CNS that lack the blood–brain barrier). Circumventricular organs are critically involved in the regulation of many homeostatic processes, including the control of cardiovascular functions, hydromineral balance, body temperature, and hormone secretion [6]. The action of peripherally generated Ang II at these sites is believed to influence classical behavioral (drinking), endocrine (vasopressin, oxytocin, and adrenocorticotrophic hormone secretion), and autonomic functions [7, 8]. Ang II belongs to the group of peptides known to stimulate dopamine (DA) release [9]. Furthermore, Ang II receptors are located in DA-rich brain areas [10]. Central actions of Ang II are not exclusively associated with their traditional roles. Indeed, several studies have shown that central Ang II is also involved in sexual behavior, stress, learning and memory [11], and included in drug abuse induced effects such as psychostimulants and alcohol.

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Brain Renin-Angiotensin System: Distribution and Functions

In the brain, the angiotensinogen (AOGEN), synthesized by astrocytes [12] and also present in neurons, is cleaved by renin, which is present in the brain in very low concentrations [13], to generate the inactive decapeptide angiotensin I. By the activity of the angiotensin-converting enzyme (ACE), widely distributed in the brain [14], angiotensin I is hydrolyzed at its carboxy-terminus,

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which leads to generation of the active octapeptide Ang II. Ang II seems to represent the first neuroactive form of the angiotensins [15] and it is not only generated in the brain via this classical pathway, involving renin and ACE, but can also be produced directly from AOGEN by cathepsin G or tonin [16]. Subsequently, Ang II is metabolized to Ang III, which is itself converted to Ang IV by aminopeptidases. There are further hypotheses that the brain processes alternative enzymatic mechanisms for the formation of neuroactive forms of angiotensin that are distinct from those involved in the classical pathway [17].

The biological actions of Ang II are mediated by seven specific transmembrane-spanning G protein-coupled angiotensin receptors. Studies of non-peptide antagonists have led to the identification of two pharmacologically distinct Ang II receptor subtypes: AT1 and AT2 [18].

The distribution of angiotensin-like immunoreactivity in nerve terminals is well defined [19] and has a good correlation with angiotensin AT1 and AT2 receptors, defined by in vitro autoradiography with 125I-Ang II, or by in situ hybridization histochemistry [2, 20]. In addition, angiotensin receptors and angiotensin-like immunoreactive nerve terminals are present in sites where microinjections of Ang II produce changes in physiological parameters such as blood pressure, drinking behavior, salt appetite, and neuroendocrine function [19]. These observations provide strong support for the hypothesis that angiotensin acts as a neurotransmitter or neuromodulator in the brain. Furthermore, the discovery of non-peptide and selective Ang II receptor antagonists (losartan, PD 123177, candesartan, between others), in addition to the known ACE inhibitors, have helped in understanding some of the central RAS functions.

Brain Ang II is involved in fluid and salt ingestion, neuroendocrine system modulation including vasopressin and corticotrophin-releasing factor release, and interaction with the autonomic control of the cardiovascular system to influence blood pressure [21, 22]. In many instances, these effects are complementary to those of the systemic peptide on peripheral target organs. Thus, systemic Ang II affects the brain through AT1 receptors located in the circumventricular organs: subfornical organ, vascular organ of the lamina terminalis, median eminence, anterior pituitary, and the postrema area of the hindbrain [2, 23]. In addition, endogenous neurally-derived Ang II appears to act at many CNS sites behind the blood–brain barrier [24, 25] such as the median preoptic nucleus, hypothalamic paraventricular nucleus, anteroventral preoptic, suprachiasmatic and periventricular nuclei, and discrete regions of the lateral and dorsomedial hypothalamus. Most of the classical actions of Ang II are mediated via the AT1 receptors present in large amounts in these areas, whereas AT2 receptor stimulation may cause opposite effects.

Ang II generated within the brain can act on AT1 receptors as a neurotransmitter or neuromodulator in neural pathways, influencing the cardiovascular system and fluid and electrolyte balance. Angiotensinergic neural pathways within the brain may have important homeostatic functions, particularly related to the control of arterial pressure, fluid and electrolyte homeostasis, and thermoregulation.

The brain RAS is also involved in the modulation of multiple additional functions, including processes of sensory information [17, 26], learning and memory [27, 28], and the regulation of emotional [26] and behavioral responses [29, 30]. Researchers have reported that Ang II influenced rat behavior in an open field [29], locomotion, and stereotypy [31, 32].

Brain Ang II was found to regulate some responses induced by drugs of choice for abuse such as cocaine, amphetamine, alcohol, as well as others. It was also found that Ang II enhanced the stereotypy induced by apomorphine (APO, D1 and D2 dopaminergic agonist), and this response was blocked by Ang II AT1 receptor antagonists [33]. The presence of Ang II AT1 receptors has been described in pre- and postsynaptic dopaminergic neurons [9] which are involved in behavioral and rewarding responses induced by psychostimulants and alcohol, as well as their modulator action on noradrenergic [34], serotoninergic [35], gabaergic, and glutamatergic neurotransmission [36, 37].

Our goal in this chapter is to present and discuss the evidence supporting an important role of

brain RAS in neuroadaptive responses induced by two of the most abused drugs: amphetamine and alcohol, proposing this system as a potential therapeutic target in the treatment of disorders related to these drugs of choice for abuse.

Renin-Angiotensin System and Dopamine

Increasing ontogenetic, anatomic, and functional evidence has indicated the existence of a brain RAS and its interaction with other putative neurotransmitters and their receptors. During the embryologic period, it was shown that Ang II increased the differentiation of mesencephalic precursors toward the dopaminergic phenotype [38]. Moreover, all RAS components have been observed in the caudate putamen (CPu), as well as in the other basal ganglia structures. AT1 receptors were observed in the cell body in the substantia nigra (SNi) pars compacta, and at the presynaptic terminal in the CPu [39, 40] and in motivated circuitry key areas, such as nucleus accumbens (NAc) and tegmental ventral area (VTA), [41, 42]. Studies in adult human brain revealed the same localization in these structures [43, 44]. Despite the fact that AT1 receptor density is low in the rat CPu and NAc, other authors found that Ang II acts presynaptically in the rat CPu and NAc to potentiate DA release [9, 45]. In human basal ganglia, ACE was located in the SNi pars reticulata and enriched in striosomes of the striatum, which regulates the DA turn-over in CPu [46].

There is evidence that indicates a role of Ang II, through AT1 receptors, in functions mediated by dopaminergic system, such as locomotor and stereotypic behaviors [31]. In this sense, it showed an increase in rat exploratory activity induced by Ang II intracerebroventricular (ICV) administration, which was higher by administration of APO and decreased by dopaminergic antagonists [47, 48]. Other experimental studies have shown that Ang II increased the stereotypy induced by APO, and it was blocked by ACE inhibitor administration [33] or by Losartan, an AT1 receptor blocker [31, 32]. Furthermore, the Ang II induced rotation behavior in 6-hidroxydopamine lesion rat striatum and was reversed by Losartan or dopaminergic antagonists [49].

In addition, ICV Ang II administration increases extracellular DA in the NAc which is related to Ang II-induced drinking [50]. This is in accord with the findings of Nicolaidis, who in 1974 found that rats submitted to extracellular dehydration were able to self-inject intracerebral Ang II [51]. These data support the concept that Ang II can contribute to the reinforcement effects of drinking behavior and add to the increasing body of evidence implicating the mesolimbic dopaminergic system in reward-relating behaviors.

On the other hand, new outcomes show that Ang II participates in neuroplastic processes. In that regard it was demonstrated that the sensitization to the hypertensive effect to systemic Ang II was induced by repeated central administration of Ang II [52]. Moreover, there is evidence that RAS is involved in neuroadaptive changes related to behavior and neurochemical sensitization to natural reinforcements and drugs of choice for abuse [53].

The RAS system is involved not only in dopaminergic system regulation, but there is evidence that through AT1 receptors, Ang II mediates the noradrenaline transport increase and the tyrosine hydroxylase and dopamine beta hydroxylase enzymes transcription [34]. It is necessary to clarify that mainly dopaminergic areas of the brain, CPu and NAc, receive projections from other different neurotransmission systems such as the noradrenergic system from locus coeruleus, the serotoninergic system from dorsal raphe nucleus, and the glutamatergic system from the cortex.

RAS and Psychostimulants

There is considerable evidence that DA neurotransmission in the CPu and NAc plays a key role in long-term neuroadaptive changes induced by psychostimulants such as cocaine or amphetamine. Repeated exposure to amphetamine, as with most addictive drugs, results in a progressive

and enduring enhancement of its psychomotor and positive reinforcing effects. The enhanced response to psychostimulants, a phenomenon termed behavioral sensitization, relies on time-dependent neuroplastic changes in the brain circuitry that are involved in motivational behavior [54, 55]. These changes are associated with long-lasting hyperactivity of the mesolimbic dopaminergic pathway [56, 57]. The evidence indicates that exposure to a drug of choice for abuse is not needed to be repeated to induce locomotor sensitization; thus studies in mice and rats showed that a single exposure to psychostimulants (amphetamine or cocaine) induced behavioral sensitization [58, 59]. The sensitization process encompasses two temporally distinct phases: induction and expression [56, 60]. Neuroadaptive changes in mesotelencephalic dopaminergic projections play a key role in the induction and expression of amphetamine sensitization. Sensitization can be induced by microinjection of amphetamine into the VTA; meanwhile its expression is associated with time-dependent adaptations in forebrain DA-innervated areas, such as the NAc and CPu.

Behavioral sensitization is not limited to addictive drugs and can also be induced by strong motivational or affective states (thirst or hunger) associated with natural reward stimuli, such as water, salt, food, etc. [52]. In this sense, repeated sodium depletion was able to induce RAS activation and Ang II synthesis, producing an increase in sodium intake. The increase in sodium intake was parallel to neuronal activation (Fos-ir) in brain nucleus involved in motivation and reward [61]. Moreover, increased Fos expression in the NAc core and shell has been described in animals with sodium depletion submitted to a sham-drinking paradigm, in which the persistent appetitive behavior and prolonged ingestion are similar to the behavior of animals responding to drugs of choice for abuse [62].

In this sense, Roitman and colleagues found that the medium spiny neurons within the shell of the NAc of rats that had experienced sodium depletions had significantly more dendritic branches and spines than controls [63]. Behavioral cross-sensitization between sodium depletion and cocaine has also recently been described [64]. The results from these experiments indicate that treatments generating a sustained salt appetite and producing cocaine-induced psychomotor responses show reciprocal behavioral cross-sensitization, similar to results found using amphetamine [53].

There is evidence that supports a direct relationship between RAS and behavioral sensitization. In our laboratory it was found that Ang II AT1 receptors are involved in the neuroadaptive changes induced by a single exposure to amphetamine and that such changes were related to the development of behavioral and neurochemical sensitization. The study examined the expression of amphetamine (0.5 mg/kg, i.p.)-induced locomotor activity in animals pretreated with an AT1 receptor antagonist, candesartan cilexetil (3 mg/kg, p.o.×5 days) 3 weeks after an injection of amphetamine (5 mg/kg, i.p.) [65]. The AT1 blockade effects became evident 3 weeks after pretreatment with a single exposure to amphetamine, when the adaptive changes in behavioral response had been described to be more pronounced [59]. The dopaminergic hyperactivity associated with sensitization was also tested by measuring ^{3}H-DA release in vitro from CPu and NAc slices, induced by K+ (28 mM) stimulus. The behavioral and neurochemical sensitization to amphetamine was confirmed with this two-injection protocol, and pretreatment with the AT1 blocker, candesartan, blunted these responses [65]. With the same purpose, the involvement of brain Ang II AT1 receptors was studied in the development of neuronal activity changes, and so the immunoreactivity of CPu neurons to FOS antibody (FOS-ir) was measured after 3 weeks of the same treatment described above. There are no previous studies showing a neuronal hyperactivity in CPu and NAc induced by a two injections protocol and the results also showed that the AT1 blocker pretreatment prevented this neuronal hyperactivity [66]. Furthermore, we observed that the same AT1 blocker pretreatment attenuated the phosphorylated extracellular regulated kinase (p-erk) immunostaining increase in NAc induced by amphetamine (unpublished data, Fig. 7.1). These experimental approaches provide evidence that supports the involvement of

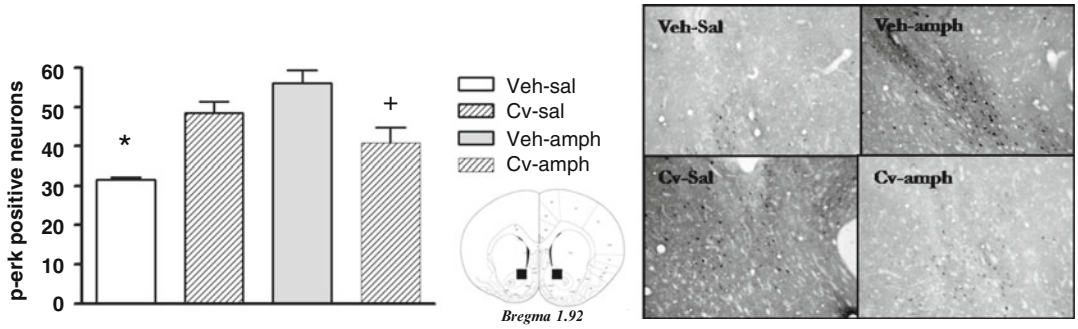

Fig. 7.1 *Left panel*: Average number of p-erk immunoreactive neurons in nucleus accumbens (NAc, *Bregma*: 1.92) in response to a challenge injection of amphetamine (0.5 mg/kg, i.p.), 21 days after a pretreatment with candesartan (Cv, 3 mg/kg, 5 days, p.o.) or vehicle (Veh) and a treatment with amphetamine (amph, 5 mg/kg, 1 day, i.p.) or saline (Veh-sal, Cv-sal, Veh-amph, and Cv-amph). Values are means ± SEM. $*p < 0.05$ significantly different from the other amphetamine-challenged groups, $+p < 0.05$ significantly different from Veh-amph, (1-way ANOVA, post hoc Newman-Keuls). *Right panel*: Photomicrographs ×200 magnifications showing the pattern of p-erk immunoreactive neurons

brain Ang II AT1 receptors in the development of amphetamine-induced behavior sensitization. A new role of brain RAS may be indicated because it has been suggested that the phenomenon of behavioral sensitization is an adaptive process within addiction to psychostimulants and other drugs of choice for abuse [67].

Recent results from our laboratory have showed an increase in the AT1 receptors protein expression in CPu and NAc, 7 and 21 days after the amphetamine treatment (5 mg/kg, i.p., 1 day), and a decrease in AOGEN RNAm and protein expression in CPu, 21 days after the amphetamine treatment, indicating that amphetamine induced long-lasting changes in brain RAS system [68]. Moreover, in another experiment the functional role of AT1 receptors in the expression of sensitization to amphetamine was studied. The AT1 receptors were blocked (Losartan 8 μg/μL by brain side) in CPu and NAc, 5 min before an amphetamine challenge (0.5 mg/kg, i.p) 21 days after amphetamine (5 mg/kg, i.p) administration. These results showed that the expression of amphetamine-induced sensitization was blunted after the blockade of AT1 receptors in CPu [68].

The experiments provide evidence supporting the brain RAS involvement in behavior sensitization induced by amphetamine, contributing to the knowledge of neurobiological mechanisms involved in the psychostimulant drug effects.

The aforementioned evidence points to a new role of the brain RAS in long-lasting effects induced by psychostimulant drugs.

RAS and Ethanol

Alcohol is another of the major drugs abused today, and alcoholism and alcohol-related disorders are disturbingly prevalent in contemporary society. Despite the ever-increasing contributions to the field of alcohol research, a clinically effective pharmacological treatment for alcohol abuse has yet to be developed [69]. It was extensively studied in animals the relationship between alcohol intake and the RAS [70, 71]. In this sense, it was also found that Ang II induced alcohol consumption throught AT1 receptors activation [71]. In 1988, Spinosa and co-workers assessed the ability of the ACE inhibitors (captopril and enalapril) to change alcohol consumption in laboratory rats. The drugs were found to produce a marked reduction in voluntary alcohol consumption independently of changes in blood pressure and without altering alcohol pharmacokinetics [72]. As was observed with psychostimulant drugs, the mesolimbic DA system had been hypothesized to mediate the reinforcing actions of other drugs such as ethanol. Acute administration of ethanol directly alters the DA neurotrans-

mission. Alcohol-preferring animals exhibited a greater dopaminergic response to acute ethanol administration than alcohol–non-preferring animals [73]. Electro-physiological studies demonstrated that ethanol administration increased the firing rate of VTA DA neurons in vivo [74] and in vitro [75, 76]. Systemic administration of ethanol has been shown to increase extracellular DA levels in the NAc and VTA [77–80]. Additionally, microdialysis studies demonstrated an increase in DA release in the NAc following oral self-administration of ethanol [81–83]. Finally, previous data indicate that microinjections of ethanol matabolite into the posterior VTA increase dopamine release in the NAc shell [84].

Involvement of brain RAS components in drug-induced responses has been investigated by correlating altered RAS function and ethanol consumption. Increased expression of AOGEN was found in microarray studies of brains from different rodent lines selectively bred for high ethanol preference (HAP mice) compared with their respective controls [85]. Supporting this idea, chronic ethanol consumption tended to increase AT1 binding in CPu and NAc in C57BL/6 mice, an ethanol-tolerating strain [10].

The studies performed in animals with genetic modification in several components of the RAS demonstrated that Ang II via AT1 receptor action is a positive modulator of spontaneous ethanol consumption in rodents [86, 87]. Transgenic rats expressing a specific AOGEN antisense RNA in the brain [TGR(ASr-AOGEN)680] present angiotensin generation and drastically reduced and modified levels of AT1 receptors in the CNS. These animals show lower ethanol consumption and altered responses after ethanol intoxication compared with controls. Supporting the idea that angiotensin-mediated DA release plays an essential role in Ang II-triggered regulation of alcohol intake, altered DA concentrations were found in relevant brain areas. Indeed, concentrations of DA as well as its principal metabolite (DOPAC) were found to be strongly reduced in a region covering the VTA of TGR(ASrAOGEN)680 rats [88]. It is interesting to note that mice lacking the D2 receptor gene were less sensitive to alcohol-induced ataxia than their wild type littermates while they ingested lower amounts of alcohol in free choice experiments [89]. It is believed that Ang II stimulates DA release in NAc and CPu [9], and angiotensin receptors are expressed in brain areas such as the NAc, where dopaminergic transmission has been strongly implicated in alcohol self-administration and sensitivity [10, 46]. Evidence has shown an increase in the voluntary consumption of alcohol in the transgenic mice expressing a rat angiotensinogen transgene (TGM123). These animals have elevated levels of Ang II resulting from additional expression of the AOGEN transgene. Consumption was significantly reduced by administration of fluphenazine, a DA receptor antagonist. Thus, increased alcohol intake in mice over-expressing angiotensin may relate to an interaction of Ang II with dopaminergic systems. Furthermore, the knockout mice lacking the AOGEN gene (TLM), drank even less alcohol than the controls. Furthermore, it was found that the ACE inhibitor Spirapril, known to cross the blood–brain barrier and consequently lower the Ang II levels in brain and blood circulation, suppressed alcohol intake in the TGM123 mice [87]. Ang II ICV infusion in the ethanol-tolerating mice, C57BL/6J, stimulated the intake of 4 % ethanol solution and caused a transient increase in the intake of 10 % ethanol solution. The increase in ethanol solution intake that occurred did not increase progressively over the 3 days of Ang II treatment, as is usually observed when water is available [90], probably indicating a response to hedonic stimulus of alcohol more than to homeostatic deregulation by Ang II infusion.

The stimulation of brain RAS by food depletion also modifies alcohol consumption. Animals deprived of ad libitum food access had higher ethanol consumption than controls during treatment, and the behavior was reversed when free access to rat food was restored [91].

Controversial results have been reported on ICV infusion of Ang II in rats. An increased alcohol intake has been described by Fitts [92], but most experiments indicate no functional effects for this type of Ang II administration [90, 91, 93]. It is important to highlight that these experiments

are based on invasive techniques with unpredictable effects on drinking behavior. Functional stimulation of Ang II receptors in the vicinity of the lateral ventricle does not exert an influence on alcohol consumption. Furthermore, as alcohol consumption does not decrease at the expense of a robust increase in water consumption, it is possible to assume dissociation between the effects of Ang II on alcohol and water intake [93].

Experiments have been conducted with pharmacologic modification of RAS activity. The blockade of the receptors with different doses of the Ang II antagonist SarThr-Ang II does not modify alcohol consumption. Interestingly, ACE inhibitors reduce alcohol intake in a dose-dependent manner in genetically selected alcohol-preferring and –non-preferring rats, as well as in Wistar rats [94–96]. This may indicate that ACE inhibitors are not acting through peripherally based Ang II-related processes to reduce alcohol consumption. Because peripheral administration of ACE inhibitors can elevate central RAS activity, the present findings indicate that if the reduction in alcohol intake produced by ACE inhibition is mediated through the RAS, the locus of this effect is likely to be at a central site not accessible to a peripherally administered Ang II antagonist [95].

These results clearly support the hypothesis that the central RAS, through AT1 receptors, are involved in the control of alcohol intake behaviour, modulating the DA system.

Conclusion

It is widely known that the high incidence in health costs and in patient welfare and its environmental deterioration are a result of drug abuse and alcohol-related diseases worldwide. In this context it is very important to study new targets in order to provide new pharmacological tools for the treatment of these pathologies. A group of drugs that interfere with the RAS, widely used in clinical practice for hypertension treatment and cardio protection, have particular advantages because they are nearly free of severe side effects. Therefore, the results presented in this chapter regarding the key role of the brain RAS in the neuroadaptative response to drugs of choice for abuse show a new therapeutic application for AT1 blockers. More research is needed to reveal the effectivity of long-term treatment of available ACE inhibitors or AT1 blockers that could be used in the treatment of drug abuse disorders.

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Role of the Neuropeptide Angiotensin II in Stress and Related Disorders

8

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The Renin-Angiotensin System

Angiotensin II (Ang II) was discovered in 1940 and described as a peripheral hormone. Later its synthesis and metabolism were characterized, currently known as the renin angiotensin system (RAS) [1]. The precursor molecule is the angiotensinogen synthesized in the liver and cleaved by a renal protease, giving an inactive decapeptide, angiotensin I. This is converted into the active octapeptide ANG II, by action of a circulating enzyme called angiotensin converting enzyme (ACE), which is also responsible for inactivating bradykinin. The octapeptide hormone was subsequently found to be produced in numerous tissues, including the adrenal glands, heart, kidney, vasculature, adipose tissue, gonads, pancreas, prostate, eye, placenta. and brain [2].

The principal actions related to Ang II are vasoconstriction, aldosterone release, sodium retention, and a key role in blood pressure control and fluid homeostasis.

All the components of the RAS, including the receptors, have been found in brain tissue, indicating a role as a hormone or neuromodulator in the central nervous system [2, 3]. Ang II exerts its principal known effects acting through the AT1 receptor. The actions of Ang II related to AT2 receptors are controversial and associated with AT1 opposite effects, although there is evidence showing cross-talk between both receptors.

Angiotensin II Receptors

It has been initially described that there are two principal subtypes of Ang II receptors named AT1 and AT2. The characterization was first made based on their affinity to specific ligands and later by molecular cloning. The AT1 receptors were designed AT1A after the discovery of other subtype of receptor designed as AT1B cloned in rats [4, 5], mice [6], and humans [7]. Although the isoform AT1A is responsible for the associated functions of the brain Ang II system [8, 9], we will refer to it as AT1 receptor. The AT1 receptor is a typical heptahelical G protein-coupled receptor and is made up of 359 amino

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acids with an unmodified molecular weight of 41,000 [10]. AT1 receptor stimulation induces multiple cellular responses, predominantly via coupling to Gq/11 stimulates phospholipases A2, C, and D and activates inositol trisphosphate/Ca2+ signalling, protein quinase C isoforms, and mitogen-activated protein kinases (MAPKs), as well as several tyrosine kinases (Pyk2, Src, Tyks, Fak), scaffold proteins (G protein-coupled receptor kinase-interacting protein 1, p130Cas, paxillin, vinculin), receptor tyrosine kynases, and the nuclear factor-κB pathway. The AT1 receptor also signals via G12/13 proteins, and Gi/o in rodents and stimulates G protein-independent signalling pathways, such as β arrestin-mediated MAPK activation and the janus kinase/signal transducer and activator of transcription [11, 12]. The AT1 receptor is responsible for most of the known biologic effects of Ang II, including those of the central nervous system [13, 14]. Alterations in homo- or heterodimerization of the AT1 receptor may also contribute to its pathophysiological roles. Many of the deleterious actions of AT1 receptors are initiated by locally generated, rather than circulating Ang II [12]. The AT1 and AT2 receptors have a similar binding affinity for Ang II although they only share a 32–34 % identity at the amino acid level [15, 16].

Brain Ang II

It is generally accepted that the Ang II from the periphery does not cross the blood–brain barrier (BBB) but stimulates the AT1 receptors located in the circumventricular organ outside the BBB [8]. This stimulation increases the intake of fluids and salt. The brain RAS generates the Ang II which stimulates receptors inside the BBB [17]. The neuroanatomical localization of Ang II, AT1, and AT2 receptors has been precisely described in different brain areas placed on neurons, astrocytes, and oligodendrocytes [8, 18, 19]. It has also described the presence of components of RAS in glial cells suggesting a more important role for these that was previously postulated [20].

Both subtypes of receptors were found to have a similar, but not identical, distribution in all the mammalian species studied, including humans [8]. While AT1 receptors predominate in adult animals, AT2 are expressed in the developing brain [8]. AT1 receptors are located in brain areas related with the control of neuroendocrine functions and the autonomic regulation of limbic and cardiovascular systems, while AT2 receptors are involved in organogenesis and in the functions of motor and sensorial systems [21].

The role of brain Ang II is complex and is related by control of the autonomic, hormonal system, and sensorial and cognitive processes including regulation of cerebral blood flow [16].

AT1 and AT2 Receptors in Stress-Involved Brain Areas

The AT1 receptors are distributed throughout the brain, including the key areas regulating stress response such as the hypothalamus–pituitary–adrenal (HPA) axis [22]. The medial, basomedial, lateral, and basolateral nuclei of amygdala control the emotional responses such as fear conditioning and adaptation to danger, and these nuclei are enriched with AT1 receptors [23, 24]. The other stress responsive brain regions include the cortex, hippocampus, locus coerulus (LC), median eminence (ME), subfornical organ (SFO), dorsomedial hypothalamus (DMH), and nucleus tractus solitarius (NTS), and are also enriched with Ang II and its receptors. The different sections of cortex such as the prefrontal cortex, entorhinal, piriform cortex and neocortex control cognition and emotional behavior [22, 25]. The hippocampus is an important structure for storing memory processes during stress. The LC region is located in the pons (a part of the brainstem). It is actively involved in controlling the physiological response to stress. Additionally, LC is a site of origin of sympathetic innervations to the cortex involved in stress-induced central sympathetic stimulation. The DMH is a nucleus of the hypothalamus that regulates panic-like responses [26, 27]. The paraventricular nucleus (PVN) of the hypothalamus is also an important stress-involved brain region; it is activated by a variety of stressful and/or physiological changes.

The SFO is a sensory circumventricular organ and is also involved in regulating the stress response. NTS is a group of cells in the brainstem that receive viscera sensory information and send it to the basal forebrain, actively involved in regulating cortical processing of anxiogenic stimuli. Moreover, additional nerve projections from the NTS to the LC, the bed nucleus of stria terminalis and the amygdaloid structures, influence the processing of anxiogenic stimuli [28]. The ME is an integral part of the hypophyseal portal system, which connects the hypothalamus to the pituitary gland. The projections of neurons from median preoptic hypothalamic nucleus to the ME regulate the stress response by controlling the release of stress hormones [29–31].

Functional and anatomical studies have shown the abundant presence of AT2 receptors in neonates, where these are involved in the development of central nervous structures [8, 22]. Although, their number is significantly reduced in the adult tissues, including the brain [32], and is restricted to the inferior olivary complex, thalamic nuclei and LC to control sensory, motor, and behavioral functions [22]. Interestingly, it has been proposed that the AT2 receptors exhibit their functional role only after their up-regulation under pathologic conditions [33], but it has been found a physiological role in mice lacking AT2 receptors (knock out), suggesting that these receptors are also functional during normal non-pathological conditions [28, 34, 35].

Ang II as a Stress Mediator

There is a large body of evidence at pharmacological, neuroanatomical, and physiological levels, supporting a key role for Ang II in the stress response, including regulation of the sympathetic and neuroendocrine systems [16, 36–38]. The presence of AT1 receptors has been shown at all levels of the HPA axis, with a higher concentration in key areas for the control of stress response, such as the PVN [39], ME, anterior pituitary, zona glomerulosa, and adrenal medulla [40]. Exposure to stress induces an increase in circulating and brain Ang II levels [41, 42]. Brain Ang II stimulates local receptors in the PVN and LC, among other nuclei, while circulating Ang II also stimulates the AT1 receptors in the subfornical organ, to which it is connected through the ME and PVN [40, 43].

Castren and Saavedra found that exposure to acute stress induced an increase in AT1 receptor density in the anterior pituitary, although exposure to repeated stress sessions increased AT1 receptor density in the PVN [44], expressed in the cellular body of neurons that synthesize corticotrophin releasing hormone (CRH) [40, 43]. In agreement with this, it has been found that AT1 receptor stimulation by Ang II induced an increase in the production of CRH [45, 46]. CRH is a hormone released to the circulation that increases adrenocorticotropic hormone (ACTH) release from the pituitary. It has been found that due to stress, high levels of adrenal glucocorticoids induced an increase in the expression of AT1 receptors in the PVN [45]. Moreover, there is local production of Ang II in the anterior pituitary which, acting together with circulating Ang II, induces an increase in ACTH secretion [38].

The PVN is an important area in the processing and integration of many different stress signals [47]. This nucleus receives noradrenergic input from the LC and serotoninergic input from the dorsal raphe nucleus, and there are reciprocal interactions between these two regions and the PVN [48, 49]. In addition, there are reciprocal neural connections between CRH neurons from the PVN and noradrenergic neurons from the LC. It has been shown that both adrenergic receptor subtypes regulate the ACTH secretion, and CRH controls central noradrenergic activity. Most of the available evidence suggests that CRH acts as a neurotransmitter in the LC modulating the noradrenergic activation in response to stress [50].

Exposure to one session of social isolation for 24 or 2 h of cold restraint induced an increase in the enzyme tyrosine hydroxylase (TH) mRNA in the LC and, in both cases, this increase was prevented by previous administration of an AT1 receptor blocker (ARB) [31, 51]. This evidence suggests that AT1 receptors are involved in the control of central sympathetic activity through the regulation of TH transcription. However, it should

be taken in consideration that influences of AT2 receptor activation in the LC respect the TH regulation. This last is based on results showing dual control by AT1 and AT2 receptors in TH transcription and in the synthesis of catecholamine at the adrenal medulla [52].

The evidence obtained using ARBs gives support for a key role for Ang II in the stress response. It is important to highlight that their actions may not be limited to the HPA axis only [16, 30, 31, 51, 53]. Supporting the extra-hypothalamic influence of Ang II in the stress response are the results obtained with candesartan, an ARB, showing a prevention of isolation-induced decrease in CRH1 receptors and the GABAA complex in the brain cortex [51]. Moreover, when the animals were tested in the plus maze they exhibited an increase in the parameters associated with anxiolytic effects. Altogether, this strongly suggests a role for AT1 receptors not only in autonomic and hormonal response, but also in behavioral response to stress [51, 54].

Ang II and Stress-Related Disorders

Anxiety

The HPA axis has a major role in stress response being mediated by CRH, although the behavioral stress response mediated by CRH occurs in a manner independent of the HPA axis. This is based, among other evidence, on the fact that hypophysectomy and the blockade of the HPA axis response with dexametaxone do not alter the stress response induced by brain CRH administration. This strongly suggests a central action responsible for the coordination of stress-related behaviors [55].

It has been found that isolation stress [51], electric shock [56], and chronic unpredictable stress [57] produce, among others, a decrease in brain CRH1 density. This can be reproduced in the prefrontal cortex by intracerebral CRH administration, and a decrease in mRNA levels of CRH1 receptors has been shown in vitro in a cell line derived from CRH neurons incubated with CRH. These results support the idea of down-regulation induced by the CRH increase [58]. Interestingly, candesartan, an ARB that crosses the BBB was found to prevent a decrease in brain cortex CRH1 induced by isolation stress [51], indicating that cortical CRH activation is positively regulated by AT1 receptors, similar to what occurs at the hypothalamic level. Even so, the presence of AT1 receptors has been determined in the piriform and entorhinal cortex [22] but only of the mRNA of AT1 receptors in the neocortex [25]. This suggests that AT1 receptor blockade could reduce the decrease in cortical CRH1 receptors [51]. There is reciprocity between CRH and noradrenergic systems during stress: the LC is activated by CRH [27] and stress induces an increase in CRH in this brain area [59]. In this sense, it has been found that CRH injected into the LC induced behavioral activation and noradrenaline release in the prefrontal cortex and these two responses were blocked by a CRH receptor antagonist [60]. However, the AT1 receptors seem to modulate only the CRH1 receptors because there is no evidence showing any action on the CRH2 receptors [51].

The CRH is also related to the GABA system because GABAA receptors are located in CRH neurons. In this respect, it has been shown that the exposure to different kinds of stressors induced a decrease in the benzodiazepine binding in the frontal cortex, increasing the anxiety behavior [61, 62]. The administration of ARBs decreased the CRH release, and this could explain the prevention of the GABAA binding decrease in animals exposed to isolation stress [51].

There is much evidence showing the anxiolytic effect of ARBs described by different authors, administered orally or intracerebrally [16, 24, 63, 64].

Gastric Ulcerations

Gastric ulcerations are largely associated with stress exposure through the development of gastric ulcers or ulcerations [65] as a result of complex psychological factors influencing individual vulnerability, the stimulation of brain specific pathways regulating autonomic function, decreased

blood flow to the mucosa, increase in muscular contractility, mast cell degranulation, leukocyte activation, and increased free radical generation, resulting in increased lipid peroxidation [65–68].

The events association with gastric lesion formation are sudden blood flow reduction to the gastric mucosa and increased free radical formation [67]. For this reason, the maintenance of gastric blood flow is important to protect the mucosa from endogenous and exogenous damage factors. It has been described that Ang II levels increase during stress in plasma and tissues, including the stomach tissue [41]. The role of Ang II in the stomach is the regulation of gastric vascular tone though AT1 receptor stimulation [69]. It is already known that Ang II generates reactive oxygen species with cellular damage and inflammation [70]. The mucosal vasoconstriction and proinflammatory effects of Ang II could contribute to the production of stress-induced gastric ulcers.

An experimental model commonly used to induce acute gastric damage is cold-restraint stress, which is also clinically relevant [71]. Using this stress model in male spontaneously hypertensive rats (SHRs), it was found that AT1 receptor inhibition prevented gastric lesions by combined local and systemic mechanisms, including gastric blood flow maintenance, inhibition of proinflammatory cascade activation, preventing the gastric ischemia and inflammation characteristic of a major stress response, and resulting in the protection of the gastric mucosa from stress-induced ulcerations [53]. The experiment was carried out in SHRs because these animals have an increased expression of the brain RAS components and have been described as stress-prone animals. More specifically it was found that blood flow to the stomach was significantly increased in animals treated with the ARB compared with vehicle-treated controls [53]. Interestingly, the maintenance of a normal pituitary–adrenal response to stress is a phenomenon that runs parallel with protection from gastric injury. This view is supported by the fact that endogenous corticoids contribute to protect the gastric mucosa from ulceration during stress, probably by contributing to an increase in blood flow and bicarbonate secretion [72].

The presence and density of Ang II receptors in the stomach was analyzed by autoradiography and showed the presence of AT1 receptors and a lower number of AT2 receptors, in all layers of the stomach and furthermore, the ARB treatment decreased the AT1 receptor binding [53]. This last could be the result of receptor occupancy with the insurmountable antagonist candesartan or of receptor down regulation [73]. Therefore, the decreased number of gastric AT1 receptors after stress may be related to receptor occupancy by the increased Ang II levels or to a receptor compensatory mechanism due to Ang II increased stimulation [37].

The exposure to cold restraint in rats induced a marked increase in the expression of the intercellular adhesion molecule 1 (ICAM-1), the proinflammatory cytokine tumor necrosis factor alpha (TNF-α), and the number of infiltrating neutrophils in the gastric mucosa [53] and it is known that these components play crucial roles in the progression of gastric injury [74]. It is also known that activated neutrophils release inflammatory mediators, capable of damaging endothelial cells and inhibition of neutrophil infiltration prevents the stress-induced reduction of mucosal blood flow and the production of gastric lesions [75]. Likewise, it has been described that Ang II promotes tissue inflammation through AT1 receptor stimulation, enhancing neutrophil infiltration [76, 77], increasing the expression of TNF-α [76, 78, 79] and ICAM-1 [80]. It was reported that TNF-α down regulated AT1 receptors [81, 82], and that TNF-α, acting with other proinflammatory cytokines, up regulated AT1 receptors and increased the profibrotic effects induced by Ang II [83, 84]. The increase of TNF-α and neutrophil infiltration in the gastric mucosa induced by cold restraint was prevented by ARB [53].

The increased ICAM-1 expression induced by cold restraint exposure in the endothelium of arteries of the gastric mucosa and submucosa, and in venules of the submucosa (where AT1 receptors are located), was also prevented by the AT1 receptor blockade [53]. The anti-inflammatory effects of AT1 receptor blockade could thus be important for protection against stress-induced gastric ulcers.

Posttraumatic Stress Disorder

Posttraumatic stress disorder (PTSD) is a debilitating stress-related illness associated with exposure to trauma. The peripheral and central mechanisms mediating stress response in PTSD are incompletely understood. The renin-angiotensin pathway is essential to cardiovascular regulation but as it was described above is also involved in mediating stress and anxiety. Based on these data, Khoury et al. examined the relationship between active treatment with blood pressure medication, including ACE inhibitors and ARBs, and PTSD symptom severity within a highly traumatized civilian medical population [85]. This study was a larger study performed in patients recruited from Grady Memorial Hospital's outpatient population from 2006 to November 2010. Multivariable linear regression models were fit to statistically evaluate the independent association of being prescribed an ACE inhibitor or ARB with PTSD symptoms, using a subset of patients for whom medical information was available ($n=505$). Categorical PTSD diagnosis was assessed using the modified PTSD Symptom Scale (PSS) based on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria, and PTSD symptom severity (the primary outcome of interest) was measured using the PSS and Clinician-Administered PTSD Scale [85]. The authors found a significant association between presence of an ACE inhibitor/ARB medication and decreased PTSD symptoms. Meanwhile, other blood pressure medications, including β-blockers, calcium channel blockers, and diuretics were not significantly associated with reduced PTSD symptoms. The authors provide the first clinical evidence supporting a role for the renin-angiotensin system in the regulation of stress response in patients diagnosed with PTSD. Further studies need to examine whether available medications targeting this pathway should be considered for future treatment and potential protection against PTSD symptoms.

Drug Abuse

The hormonal changes, involving increased peripheral glucocorticoid levels and CRH release in different brain sites, initiate a cascade of biological responses to counteract the altered homeostatic balance of the organism in response to stress. The modification in the brain physiology induced by stress triggers the release of neuroactive hormones such as biogenic amines and adrenal steroids, which activate the same neuronal circuit as the psychostimulant drugs, cocaine or amphetamine. Many years ago, clinical studies of methadone-treated heroin addicts [86] showed atypical stress response in both active and long-term abstinent heroin addicts, similar to the atypical stress response of the HPA axis that has been found in abstinent cocaine addicts [87]. Thus, it has been hypothesized that an atypical response to stressors may contribute to compulsive drug use [88]. Furthermore, it have been demonstrated in a series of studies that rats with higher levels of behavioral and neuroendocrine response to stress develop psychostimulant drug self-administration more rapidly than low responders [89, 90]. In conjunction with other evidence, this supports a major role for stress in individual vulnerability to self-administer drugs of choice for abuse. In addition, corticosterone, the major glucocorticoid end-product of HPA axis activation in rodents, was shown to be self-administered in rats [90], and pharmacological manipulation of the circulating corticosterone levels altered cocaine self-administration behavior [91]. These results and many others suggest that the activity of the HPA axis may play a role in different phases of drug addiction.

Brain Ang II was found to regulate some responses induced by drugs of choice for abuse such as cocaine and amphetamine, among others [92–95]. The presence of Ang II AT1 receptors has been described in pre- and postsynaptic CPu dopaminergic neurons [96], which are involved in the motor and behavioral responses induced by psychostimulants, as well as their modulatory action on noradrenergic [97], serotoninergic [98], glutamatergic, and gabaergic neurotransmission [99, 100]. It has been described that Ang II modulates the neuronal response to glutamate via both AT1 and AT2 receptors possibly at the postsynaptic level in the superior colliculus, locus coerulus, and dorsal lateral nucleus in addition to other areas [101–103].

There is indirect evidence of RAS involvement in neuroadaptative changes induced by

psychostimulant drugs. In this sense, a history of sodium depletion, which activates RAS and Ang II synthesis, was found to develop cross-sensitization effects leading to enhanced locomotor activity responses to amphetamine or cocaine [104, 105]. Evidence was recently found in our laboratory involving the activation of brain AT1 receptors in the development [93, 94] and expression [106] of behavioral and neurochemical neuroadaptations induced by amphetamine in rats.

Hypertension

It has been found that repeated stress in rats significantly increased blood pressure and noradrenaline and adrenaline levels, and these effects were attenuated by adrenalectomy [107].

The stress response increases sympathetic nervous activity, which can adversely affect the cardiovascular system [108]. Cardiovascular disease is in part a result of stress-induced mechanisms mediated primarily through increased adrenergic stimulation. These stress-induced mechanisms include elevation in serum lipid levels, alterations in blood coagulation, atherogenesis, vascular changes in hypertension, and myocardial ischemia. Stress management interventions for hypertension are controversial; however, interventions for coronary heart disease-prone behavior patterns have proved successful. Stress management interventions have also reduced cardiovascular events, mortality, and coronary atherosclerosis. Assessment of stress includes individual interviews which can be complemented by information derived from questionnaires and mental stress testing. Educational and relaxation strategies can prepare patients to understand and cope with stress. These approaches will hopefully decrease the occurrence of stress and, ultimately, the risk for cardiovascular disease [109].

Circulating and locally formed Ang II controls cerebral blood flow by AT1 receptor stimulation in cerebral vessels and sympathetic nerves. Brain Ang II and sympathetic systems are stimulated in spontaneous hypertensive rats, producing increased vasoconstrictor tone and arterial thickness with smooth muscle proliferation, decreased vascular compliance, and decreased ability of cerebral vessels to dilate during hypoperfusion. Blockade of Ang II formation by ACE inhibitors inhibits cerebrovascular tone, and cerebral blood flow is maintained by compensatory small resistance artery vasoconstriction, improving tolerance to hypotension and increasing adaptation to the reduction in blood flow during stroke. In this sense, it was found the protective effect of chronic administration of candesartan during ischemia and the improvement of cerebral blood flow in spontaneous hipertensive rats by chronic pretreatment with candesartan [110]. Moreover, Ang II system inhibition protected against neuronal injury more effectively than other antihypertensive drugs such as calcium channel blockers. The protection after AT1 receptor blockade is not directly correlated with blood pressure reduction but with normalization of middle cerebral artery media thickness, leading to increased arterial compliance and reduced cerebral blood flow decrease during ischemia at the periphery of the lesion [110].

Conclusions

The results presented from studies on the physiological and pathological role of brain Ang II aim to encourage the study of this system in the context of the search for new pharmacological tools in the treatment of stress-related disorders (Fig. 8.1). Moreover, the advantage of the available compounds that interfere with the RAS, ACE inhibitors and ARBs, is that they are tolerated well

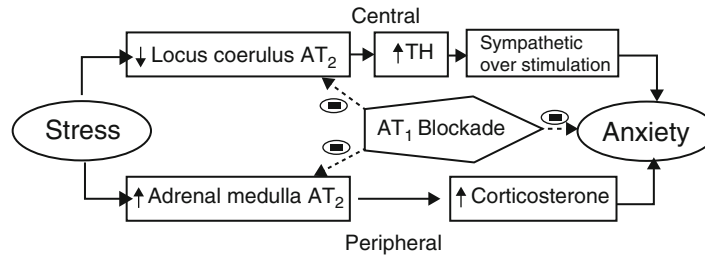

Fig. 8.1 Events related with stress response involving the central and peripheral angiotensin II AT1 receptor activation

and widely used in the treatment of hypertension. Interestingly, the ARBs do not modify the blood pressure in normotensive individuals.

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Neurovascular Cognitive Alterations: Implication of Brain Renin–Angiotensin System

9

Therapeutic Opportunities and Risk Factors

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Brain and Cerebral Vasculature

Brain vascularization is responsible for normal brain functioning through the continuous supply of glucose and O_2 necessary for its elevated metabolic demand. Even though the human brain comprises only 2 % of the body's mass, its activity during the resting state demands 17 % of the heart blood flow and consumes 20 % of the energy produced [1, 2]. This high consumption of energy is used mostly to reverse the ion influxes that underlie excitatory postsynaptic currents and action potentials, thus adequate cerebral blood flow (CBF) comprises not only neuron and glia viability, but also its communication [3]. Cessation or reduction of regional CBF leads to time-dependent decrease in membrane electrical activity, neuron viability and tissue integrity [4].

The intracranial cerebral arteries initiate in the circle of Willis at the base of the brain and give rise to progressively smaller vessels traveling on the brain surface (pial arteries) that also branch out into smaller vessels which penetrate into the substance of the brain and give rise to arterioles [1]. CBF is highly regulated by coordinated responses that integrate regional and segmental changes in vascular tone. Three regulatory mechanisms have been identified to ensure that brain perfusion is maintained at all times [5].

The first involves brain control over systemic circulation through its humoral and neural influence over the cardiovascular system. The second is known as cerebrovascular autoregulation. It involves cerebral arteries and cerebral arterioles on the surface of the brain that are responsible for two thirds of the total cerebral vascular resistance. Changes in resistance of large arteries counteract the cerebrovascular effects of the normal fluctuations in arterial pressure. In this way cerebral arteries relax when arterial pressure decreases and constrict when arterial pressure rises [6, 7]. The third mechanism, functional

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hyperemia, controls substrate delivery and removal of the by-products of metabolism.

Hyperemia utilizes local changes in the microvasculature in order to match CBF to the functional activity of the different brain regions so that when the activity of a brain region increases, flow to that region also increases [8–10]. As functional brain imaging signals are based on mapping the changes in CBF and matching them with neural activities, understanding the mechanisms that allow functional hyperemia is an important field of investigation.

The Neurovascular Unit

The notion of changes in blood flow in response to evoked neuronal activity after an emotional stimulus was proposed in the literature in the late nineteenth century. The traditional explanation sustained that the energy demand of the active tissue directly induced the modification in CBF in the area. However, O_2 consumption and CBF changes occur in parallel, but as dissociated processes. The fractional increase in blood flow induced by sustained neuronal activity is at least fourfold greater than the increase in adenosine triphosphate (ATP) consumption by the neurons. This is consistent with the idea that blood flow is regulated mainly by feed-forward neurotransmitter-mediated mechanisms rather than by a negative-feedback loop driven by energy demand [3]. It is now known that the hemodynamic response is coupled to neurosignaling events and this interaction is crucial in maintaining the homeostasis of the cerebral microenvironment; then, its alteration may lead to brain dysfunction and disease [5, 11–13]. The coordinated responses between neural activity and CBF observed in hyperemia are achieved through a close spatial and temporal relationship, termed neurovascular coupling, resembling an integrated unit that comprises neurons, glial cells (astrocytes), and vasculature (endothelial cells and vascular muscle cells) closely related developmentally, structurally, and functionally [11, 14] (Fig. 9.1).

Role of Vascular Endothelium

Once pial arteries penetrate into the brain they branch into smaller arteries and arterioles. These resemble peripheral vasculature, consisting of an endothelial cell layer, a smooth muscle cell layer

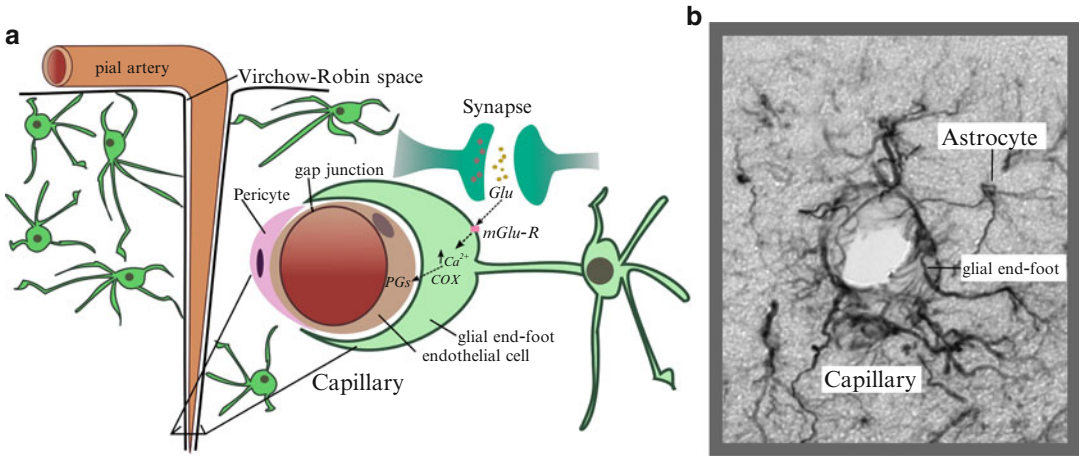

Fig. 9.1 The neurovascular unit. (**a**) Astrocytes are closely related to cerebral blood vessels and synapses. Astrocytes endfoot surround penetrating arterioles and fine processes are in close proximity to synapses. Activation of metabotropic glutamate receptors (mGluR) during synapse activity and propagation of Ca^{2+} waves from neighbouring astrocytes increase intracellular [Ca^{2+}]. The vasodilation associated with neural activity could be promoted by astrocytic lipoxygenase products. (**b**) Microphotograph (400× magnification) showing glial fibrillary acidic protein (G-FAP) immunoreactive astrocytes surrounding the microvasculature in rat prefrontal cortex

(myocytes), and an outer adventitia layer, containing collagen, fibroblasts, and perivascular nerves [15]. The Virchow-Robin space, which contains cerebrospinal fluid, separates the vasculature from the glia limitans until arterioles and capillaries reach deeper into the brain and become smaller. Capillaries consist of endothelial cells, pericytes (with contractile properties), and the capillary basal lamina on which the astrocytic feet are attached [11].

The Endothelial Cells

Endothelial cells play a critical role in the regulation of both vascular tone and changes in the growth and morphology of the vessel wall by maintaining a vital balance between the various dilating and constrictor processes which influence many other cell activities. These actions are achieved by releasing diffusible factors such as nitric oxide (NO), free radicals, prostanoids, endothelium-derived hyperpolarizing factor, and endothelin [16]. Thus, endothelium cells are not only a simple monolayer barrier dividing different tissue, as they play an important role in several vascular events.

NO is produced following the conversion of L-arginine to L-citrulline by the catalytic action of the enzyme endothelial NO synthase (eNOS). This enzyme is constitutively expressed and its activity is modified by factors such as the shearing stress of flowing blood, bradykinin, and by exogenous application of acetylcholine. NO diffuses to stimulate soluble guanylate cyclase in vascular muscle, resulting in an increase in the intracellular concentration of cyclic guanosine monophosphate (cGMP), subsequent lowering of intracellular Ca^{2+} and muscle relaxation [16, 17]. NO also provides anticoagulant activity by inhibiting platelet activation as well as exerts anti-inflammatory effects, reducing vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) expression and inhibiting release of chemokines, such as monocyte chemoattractant protein 1 (MCP-1) [17]. NO levels can be diminished either by its synthesis interference or by direct destruction when it reacts with superoxide anion. The reaction of NO with superoxide anion results in the formation of peroxynitrite, a potent oxidant that can produce cytotoxicity, including nitrosylation of proteins and damage to DNA [17].

Similar to NO, prostacyclin has vasodilator and anti-platelet aggregator functions and is viewed as an important agent of vascular protection. Prostacyclin is the prostanoid produced in the greatest amounts in the endothelium, which has enriched constitutive expression of cyclooxygenase (COX)-1. Prostacyclin receptor activation in muscle cells causes enhanced adenylate cyclase/cyclic adenosine monophosphate production resulting in vasodilatation. Additionally, under pathological microenvironmental conditions, in addition to other prostanoids, prostacyclin can be generated by COX-2 isoform [17].

The Blood–Brain Barrier

Brain endothelial cells from capillaries are unique because they are not fenestrated and are sealed by tight junctions, features that underlie the BBB. The combined surface area of these microvessels constitutes the largest interface for blood–brain exchange by far. The surface area, depending on the anatomical region, gives a brain exchange total surrounding area between 12 and 18 m^2 for the average human adult [18].

Its particular characteristics provide a stable environment for neural function and synaptic activity by (1) controlling ion movements; (2) keeping separate pools for central and peripheral transmitters, minimizing cross-talk; (3) preventing the entrance of many circulating macromolecules; (4) protecting neurotoxic substance to reaching brain tissues; and (5) allowing passive transport for many essential nutrients and metabolites [18].

The peculiarity of BBB is given by the specific phenotype of the endothelial cells, with adherences junctions as cell–cell interaction stabilizers, and tight junctions (TJs) that reduce permeation. The latter limit the paracellular flow of water, ions, and larger molecules into the brain (gate function) from the blood plasma to the brain extracellular fluid, and organize the cell membrane in apical–basal domains (fence function). The extreme effectiveness of the TJs in the impediment if ion

movement results in the high in vivo electrical resistance observed for the BBB [18, 19].

The current statement is that under normal circumstances the tightness and transport properties of the BBB are relatively constant, which is in agreement with the need for a conserved extracellular milieu allowing normal central nervous system (CNS) functioning. However, many of the cell types associated with brain microvessels, including microglia and astrocytes and nerve terminals adjacent to the endothelial extracellular matrix/basal lamina release vasoactive agents and cytokines that can modify TJs assembly and barrier permeability [18, 19].

Role of Astrocytes

Direct contact between central neural terminals and vascular smooth muscle is infrequent and a process of extracellular diffusion of vasoactive agents released from active synapses in the brain parenchyma to local arterioles cannot account the temporally coordinated vascular changes that underlie CBF increases. In this sense, functional hyperemia cannot be explained solely by an interaction between neurons and local vessels, other cells must also be involved [10].

Gray matter astrocytes (protoplasmic astrocytes) have a polarized anatomical structure and entail two types of processes: fine perisynaptic processes that cover most synapses, providing optimal support for synaptic transmission; and large diameter vascular processes (end feet) that are near in the vicinity of the vessel wall, covering >99 % of the vascular surface facing endothelial cells or pericytes [10]. Furthermore, astrocytes can produce a great variety of vasoactive substances such as NO, ATP and cyclooxygenase, and epoxygenase activity derived products [20].

The first proposed mediator was potassium ions (K^+). As modest increases in extracellular K^+ concentration can hyperpolarize smooth muscle cells, it was postulated that K^+ released from active neurons depolarized astrocytes, leading to K^+ efflux from astrocyte end feet [3].

Nevertheless, evidence accumulated over the years has led to the proposal of a different main mechanism, taking into account Ca^{2+} signaling in astrocytes. These ion oscillations are known to be restricted to microdomains in the processes of individual astrocytes and are observed when synaptic activity occurs [20]. Furthermore, stimulation of neuronal afferents, direct mechanical stimulation of individual astrocytes and mGlu-receptor agonist were found to trigger temporally correlated Ca^{2+} elevations in astrocyte end-feet and dilation of cerebral arterioles [21]. Neuronal activity-dependent dilation of cerebral arterioles was reduced either when Ca^{2+} oscillations evoked in astrocytes by synaptic glutamate were inhibited using mGlu-receptor antagonists, or when COX inhibitors that block prostaglandin synthesis were used. These results are further validated as in vivo records of blood flow in the somatosensory cortex by laser Doppler flowmetry. The hyperemic response evoked by forepaw stimulation was markedly reduced after the systemic application of mGluR antagonists [21]. Additionally, subsequent experiments indicate that vasodilation of the microvasculature could be mediated, at least in part, by prostaglandin E_2 (PGE_2), because astrocytes in culture were observed to release this powerful dilating agent in a pulsatile manner according to the pattern of mGlu-receptor stimulation mediated Ca^{2+} oscillations [22].

A model is then proposed where astrocytes can encode different levels of neuronal activity into defined Ca^{2+} oscillation frequencies that, at the level of perivascular end feet, mediate the release of COX-derived vasoactive agents. The prostaglandins, mediating physiological vasodilation in response to a rise in astrocyte Ca^{2+}, are mainly produced by COX-1, which is expressed in astrocyte. Neuronal activity-dependent Ca^{2+} oscillations may ultimately represent the signaling system that allows blood flow to vary in a manner proportional to the intensity of neuronal activity [20] (Fig. 9.1).

However, in pathological conditions, expression of COX-2 is upregulated in astrocytes and this might also contribute to prostaglandin synthesis.

Considering this new information, it is now given to understand the role of the different isoforms of the enzymes that synthesize the vasoactive messengers [3].

Vascular Pathologies and Cognitive Impairment

Endothelial Dysfunction

The endothelium plays a central role in the regulation of vascular tone through timely and balanced production and release of endothelial relaxing factors, as well as endothelium-derived contracting autacoids, and also by detection of diverse physical, chemical, or mechanical stimuli. Moreover, healthy endothelial cells continuously adapt to local requirements and are essential for the maintenance of the entire vascular homeostasis involving antioxidant, anti-inflammatory, profibrinolytic, and anticoagulant effects, in addition to trophy effects in surrounding cells [23].

Endothelial dysfunction is primarily characterized by the loss of NO bioavailability and contingent with elevated levels of reactive oxygen species (ROS). These events have far-reaching implications, not only because the imbalance promotes smooth muscle cell activation, which leads to proliferation and migration; but also upregulates the endothelial expression of adhesion molecules, which induces immune cell recruitment to the vascular wall. Inflammation, in turn, enhances oxidative stress by upregulating the expression of free radical-producing enzymes and by downregulating antioxidant defenses. Finally, endothelial dysfunction is also characterized by increased production of endothelium-derived contracting factors. All these events are involved in an impaired regulation of vascular tone [23–26].

Oxidative Stress

Oxidative stress in the vasculature appears to be a common feature in diverse models of cerebral vascular disease and injury. This pathological state occurs as the result of an imbalance that favors the generation of ROS over its metabolism by various antioxidant defense mechanisms. There are multiple sources of the main ROS, superoxide, in the vasculature; including mitochondria, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, COX, and xanthine oxidase. Cerebral endothelial cells are metabolically active and the number of mitochondria present in cerebral endothelium is high, thus generating high levels of superoxide during oxidative phosphorylation. The effects of ROS are prominent in the cerebral circulation because cerebral blood vessels have the capacity to generate high levels of superoxide and are particularly sensitive to the effects of ROS. Low concentrations of ROS function as mediators and modulators of cell signaling. By contrast, higher levels of ROS commonly contribute to vascular disease. The overall effects of ROS depend on local concentrations, subcellular localization, and the proximity of ROS to other target molecules [7, 25].

Vascular Effects

Superoxide can have direct effects such as reacting with enzymes containing iron–sulphur centers resulting in release of free iron and subsequent formation of the highly reactive hydroxyl radical. Additionally, the formation of multiple other biochemical species such as superoxide radical, hydrogen peroxide, and reactive nitrogen species is dependent on production of superoxide [7, 25].

Besides responses that are dependent on endothelial cells, other vasodilator mechanisms are inhibited by ROS. Similarly to endothelium-dependent vasodilation, neurovascular coupling is also impaired by superoxide. Reductions in resting blood flow and impairment of vasodilator responses as a result of oxidative stress may result in a mismatch between energy requirements, substrate delivery, and clearance of cellular by-products [7, 25]. Furthermore, oxidative stress attenuates the growth factor support provided by endothelial cells to oligodendrocyte precursors. Loss of trophy support may impede the proliferation, migration, and differentiation of oligodendrocyte progenitor cells and compromise the repair of the damaged white matter [26].

Many studies have shown that ROS affect vascular structure or growth. ROS might produce hypertrophy by inactivation of NO that normally inhibits vascular growth, or through direct activation of signaling cascades involved in growth of vascular muscle including growth factors, kinases, and transcription factors. Such structural changes can have functional consequences because remodeling and hypertrophy also contribute to the shift in autoregulation by reducing the vascular lumen and increasing cerebrovascular resistance. Alterations in autoregulation increase the susceptibility of the brain to cerebral ischemia when blood pressure drops because cerebral blood vessels fail to compensate for the reduction in perfusion pressure [10].

Blood Brain Barrier: Alterations

Superoxide or other ROS increase permeability of the BBB. Dysfunction of endothelial cell-to-cell junctions, which disrupts the endothelial barrier and increases vascular permeability, appears to be also involved in the pathogenesis of vascular failure [24, 27]. As senescence animal show increased levels of oxidative stress, it has been proposed that ROS that are locally produced in brain parenchyma can trigger alterations of the BBB, possibly by cell death, gliosis, and signaling changes. In aged Wistar rats, which show impairment in short term-memory, leakage through the BBB was found to be associated with microglial activation, which are a known source of oxidative damage. Leakage of BBB can induce microglial activation by letting abnormal molecules pass into the brain parenchyma and in turn free radicals released from the microglia may further alter the BBB, in a vicious cycle leading to perivascular edema and axonal demyelination. Demyelination slows the transmission of nerve impulses and might contribute to the neural dysfunction. More importantly, in a rodent model of senescence, morphological changes of BBB and leakage of endogenous albumin and immunoglobulin G (IgG) to the brain parenchyma were selectively demonstrated for brain regions involved cognition, such as the hippocampus [19, 26].

Vascular Cognitive Impairment

In the past years the idea that the cerebrovascular dysfunction often precedes the onset of cognitive impairment has been extensively debated, taking into account that alterations that disrupt the homeostasis of the cerebral microenvironment could promote the neuronal dysfunction underlying the impairment in cognition. Structural and functional integrity of cerebral blood vessels is vital for the preservation of cognitive function. To a great extent, cognitive health depends on cerebrovascular health [11, 26, 28].

Vascular cognitive impairment (VCI) refers to the broad spectrum of cognitive deficits associated with cerebrovascular diseases. In 2011, The American Heart Association/American Stroke Association presented a statement to define and understand the rationale behind the use of the term VCI [29]. There has been significant growing terminology to characterize the cognitive syndrome associated with risk factors for cerebrovascular disease and its manifestations, especially in the description of dementia. In the past years the term vascular dementia (VaD) has been used, regardless of the pathogenesis of the vascular lesion—ischemic or hemorrhagic or single or multiple infarct (s). Moreover, cerebrovascular disease can affect multiple cognitive functions and vascular mild cognitive impairment (VaMCI) has been proposed as the "vascular" equivalent of the known mild cognitive impairment. In this context, VCI encompasses all the cognitive disorders associated with cerebrovascular disease, from frank dementia to mild cognitive deficits. Thus, VCI is a syndrome with evidence of clinical stroke or subclinical vascular brain injury and cognitive impairment affecting at least one cognitive domain, VaD being the most severe form of VCI [29].

Vascular-related structural pathology may produce so-called vascular dementia, including multiple infarcts, hemorrhagic events, focal infarcts, subcortical white matter disease or hemispheric ischemia, and hemorrhagic stroke [30–32]. Beyond the impact of these fixed structural lesions, hemodynamic dysfunction of CBF, which includes hypoperfusion and altered cerebral

autoregulation, may be independently associated with cognitive decline. Hemodynamic effects may occur at the level of large vessels at the neck or head, at a global level in the setting of cardiac failure, or intrinsically as a result of dysfunction of the endothelium in the microvasculature [33].

Currently, all diagnostic criteria to characterize cognitive syndromes associated with vascular disease should be based on two factors: demonstration of the presence of a cognitive disorder (dementia or VaMCI) by neuropsychological testing and history of clinical stroke or presence of vascular disease by neuroimaging that suggests a link between the cognitive disorder and vascular disease. Clinical studies have shown that subjects with VaMCI can present a broad cognitive impairment, which can also include memory deficits [29]. Patients with VaD show mild impairment of memory with impairments in executive function such as judgment. Those who have memory problems tend to have more difficulty with encoding new information, thereby limiting acquisition rather than the pattern of rapid forgetting seen in Alzheimer Disease (AD), which is related primarily to poor retention. In order to determine the relationship of cerebrovascular disease to the cognitive symptoms, it is critical to identify the presence of cortical or subcortical infarcts or other stroke lesions with neuroimaging, and these should be associated with clinical symptomatology. It may also be important to consider the source of the cardiac or vascular pathology that underlies the cerebrovascular disease associated with VCI to provide a more specific clinical pathologic relationship [29, 32, 34].

It is important to highlight that after AD, VaD is the second most common form of dementia, comprising 10–20 % of all dementias. Data suggest that the annual incidence of VaD may range from 20 to 40 per 100,000 in people between 60 and 69 years of age to 200–700 per 100,000 in people over 80 years of age, with prevalence rates doubling every 5 years. As with cerebral ischemic disease, the risk of VaD is somewhat higher for men than it is for women [32].

Additionally, there is a decreased survival rate for patients with VaD as compared with patients with AD, presumably related to underlying cardiovascular disease risk factors. Risk factors for VaD reflect the general risk factors associated with cerebral ischemia: age, hypertension, diabetes, obesity, cigarette smoking, hyperlipidemia, and cardiac disease (including coronary artery disease and cardiac arrhythmias) [32, 35]. Association of vascular risk factors with cognitive decline and dementia are probably mediated largely by cerebrovascular disease, which can have additive or synergistic effects with coexisting neurodegenerative lesions [36].

Brain Renin–Angiotensin System Modulates Vascular Events: Therapeutic Opportunities

The Renin–Angiotensin System

The classical effects of the renin–angiotensin system (RAS) were related to its endocrine role in the electrolytic homeostasis and control of the blood pressure. Angiotensin II (ANGII) acts by rapidly increasing vascular resistance and through long-term effects acting on vasculature, heart, kidney, sympathetic output, and the CNS, promoting vasopressin and aldosterone actions and regulating thirst and water intake [37–40].

Several receptors have been identified to be activated by ANGII, among them the AT_1 receptor (AT_1-R), which is the one mediating most of the ANGII physiological and pathological functions. This surface receptor belongs to the G-protein-coupled receptor family and has a seven transmembrane domain conformation [40–42]. AT_1-R activates multiple intracellular signaling pathways. Mainly, it promotes inositol trisphosphate (IP3) formation and Ca^{+2} releases from intracellular compartments, adenylcyclase inhibition, modulation of voltage-dependent Ca^{+} channels, or activation of phospholipase C (PLC). Secondary pathways involving mitogen-activated protein kinase (MAPK), extracellular-signal-regulated kinase (ERK) or c-jun N-terminal kinase (JNK) activation have also been described, this way participating in trophy events. Independent G-protein activation pathways involve later

desensitization and even internalization by endocytosis mediated by β-arrestins [40].

Over the years it has become apparent that a local autocrine or paracrine RAS may exist in a number of tissues, implying new roles for this system [43]. Moreover, the functions of the tissues and systemic activities show significant differences. Even though circulating ANGII levels may not be intensively high, AT_1-R expression in different organs is 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 [40]. In particular, brain microvessels have all of RAS components (precursors, enzymes, and receptors) indicating the presence of a local RAS [44]. This evidence suggests that cerebral circulation is particularly sensitive to ANGII. Cerebral blood vessels are exposed to ANGII from both circulating and local (formed locally within the vessel wall and brain) sources, which play an active role in cerebrovascular events. There is much evidence supporting the idea that most ANGII effects occur independently of the effects of ANGII on arterial pressure [45, 46].

Renin–Angiotensin System and Oxidative Stress

Activation of ANGII AT_1-R leads to activation of protein kinase C (PKC), which in turn phosphorylates p47 phox, leading to the assembly of the enzyme and ROS production. ANGII also increases vascular expression of components of NADPH oxidase and levels of superoxide. Contributions of ANGII to oxidative stress are due in large part to activation of NADPH oxidase. ANGII-induced endothelial dysfunction is prevented by inhibition or genetic deletion of AT_1-R, pharmacological scavengers of superoxide or genetic deletion of NADPH-oxidase isoform 2 (Nox 2) [7, 12]. Spontaneously hypertensive rats (SHR), an animal model for studying hypertension, display elevated levels of all of RAS components in brain microvessels and allow the evaluation of ANGII-action overexpression [44]. These animals present a role switch in cerebrovascular NOS isoenzymes, from physiologic and protective, to deleterious. While overproduction of NO by the inducible form (iNOS) promotes neurotoxicity, the diminished production of NO by the eNOS alters regional blood flow. The imbalance observed in NO roles is partly a consequence of AT_1-R activation, given that blockade of these receptors restored the normal proportions of NOS isoenzymes. The final result obtained is that eNOS activation is restored, and vasodilatation and reversed pathologic arterial remodeling is improved. Furthermore, iNOS inhibition reduces ANGII-induced ROS production, NO scavenging, cellular damage, and inflammation [47].

A key feature of oxidative stress is that once it is initiated, ROS or peroxynitrite can feed forward, promoting additional oxidative stress. RAS may be one of the mechanisms that contribute to these effects. Oxidized angiotensinogen is more readily cleaved than the non-oxidized form. Thus, in an oxidative environment, the oxidized form of angiotensinogen can be more prevalent. In this form, ANGII-induced oxidative stress may feed forward, promoting further production of ANGII [7].

RAS and Neurovascular Coupling

Evidence relating to ANGII and neurovascular coupling first indicated that ANGII does not affect the neural processes that generate the vasodilator response, but inhibit their vascular effects, resulting in attenuation of the vascular response associated with neural activation. Systemic administration of ANGII elevates medial arterial pressure (MAP) and attenuates the increase in CBF produced by whisker stimulation in the somatosensory cortex. Nevertheless, this ANGII-induced attenuation of functional hyperemia is independent of blood presssure elevation, as still persists even when the elevations in MAP are offset by removal of small amounts of arterial blood or if the systemic effects of ANGII are prevented by direct application of the peptide to the somatosensory cortex. However, the effects of systemic ANGII on functional hyperemia were not associated with attenuation of the field potentials evoked by whisker stimulation in the same area [46].

Consequently, much evidence suggests a role for ROS in this pathological role of ANGII. The attenuation of functional hyperemia induced by acute or chronic (7 days) administration of ANGII can be reversed by ROS scavengers, genetic deletion, or pharmacological intervention of NADPH-oxidase subunits. All of these manipulations also reverse ANGII-induced ROS production in these vessels. Moreover, it was found that NADPH-oxidase subunits and AT_1-R are present in the same endothelial cells and in close proximity [48]. In mice lacking the NADPH oxidase subunit Nox-2, nonselective NOS inhibition or selective inactivation of eNOS prevents the ANGII-induced extensive nitration of cerebral blood vessels. These findings provided new evidence suggesting that the effects of ANGII on CBF responses to acetylcholine and whisker stimulation also requires peroxynitrite, which is formed mainly from eNOS-derived NO and Nox-2-derived superoxide [11]. Endothelial function is greatly impaired in a genetic model of chronic ANGII-dependent hypertension via a mechanism that involves increased oxidative stress. The mechanism which accounts for this dysfunction involves superoxide, as treatment with polyethylene glycol superoxide dismutase (PEG-SOD) completely restored vascular responses [49].

Further studies have demonstrated that the cerebrovascular oxidative stress and the attenuated endothelium-dependent response induced by systemic administration of ANGII can be blocked by AT_1-R inhibition, free radical scavengers, NADPH oxidase peptide inhibitors, and is not observed in Nox-2-null mice [49].

The vascular dysregulation induced by ANGII also requires constitutive levels of COX-1-derived prostaglandin E receptor type 1 (PGE_2) acting on EP1 receptors in order to achieve NADPH oxidase-dependent ROS production. Based on the localization of COX-1 in microglia and of EP1 receptors in cerebral arterioles, PGE_2 could originate from microglia and act on vascular EP1 receptors to enable ANGII-induced vascular oxidative stress [50].

All of the above-mentioned findings support the concept that cerebrovascular oxidative stress mediates the powerful effects of ANGII on the cerebral circulation, which may contribute to the susceptibility to ischemic injury and dementia associated with hypertension [50].

RAS and Inflammation

Two key mechanisms appear to underlie the classical ANGII-induced inflammatory response: (1) generation of ROS and (2) production of nuclear transcription factor kappa-B (NF-κB) [17].

As previously assessed, ANGII is a powerful modulator of ROS production in endothelial cells and vascular smooth muscle cells. The ANGII-induced oxidant stress by AT_1-R activation is closely associated with elevation of agents such as VCAM-1, ICAM-1 and MCP-1 and thus initiation and progression of vascular inflammation [17]. In SHR, increased endothelial AT_1-R correlates with increased ICAM-1 expression, higher numbers of endothelium-adhering macrophages in cerebral microvessels and carotid artery, and an increased number of perivascular infiltrating macrophages in microvessels [51]. Chronic infusion of ANGII at the slow-pressor dose has been shown to cause significant increase of leukocyte adhesion on brain venules at the same time it promotes oxidative stress development [52, 53]. Moreover, systemic injection of ANGII in rats produces arteriolar leukocyte adhesion and increases P-selectin, E-selectin, ICAM-1 and VCAM-1 expression in arterioles and venules [53]. However, it is not entirely clear if enhanced ROS generation is the primary event in ANGII-mediated vascular dysfunction or if the oxidant stress and inflammatory response act synergistically [17].

ANGII, working via AT_1-R, has been shown to activate NF-κB in vascular tissue. NF-κB is the primary transcription factor responsible for regulating transcription level of pro-inflammatory genes in vascular tissue and has been shown to mediate the enhanced expression of Interleukin-6 (IL-6), VCAM-1, and MCP-1. Treatment with ANGII increases vascular expression of IL-6, tumor necrosis factor-alpha (TNF-α), and iNOS [54]. In the same direction the strong inflammatory response via the NF-κB

pathway, as well as other pro-inflammatory mediators such as TNF-α and IL-1, in cerebral microvessels of SHR is dependent on ANGII stimulation, given that its elevated levels can be suppressed by blockade of its AT_1-R. Many genes of the heat shock protein (HSP) family are upregulated in this animal model, such as HSP60, HSP70, and HSP90 in the microvessel endothelium, as confirmed by gene upregulation and protein overexpression [55].

Additionally, NF-κB activates gene encoding angiotensinogen, thus leading to a positive feedback loop and amplifying the ANGII-mediated inflammatory response. Furthermore, ROS has been shown to positively regulate NF-κB levels and therefore oxidant stress, NF-κB and inflammatory mediators may constitute a triumvirate responsible for ANGII/AT_1-R-mediated vascular dysfunction [17].

Microglia and astrocytes are the main resident immunomodulatory cells in the CNS and during neuroinflammation are stimulated by ANGII via the AT_1-R activation. In microglial cells, this initiates the production of transforming growth factor beta (TGF-β), whereas in astrocytes, ANGII mainly upregulates TSP-1, which in turn activates latent TGF-β. An increase in active TGF-β levels in the brain creates a permissive niche in the CNS, allowing T cells to obtain a more inflammatory phenotype. As a result of the multifunctional character of ANGII, this mechanism likely occurs synergistically, with the canonical NF-κB1 pathway and ROS production [56].

In conjunction, it is clear that ANGII can initiate inflammatory processes by increasing vascular permeability, recruiting inflammatory cells trough the regulation of adhesion molecules and chemokines by residents cells [57].

RAS and BBB Dysfunction

ANGII was shown to increase BBB permeability as observed with the Evans Blue extravasations assay in association with abnormally increased leukocyte adhesion and rolling. Oxidative stress may be the mediator in the cerebrovascular dysfunction in ANGII-infused mice, as treatment with Tempol corrected both events [58]. In vitro results indicated that ANGII modulates the BBB endothelial cells, involving the AT_1-R activation by altering both transcellular and paracellular permeability. The cellular mechanisms are not quite clear. However, they may involve PKC because ANGII stimulates PKC activity in these cells [59].

RAS effects in BBB may also implicate astrocyte activity as it was demonstrated that astrocyte-dependent angiotensins are crucial in the maintenance of BBB function. The astrocytes of angiotensinogen knockout mice had attenuated expression of glial fibrillary acidic protein and decreased laminin production in response to cold injury, and ultimately incomplete reconstitution of impaired BBB function [60].

RAS and Vascular Remodeling

Inward remodeling and vascular hypertrophy have been related with impaired vasodilator capacity. Inward remodeling represents a rearrangement of the vessel wall around a smaller lumen and has the greatest impact on lumen diameter. This vascular abnormality increases minimal resistance, resulting in reduced vasodilator responses, lower levels of perfusion pressure, and impaired blood flow in collateral-dependent regions [7]. Remodeling events cannot be attributed to altered distensibility of the vessel wall. Cerebral arterioles mice over-expressing angiotensinogen and renin undergo significant remodeling of the vessel wall. In contrast, cerebral arterioles in a genetic mice model of ANGII-independent hypertension, animals do not undergo remodeling. These findings provide strong support for the hypothesis that the RAS is a major determinant of vascular remodeling during chronic hypertension [61].

A non-pressor dose of ANGII was found to cause inward remodeling of cerebral arterioles in wild type (WT) mice that was prevented in Nox-2–/– mice. These results suggest that Nox-2-derived superoxide, independent of increase in

pressure, has an important role in ANGII-mediated inward remodeling. ANGII-induced superoxide production from Nox-2-containing NADPH oxidase has important role in inward remodeling, which is mechanistically different from that of hypertrophy [62]. Hyperactivation of vascular NADPH oxidase may lead to increased generation ROS, particularly O_{2-} and H_2O_2 that mediate many downstream signaling molecules, such as MAPK, protein tyrosine phosphatases, protein tyrosine kinases, and transcription factors. Activation of these signaling cascades is known to promote vascular smooth muscle cells growth and migration [63].

However, in animals submitted to a pressor dose of ANGII or in ANGII- independent hypertensive mice cerebral arterioles undergo hypertrophy, suggesting pressor effect may be mandatory for hypertrophy [60, 62].

RAS and Angiotensin Receptors Blockers Therapy

The initial pharmacological approaches to block ANGII actions were through peptide analogs of the molecule. Later, non-peptide and selective antagonist (ARBs) were synthesized from imidazol derivate that could be administered orally. The first of them, losartan, was approved for human use by the United States Food and Drug Administration in the mid 1990s. All of them show AT_1-R high affinity, 10,000 times more powerful than AT_2-R affinity. Affinity ranges are candesartan > irbesartan > telmisartan = valsartan > losartan. They are widely used in clinics as a part of anti-hypertensive treatment because they are tolerated well and have minimal secondary effects [64, 65].

Animal studies have shown that continuous increase of ANGII production could result in impairment of normal development of cognitive function and, consequently, cognitive decline. Moreover, administration of an ARB olmesartan ameliorated cognitive decline, with a reduction of blood pressure; whereas treatment with hydralazine did not, even with a similar decrease in blood pressure. Therefore, it is proposed that sustained decrease in oxidative stress by blockade of the AT_1-R could contribute to neural protection and an increase in CBF, resulting in the prevention of consequent cognitive decline [66]. Type 2 diabetic mice also showed improved performance after candesartan treatment [67]. Single and chronic candesartan treatment starting at 24 h after middle cerebral artery occlusion still results in a significant reduction of infarct size and improved neurologic outcome [68]. Moreover, RAS inhibition with the ARB olmesartan protects from cognitive deficits and BBB augmented permeability in Dahl Salt-Sensitive hypertensive rats [69]. In experimental deoxycorticosterone acetate (DOCA)-salt hypertensive animals the endothelial dysfunction and vascular dementia-like lesions can be prevented by chronic treatment with telmisartan [70].

Clinically, it has been reported that the addition of the ARB valsartan reduced 40 % in stroke onset compared with supplementary conventional treatment without ARB independent of blood pressure reduction. Furthermore, losartan has additional therapeutic effects on impaired cognitive function and restoration of CBF autoregulation beyond its antihypertensive effect, indicating a contribution of ARB to brain protection involving the prevention of cognitive impairment [71, 72].

Chronic treatment to SHR with telmisartan shifted the lower limit of CBF autoregulation toward lower pressure levels. The treatment also attenuated the superoxide levels in the aorta, carotids, and cerebral arteries and morphological analyses revealed hypertension-induced vascular morphology was reversed. Superoxide levels in the cerebral arteries were markedly inhibited in the hypertensive rats by telmisartan. Therefore, the attenuation of vascular superoxide production may at least partly improve the CBF autoregulation. The modulation of the cerebrovascular structure and superoxide generation, which was not mediated by blood pressure changes, may be a pleiotropic effect of ARB, and indicates the importance of the RAS in the cerebrovascular function [45]. Administration of olmesartan in high salt and

cholesterol fed mice increased mRNA expression of a neuroprotective factor, methyl methanesulfonate-sensitive 2 (MMS2), and attenuated the increase in superoxide anion production [73]. Administration of ARBs restores cerebrovascular autoregulatory mechanisms in hypertensive rats, facilitating vasodilatation when perfusion pressure is reduced. This protective effect is similar to that obtained after treatment with angiotensin converting enzime (ACE) inhibitors [74]. This effect is thought to be the result of a reversal of cerebrovascular remodeling and restoration of compliance and autoregulation, decreasing the loss of cerebral blood flow, and reducing the area of neuronal necrosis [75].

Widespread anti-inflammatory effects of ANGII AT_1-R blockade have been shown to occur in the periphery and brain. ARBs reverse the cerebrovascular inflammation characteristic of genetic hypertension in SHR [51, 55]. The AT_1-R blockade can significantly reduce the inflammation-induced mRNA expression of proinflammatory cytokines and their receptors, as well as prevent the inflammation-induced activation of transcription factors regulating expression of multiple inflammatory genes, including c-Fos and FosB, members of the activator protein-1 (AP-1) transcription family, and NF-κB [52, 76]. Treatment of animals in a model of sclerosis with AT_1-R inhibitors delays the onset and ameliorates brain inflammation by influencing neurons, astrocytes, and microglia to downregulate TGF-β and TSP-1, which are normally upregulated early during inflammation [56]. In the cerebrovascular endothelium of SHR, the normalization of pathologic arterial remodeling, eNOS and ICAM-1 expression, and macrophage adherence and infiltration are achieved by treatment with an AT_1-R antagonist [77]. Moreover, the ACE inhibitor Enalapril has similar results because it attenuates ANGII-induced and vascular inflammation [78]. The effects of ACE inhibitors in humans are similar to those in hypertensive animals, reducing hypertension while protecting cerebral blood flow and reducing ischemia. Furthermore AT_1-R blockade prevents ANGII-induced pathological increase in BBB permeability [59]. The property of ARBs to prevent BBB breakdown may be an important factor in their reduction of cerebrovascular inflammation [79].

Risk Factors

Stress

Stress is described as a physiological and/or psychological response to a potentially harmful stimulus. Controversial evidences over the years has led to the understanding that low concentrations of endogenous glucocorticoids are permissive of immune enhancement, while higher concentrations may inhibit the immune system. The hypothesis that repeated episodes of acute stress or chronic stress can promote vascular alterations is supported by evidence that various psychosocial stressors, including personality, job stress, and social isolation can be risk factors for the development of atherosclerosis. There is also enough evidence to suggest that chronic psychosocial stress is a specific risk factor for the acceleration of coronary artery disease [80]. The elevations in blood pressure via CNS activation, the increase in the levels of corticosteroids and catecholamines, as well as the subsequent increase in the levels of proinflammatory cytokines during the stress response are responsible for mediating induction of adhesion molecules, recruitment of leukocytes to the vessel wall, and endothelial damage. In chronic and recurrent stress, this cascade of events can lead to vascular inflammation and subsequent development of atherosclerotic plaques [81]. Pronounced effects of chronic stress have been shown in the vasculature, proved as an augmentation of post-angioplasty neointimal formation and vessel occlusion in rats without metabolic abnormalities. Exposure to cold stress caused rapid development of an occlusive neointimal atherosclerotic-like plaque, thrombotic and inflammatory lesions in carotid arteries after angioplasty [82].

The initial observations showing increased plasma renin activity and increased levels of circulating ANGII in animals and humans exposed to

various stressors date back to the 1970s. It is well established that RAS is activated under stressful situation and that it plays an active role in the subsequent response [83]. ANGII, functioning as a major stress hormone, serves to enhance both the innate and adaptive immune responses in preparation for imminent immune challenges that can occur with experience of the stressor [80]. Reversal of stress-induced inflammation in the gastric mucosa by AT_1-R antagonists suggests common pathogenic mechanisms for ischemic and stress-related disorders and similar therapeutic advantages of treatment with AT_1-R antagonists. SHR exposed to cold-restraint stress exhibit reduced gastric blood flow and acute gastric mucosal lesions, with increased expression of ICAM-1 in gastric arterial endothelium, neutrophil production of TNF- α, and neutrophil infiltration of the gastric mucosa. These effects were diminished when SHR were pretreated with the AT_1-R antagonist candesartan by a combination of gastric blood flow protection, decreased sympathoadrenal activation, anti-inflammatory effects with reduction in TNF-α, and ICAM-1 expression, leading to reduced neutrophil infiltration while maintaining the protective glucocorticoid effects and PGE_2 release [84]. Given the proinflammatory activity of the RAS and the evident increase in RAS activity during the stress response, there may be a link between recurrent or chronic stress, recurrent or chronic activation of the RAS, and vascular dysfunction [80]. Early life stress (i.e., maternal separation) increased ANGII sensitivity in adult male rats. These animals showed sensitized response to ANGII-dependent hypertension, increased heart rate, and vascular inflammation, evaluated by an increase in inflammatory cell numbers, identified by CD68 and CD3 immunostaining, in the aortic endothelium and perivascular adventitia [85]. Considering the broad range of actions described for ANGII , there are several mechanisms that could underlie RAS-mediated vascular pathogenesis during stress. For instance, recurrent or chronic activation of immune, endothelial, and vascular smooth muscle cells by direct stimulation of AT_1-R may predispose the vasculature to inappropriate inflammatory events. Other possible factors involved could be positively led back to other components of the stress response, recurrent or chronic increases in blood pressure, or blood pressure fluctuations [80].

Psychostimulants

Psychostimulants have long been described as cathecholaminergic agonists. Compounds such as cocaine, amphetamine, and methamphetamine are the most widely-used psychostimulants by the population. The classical recognized effects of these drugs are increased heart rate and blood pressure in conjunction with behavioral alterations such as wakefulness, better outcome of executive tasks, and euphoria. Moreover, the effects of these drugs may implicate other physiological consequences given that several reports have indicated vascular alterations in psychostimulant users [86, 87].

Recreational drug users have a higher risk of vascular complications ranging from vasospasm, not related to vasculitis occlusive and hemorrhagic strokes, and vasculitis. Vasculitis refers to inflammation of blood vessels with or without vessel-wall necrosis. This phenomenon in the CNS has been identified as a secondary event after sympathomimetic drug use, probably by platelet and coagulation factor abnormalities, accelerated atherosclerosis, or foreign body embolism [88].

Several doses of cocaine can sensitize the arterial and venous segments to norepinephrine; however, the sensitized response is not evidenciated in small vessels. Thus, the vascular supersensitivity to norepinephrine after cocaine use is not a uniform phenomenon but is restricted to certain vascular segments [89]. Clinical reported cerebral vasculitis after cocaine abuse was objectified by lymphocyte in perivascular collections and within the walls of several small arteries, foci of interstitial edema in the immediate vicinity of small arteries, enlarged perivascular spaces with proteinaceous material, and endothelial swelling in some arterioles. Neurological complications of cocaine use include cerebral and spinal cord infarction, transient cerebral ischemia, partial and generalized seizure, and intracranial and

subarachnoid hemorrhage [90]. Drug-associated cerebral vasculitis, as well as multiorgan necrotizing vasculitis, has been best documented with amphetamine abuse following single or repeated exposure. Studies in animals have revealed immediate angiographic changes after exposure to amphetamine. Amphetamine-affected vessels display fibrinoid anginitis, necrosis of the media and intima layers, leukocytic infiltration, intimal proliferation, and direct vessel damage with platelet aggregations and increased vascular permeability [91, 92].

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Prevention of Stress-Induced Cognitive Impairment: Today and Tomorrow

10

Emil Trofimiuk and Jan J. Braszko

It's not stress that kills us, it is our reaction to it.

Hans Selye

Introduction

It is well recognized that prolonged, particularly inescapable stress, is an important risk factor for the development of several cognitive dysfunctions. Chronic stress exposure incurs profound physiological, structural, and molecular changes in the brain. Alterations occur in brain structures such as the hippocampus, prefrontal cortex (PFC), and amygdala are responsible for the observed cognitive impairments (i.e., learning, memory, recall). In propagation of the negative effects of stress in brain neuroendocrine alterations appear to be heavily engaged. Glucocorticosteroids (GCs) play an important role in a quick, nongenomic manner, and then slowly, through their receptors. Stress-evoked variations include a deluge of neuroendocrine events, such as the hypothalamic–pituitary–adrenal (HPA) axis overexpression and excessive release of hypothalamic corticotrophin-releasing hormone (CRH), which consecutively excrete adrenocorticotrophin-releasing hormone (ACTH), then massive secretion of GCs into the blood circulation [1, 2]. Consequently, they evoke a number of negative neurochemical and neuroanatomical changes in the brain [3–5]. Accordingly, neurogenesis is limited and dendritic remodeling occurs in hippocampal areas CA1 and CA3 [6–8] in the medial prefrontal cortex (mPFC) [9, 10] and amygdala [11]. The neuronal atrophic remodeling results [5, 12] in a reduction of volume and decrease in total number of neurons and their branches [13] which in turn results not only in cognitive function impairment, but also in emotional disturbance and some autoregulatory mechanisms of neuroendocrine and autonomic functions [14]. Goldwater et al. [15] found that stress-evoked apical dendritic retraction and spine loss in the infralimbic region of the rat mPFC concurred with the receptor-mediated inhibition of catecholamine-stimulated synaptic plasticity. Post-stress recovery did not reverse distal dendritic retraction, but it did result in overextension of proximal dendritic arbors and spine growth, in addition to a full reversal of chronic restraint stress-induced impairments of catecholamine-mediated synaptic plasticity [15].

As a result of prolonged exposure to stress there is a dwindling of compensatory mechanisms of the organism. It is widely accepted that prolonged administration of the exogenous GCs can mimic effects of the stress HPA axis overactivation. Although these effects appear similar there are, however, significant differences. For example, in one of our studies [16], chronic

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corticosterone-induced impairment of spatial memory in the Barnes maze was effectively abolished by *Ginkgo biloba* extract while those caused by chronic stress was not. On the other hand, Morales-Medina et al. [17] showed that, similar to stress, 3-week corticosterone administration results in dendritic remodeling and spine density rearrangement in the rat basolateral amygdala (BLA) and pyramidal neurons of the CA1 area of the hippocampus as well as in spiny medium neurons of nucleus accumbens. Prolonged administration of corticosterone also reduced total dendritic length specific to pyramidal CA1 hippocampal neurons and decreased dendritic branching.

Over the last few decades, animal research has helped to clarify the mechanisms that underlie stress-induced impairments, revealing a complex relation between neurotransmitter signalling and hormone actions.

Stress and Classic Neurotransmitters

Stress and Serotonin

Stress-evoked disturbances occurring in the central serotonergic system are well known. Serotonin (5-HT), via 5-HT1A and 5-HT2A/2C receptors [18], modulates the HPA axis response to stress [19] in that serotonergic input stimulates CRH release, which in turn plays a fundamental role in regulating behavioral and neuroendocrine responses to stress [20] and ACTH secretion [21]. Also, there are data showing participation of 5-HT7 receptors in HPA axis regulation [22]. Therefore, the 5-HT1A/5-HT7 receptor agonist (8-OH-DPAT) may elicit neuroendocrine responses which, in the case of corticosterone secretion, are not completely inhibited after the 5-HT1A receptor antagonist administration [23]. Furthermore, there is ample evidence of the modulation of brain 5-HT receptors by chronic stress as well chronic GC administration [4, 24, 25], which further supports 5-HT receptors involvement in HPA axis functioning. Stimulation of hippocampal 5-HT1A receptors could increase hippocampal expression of the GC receptors [26], whose role is to act as a go-between GC feed-back via inhibitory pathway to the hypothalamic paraventricular nuclei (PVN) in which CRH release takes place [27]. Chronic stress disrupts cross-talk between the 5-HT1A receptors and the HPA axis elements via desensitization of the presynaptic 5-HT1A autoreceptors [28]. Decrease of hippocampal postsynaptic 5-HT1A receptor number in response to prolonged chronic stress has also been shown [29]. Furthermore, it was found clinically that 5-HT reuptake inhibitors (SSRI) may desensitize HPA axis hyperreactivity and the associated psychopathologies in stress-related disorders [30].

Stress and Dopamine

Stressors and stress response also applies to the mesoaccumbens dopaminergic system which is involved, in a complex manner, in propagation of the body's answer to stress arousal and likely plays a major role in the adaptive and maladaptive responses to it. Stress stimuli cause changes in dopaminergic transmission, but the direction of these changes (increase or decrease) strictly depends on the nature and character of stressor [31]. Stressors such as restraint are known to evoke emotional changes such as anxiety and have been associated with appreciably increased dopaminergic neurotransmission [32]. In turn, inescapable mild stress (i.e., chronic cold stress) causes behavioral signs related to depression and switching down dopaminergic activity [33, 34].

It appears that not only 5-HT has a direct link with the stress response and is involved in activation of HPA axis. Hensleigh and Pritchard [35] revealed that GC receptors were present in 47 % of dopaminergic neurons in both substantia nigra and ventral tegmental area (VTA). This may suggest that stress-induced GC release could induce functional alterations in the mesolimbic dopamine (DA) circuitry via direct modulation of GC receptors on dopaminergic neurons [35]. However, there is also evidence of an opposite

direction of this pathway: DA in response to severe stress, acting through D1 and D2 receptors, causes the activation of the HPA axis [36]. Although the sequence of events in those processes requires further research, it appears certain that there is a close relationship between DA and GC signalling.

From the perspective of behavioral changes induced by exposure to chronic stress, particular attention should be paid to cognitive impairment. It is known that DA exerts its inverted U-shaped effect on working memory performance through its actions on the D1 receptor family (D1 and D5). Namely, both excessive excitation as well as a block of D1 receptors may cause distinct working memory impairments [37, 38]. Administration of D1 receptor antagonist alleviates working memory deficits induced by excessive activation of D1 receptor seen after exposure to stress [39, 40]. Exposure to a strong stressor causes excessive HPA axis activation and leads to an arousal of the VTA, then massive DA release into the PFC [39], and consequently to memory disturbance.

Pickel et al. [41] showed that D1 receptors colocalize with glutamate receptors on dendritic spines, making them strategically positioned to modulate incoming excitatory signalling. In turn, via its D1 and D2 receptors, DA works in the PFC to modulate postsynaptic neuronal responses to glutamatergic input [42].

Stress and Noradrenaline

Investigations into the downstream stress mechanisms indicate critical roles for the DA D1, and noradrenergic alpha1 and alpha2 receptors in working memory modulation [9]. Similarly to DA, noradrenaline (NA) has an inverted U-shaped influence on working memory, whereby either too little or too much NA attenuates PFC function [43]. Matters are not so obvious in the case of NA because NA activates different types of receptors with varying levels of its release. It is known that NA has the highest affinity for alpha2-adrenergic receptors, then much lower for alpha1-, and beta-adrenergic receptors. Normal agitation, without stress, causes release of NA at the level that activates alpha-2A receptors and improves working memory [43]. In response to the strong stressor, NA activates lower-affinity receptors: alpha1 and beta1 and in turn impairs function of the PFC resulting in a decrease of working memory performance [44, 45]. This was biochemically confirmed at the beginning of the last century and has since become known as the Yerkes-Dodson law [46]. Therefore, decreased levels of NA [43] or blockade of alpha2A receptors in the PFC [47, 48] both cause working memory decline, while stimulation of postsynaptic alpha2A receptors has a stimulatory effect [43, 49]. In the case of alpha1 receptors it was demonstrated that their activation in the PFC causes fast inhibition of neuronal arousal and impairment of spatial working memory performance [50]. Moreover, an antagonism at alpha1 receptors effectively abolishes stress-evoked memory impairments [44]. This was also confirmed clinically; the alpha1 receptor antagonist prazosin was found helpful in the treatment of cognitive impairment in posttraumatic stress disorder (PTSD) [51]. Similar results were obtained after injecting a beta-receptor blocker and an alpha2 agonist to the BLA, a key structure important for the response to stress and aversive memory formation [52]. The use of beta-blocker, propranolol, as well as administration of alpha2 agonist, clonidine, resulted in a decline of arousal induced by NA in the BLA supporting a bidirectional model of NA modulation of stress response. There is also evidence to suggest that propranolol does not block stress-induced amnesia [53]. In another study, conducted in humans, beta-blocker prevented stress-induced impairment of cognitive functions [54].

DA and NA appear to play somewhat different functions in receptor activation because activation of alpha2A receptors enhances reactivity to environmental stimuli [55], while activation of the D1 receptor evokes decreased reactivity to these stimuli [56].

In summary, stress-evoked impairments in PFC associated with the decline of cognitive

functions can be decreased by blocking either D1 or alpha1 receptors. These findings are consistent with the synergistic interaction between DA and NA, and both might augment the effects of GCs on the PFC. Exposure to stress induces HPA axis response resulting in the release of GCs, and the same also applies to the release of catecholamines. Through D1 and alpha1 receptor signalling, neuronal activity in the PFC is inhibited and information crucial to correct task performance might be lacking. This disturbed PFC function can lead to a prolonged GC release, then exacerbation of working memory impairments because GCs have the potential to impair cognition per se. Those effects of GCs are rapid and take place in a nongenomic manner. It is possible that GCs exaggerate catecholamine actions in the PFC by blocking the extraneuronal catecholamine transporters in the glia that clear the extrasynaptic space of catecholamines [57].

Additionally, an adverse influence of stress on the levels of nerve growth factor and brain-derived neurotrophic factor (BDNF) [58, 59] was observed. Accordingly, BDNF signalling is negatively regulated by GCs, which results in an impairment of synaptic plasticity in the brain through negative regulation of spine density, neurogenesis, and long-term potentiation (LTP) [60].

Stress and Cognition

Over the past few decades, animal research has helped to elucidate the mechanisms that underlie stress- and GCs-induced impairments, revealing a complex interaction between neurotransmitter processing and hormone actions. From an ethological viewpoint, loss of complex processing may have once allowed more primitive behaviors to take priority in order to aid survival. Now, however, in everyday life we experience non-life-threatening stressors which can activate the same pathways, evoking dispersion of thought, decreased concentration and divided attention, incorrect assessment of incoming information, and misinterpretation of a given situation. All these cause disorganization to daily life and—in extreme cases—lead to disease.

All the changes described above cause cognitive decline, particularly deficits of spatial working memory. Spatial working memory was first defined by Honig [61] as information that the animal needs to remember in order to successfully perform on a single task trial. The information is critical for one trial and one trial only, and the animal should actively forget the information lest it interferes with performance of subsequent trials.

Exposure to strong emotions or stressors causes HPA axis activation and stimulates the adrenal cortex to a massive release of GCs which activate glucocorticoid receptors in the brain [62]. In moderation, this activation is essential for an enhancement of acquisition of information associated with the event. Positive effects of GCs activation on memory consolidation critically engage the BDNF pathway [63], but the same GC activation in the PFC may cause cognitive impairment [64]. Similar effects could be observed after administration of exogenous GCs which results in a significant impairment of working memory in rats [65]. These findings suggest that GCs can impair PFC function through direct actions at the GC receptors. GCs may also exert their effects by nongenomic actions and indirectly worsen cognitive functions by interactions with catecholamine receptor function [65].

The negative impact of chronic stress on cognition prompted several laboratories to look for effective ways of restoring the lost, and protect the remaining, cognitive abilities after stress. One should remember that an undue attempt to "repair" cognitive processes may further their deterioration instead of improving them. In view of the fact that we all experience stress which accompanies us almost every day, any drug to be used for protection against its negative impact must be free of serious side effects and be relatively inexpensive.

Old and New Drugs to Ameliorate Stress-Induced Cognitive Impairment

Benzodiazepines

Benzodiazepines are widely prescribed in the treatment of anxiety disorders, but they also indirectly affect GABAergic neurons, which in turn act on hypothalamic nuclei of the HPA axis [66, 67]. It has been demonstrated that benzodiazepines may normalize increased levels of GCs caused by exposure to stress or other factors [67, 68]. It was found in the 1980s that stress, dependably on its nature and duration, influences the number and distribution of benzodiazepine receptors in the central nervous system and periphery [69]. Some authors showed that benzodiazepine pre-treatment (clonazepam, lorazepam) abolishes stress-evoked decrease of monoamine oxidase (MAO) activity in rats [70]. It was found that endogenous benzodiazepine/GABAA system, which attenuates memory by affecting the amygdala, septum, and hippocampus can also be co-activated in response to anxiety and/or exposure to stressors [71]. In naïve rats different forms of learning cause a fast reduction of benzodiazepine-like immunoreactivity in the septum, amygdala, and hippocampus, the same effect being seen after pharmacological manipulation by microinjections of the benzodiazepine antagonist flumazenil into these regions at the time that consolidation is taking place [72, 73]. In view of the above data it appears that the use of benzodiazepines can alleviate somatic symptoms of exposure to stress and those caused by anxiety. Otherwise, their impact on the cognitive functions is unambiguously negative; the only exception might be PTSD. In addition, the side effects associated with long-term use of these drugs practically eliminate them as a potential treatment of the stress-related cognitive impairment.

Antidepressants

It has been found that exposure to repeated, prolonged stress for 3 weeks in rodents evoked changes in the hippocampus, amygdala, and prefrontal cortex, including suppression of 5-HT1A receptor binding and atrophy of dendrites, as well as impairment of learning and memory. Due to the fact that stress causes increase of 5-HT release and 5-HT mediate certain effects of stress on nerve cells, a number of researchers began to pay more attention to antidepressants in this context. Tianeptine, an atypical tricyclic antidepressant, is known to enhance serotonin uptake and its administration prevented the stress-induced atrophy of dendrites of hippocampal neurons [74, 75] and normalized disrupted glutamatergic neurotransmission [76]. Numerous data suggest that prolonged stress may enhance glutamatergic activity through post-synaptic mechanisms by regulating *N*-Methyl-D-aspartate (NMDA) receptor complex, increase of expression of the obligatory GluN1 subunit, as well as of the accessory subunits GluN2A and GluN2B at transcriptional and translational levels, particularly in the ventral hippocampus [77] and that antidepressants (i.e., tianeptine, duloxetine) may in part normalize these changes [77, 78]. Tianeptine treatment also prevented the stress-induced cognitive impairments as assessed in various behavioral tasks [53, 78, 79]. The side effects of tianeptine may not be severe, but are still troublesome; most often headache, dizziness, insomnia/nightmares, drowsiness, dry mouth, constipation, abdominal pain, weight gain, agitation, anxiety/irritability, blurred visions, palpitations, hot flushes, and myalgia occur [80].

Studies performed on animals have shown that selective serotonin reuptake inhibitors (fluoxetine, reboxetine, escitalopram) could support neurogenesis in the hippocampus decreased by chronic stress [81, 82], particularly via regulation of BDNF and cyclic adenosine monophosphate (cAMP), resulting in long-term positive effects on brain function [83]. The novel antidepressant, agomelatine, which acts as a melatoninergic (MT1/MT2) receptor agonist and serotonergic 5-HT2C receptor antagonist also showed beneficial effect on neurogenesis inhibited by stress [84]. Most studies using antidepressants for relieving stress-induced changes evaluate them at the biochemical and cellular levels, with no reference to their neurotransmitter effects related to cognition so we do not know how their latter

action contributes to the final anti-stress effect. It has been shown that fluoxetine reversed effects of the exposure to chronic mild stress in that it normalized insulin-like growth factor-1 receptor protein levels in the hippocampus as well as restored decreased extracellular signal-regulated kinase but did not affect spatial memory impaired by stress, assessed in the Morris water maze [85]. Similar results were obtained with reboxetine [86]. It has also been shown that paroxetine partially reversed impairment of recognition memory evoked by chronic mild stress, but failed to prevent the increased immobility in the forced swimming test [87]. In another study, amitriptyline and olanzapine (atypical neuroleptic) also partly abolished the negative impact of stress on the visuospatial memory [88]. There are, however, studies showing the negative effect of paroxetine, imipramine, and some other antidepressant drugs on cognitive processes impaired by chronic stress [89]. In general, antidepressants appear to be effective in the treatment of cognitive impairments associated with exposure to chronic stress but their use for prevention does not seem, at present, fully justfied.

Anticonvulsants

The anticonvulsant agent, phenytoin, was found to relieve detrimental effects of chronic stress on cognitive processes [74, 90]. Corticosterone- and stress-induced atrophy of neuronal dendrites in the hippocampus is blocked by phenytoin, which is an inhibitor of excitatory amino acids release and action [74]. It was suggested that 5-HT released by stress or corticosterone may interact pre- or post-synaptically with glutamate released by stress or corticosterone, and that the final common pathway may involve integrated effects of serotonin and glutamate receptors on the dendrites of CA3 neurons innervated by mossy fibers from the dentate gyrus of hippocampus. GC overload is associated with an impairment of declarative memory and with adverse structural changes in the hippocampus. In animals, phenytoin blocks the effects of stress and GCs on memory and hippocampal morphology [74, 91].

Brown et al. [92] demonstrated that the administration of levatiracetam attenuates declarative memory deficits and has neuroprotective properties in patients with bronchial asthma receiving GC therapy for a prolonged period of time. In another study, also performed on patients with asthma, phenytoin did not counterbalance the negative effects of GCs on declarative memory [93]. From the group of anticonvulsants with mood-stabilizing properties, topiramate relieved symptoms of extreme forms of stress, such as PTSD in a study performed on humans [94]. In that study, however, the effect of topiramate on cognitive functioning was not evaluated. Thus, it seems that at least some antiepileptics have potentially beneficial effects on cognitive impairment caused by stress, but because of the profile of action and several side effects, their use should be rather limited to treatment, not for prevention. However, this requires further detailed studies.

Sartans

For some time there has been a possibility of taking advantage of the additional effects of drugs that are already used for their specific indications but in addition they appear to ameliorate some negative effects of the exposure to chronic stress. An example of such drugs with recently discovered anti-stress effects are AT1 angiotensin receptor blockers (ARBs) known also as sartans, widely used in the treatment of hypertension. Exposure to chronic stress is a well-recognized factor inducing hypertension [95]. Angiotensin II (Ang II) and its receptors AT1 and AT2 localized on stress-responsive brain areas including the HPA axis are involved in the propagation of the body's response to stress [96, 97]. It has been revealed that candesartan is able to prevent a wide range of the stress-evoked pathological changes including gastric ulceration, adverse reorganization of the cerebrovascular structure and function, and HPA axis overactiation, independent of its antihypertensive effect [98, 99]. In addition, there are anxiolytic properties of losartan administered to the brain of animals undergoing exposure to acute stress. It was recently found

that the AT1 receptor blockade in the amygdala critically contributes to its anxiolytic action [100]. It is known that during exposure to stress stimuli, Ang II-dependent behavioral changes may be partially mediated via benzodiazepine receptors (especially a part of the GABAA receptor complex) and the candesartan pretreatment completely inhibited stress-evoked reduction in cortical benzodiazepine receptor binding and expression [101]. Therefore, restoration of the benzodiazepine receptor binding and inhibitory influence of the GABAA receptor complex may, at least in part, explain anxiolytic effects of ARBs.

The beneficial influence of sartans on the cognitive processes impaired by stress in animal models, and the possibility of prevention of the stress-induced cognitive impairments by threshold nonhypotensive doses of candesartan and telmisartan have recently been described [102, 103]. Also, candesartan prevented the stress-evoked AT2 receptor binding decrease in the ventrolateral thalamic nucleus and it is known that AT1 blockade in the locus coeruleus and adrenal medulla abolishes the stress-induced activation of tyrosine hydroxylase and decreases its mRNA level [104]. Moreover, candesartan administered orally efficiently diminished activity of AT1 receptors (overexpressed by stress exposure, [105]) and at the same time increased selectively activation of AT2 receptor in the brain [104]. It is known that stress stimuli (immobilization/restraint, cold, predator exposure, isolation, immunological) activate the renin-angiotensin system and Ang II release [97]. The effects of arousal of central Ang II systems in conjunction with the increased AT1 receptor expression, results in the activation of HPA axis, CRH increase [98, 105] and then ACTH release stimulating GCs synthesis and release into the systemic circulation [106]. In view of the above data, part of the beneficial effects of ARBs on cognitive processes may result from their direct interaction with GCs. The use of ARBs may be an excellent treatment option in patients with stress-induced hypertension associated with cognitive impairment. However, the use of sartans in normotensive patients for the prevention of cognitive decline evoked by stress may not be a valuable therapeutic option.

While the use of all the above-mentioned drugs for the treatment of memory disturbance associated with pathologies such as depression, PTSD, and hypertension appears justified, their preventive administration, taking into account the risk-benefit ratio, should not be recommended. Therefore a more secure prevention modality appears to be desirable. Formulations of natural origin containing safe natural products (e.g., medicinal plants) can be a useful alternative.

Ginkgo Biloba

EGB 761 (*Ginkgo biloba* extract) seems to be a good candidate as a drug preventing stress-related cognitive impairment [107]. In contrast to synthetic agents, with their mechanisms of action described in detail, the plant formulations have a number of natural active substances which probably contribute to the final effect. The cellular mechanisms underlying multiple effects of *G. biloba* can be attributed to the different constituents of the extract, which may act independently or in a synergistic manner. Understanding how EGB 761 can alleviate stress-induced memory impairments and how its individual components alter response to stress requires a closer look. Numerous studies have shown that *G. biloba* has neuroprotective effects [108–110] which include up-regulation of mitochondrial ND1 gene expression crucial in meeting the high-energy demands of neurons [111]. In addition, it was shown that bilobalide (one of the major active compound of *G. biloba*) exerted strong neuroprotective activity in an animal model of Alzeimer disease and protected against learning and memory impairments induced by amyloid beta via attenuation the neuronal damage and apoptosis in the PFC and hippocampus. [112]. Biological effects of *G. biloba* were found to be somewhat similar to those exerted by tyramine [113] and they include an increase in the density of muscarinic cholinergic, beta-adrenergic, and thyrotropin-releasing hormone receptors. Futhermore, it has been found that *G. biloba* extract has the potency to increase the rate of

acetylcholine (ACh) turnover and augment the binding activity of ligands to muscarinic receptors in the hippocampus and also to increase dose-dependent dopaminergic transmission and ACh release in mPFC [107, 114]. Chronic administration of EGB 761 inhibits stress-induced HPA axis overactivation that results in corticosterone hypersecretion (over 50 % decrease of GCs levels) which is done by reduction of the number of adrenal peripheral benzodiazepine receptor sites and suppression of their genes [115, 116]. It has been shown that components of EGB 761 exert specific effects on adrenocortical cells by inhibiting peripheral benzodiazepine receptor sites mRNA and protein expression, thus limiting the amount of mitochondrial cholesterol available for GCs synthesis [116]. Moreover, repeated treatment with EGB 761 and ginkgolide B reduced the ACTH-stimulated corticosteroid production without affecting basal GCs and aldosterone formation [117].

Available data show that impairments of spatial and non-spatial hippocampus-dependent memory after chronic stress or chronic administration of "equivalent" (to that observed in stress) doses of exogenous corticosterone can be prevented by EGB 761. The extract used simultaneously not only normalized stress- and corticosterone-evoked cognitive decline, but showed nootropic properties on its own [118, 119]. Similar beneficial results of *G. biloba* in an animal stress model were obtained for reference and working memory [16, 120]. Use of *G. biloba* preparations appears rather safe, but a certain amount of caution should be maintained in people on anticoagulants. Fortunately, a recently performed meta-analysis showed no increased risk of bleeding during the use of *G. biloba* extracts [121]. In view of all these data, it appears that *G. biloba* may constitute a real, effective, and foolproof alternative to antidepressants or anticonvulsants in the treatment of cognitive impairment caused by stress.

Hypercium Perforatum

Another plant deserving particular attention in analyzing stress and cognitive disorders is *Hypericum perforatum*—a well-known natural antidepressant. Administration of *H. perforatum* to naïve animals exerts certain nootropic effects [122–126]. Recent neuroendocrine studies suggest that *H. perforatum* is able to regulate genes that control functioning of the HPA axis [127], normalizing increased release of ACTH and restoring normocortisolemia [57] that may partially explain removal of the deleterious effect of stress on cognitive functions. In our earlier studies [124–126, 128, 129], preventive effects of the treatment with *H. perforatum* against negative effects of chronic administration of exogenous corticosterone (dose "equivalent" to the stress) on a number of cognitive functions were thoroughly observed.

The mechanisms of action of *Hypericum* extracts are not fully understood. It contains many active constituents that may contribute to its final pharmacological effect. *H. perforatum* (particularly hypericin) reduces DA levels and decreases its turnover in PFC [130]. Furthermore, the other components of the plant (amentoflavone and pseudohypericin) display a high affinity for D1, D2, and D4 dopaminergic receptors [130, 131]. It has been demonstrated that normal functioning of working memory depends on the optimal level of DA in mPFC [132, 133]. Adverse influence of stress on the dopaminergic system in the brain is well known [132, 134]. It can be speculated that some kind of regulation of subpopulations of the DA receptors contribute to the beneficial effects of the *H. perforatum* extracts in stress.

An exposure to high levels of corticosterone or chronic stress causes an increase in 5-HT and NA levels in the hippocampus accompanied by their decrease in the PFC [135, 136]. A moderate decrease of NA level in mPFC (10–33 %), particularly with the synchronized reduction of DA level, causes distinct impairments of cognitive functions in rats [137] and it is now known that long-term administration of *H. perforatum* may increase NA levels in the PFC [123]. Additionally, changes in the dopamine transporter function which controls the level of DA in the synaptic cleft, was influenced by prolonged administration of *H. perforatum* [138], an effect that may be partly responsible for the

positive effects of the herb on cognitive processes.

Ogren et al. [139] reported stress-induced decrease in the number of 5-HT1A receptors located in pyramidal and granular neurons and on GABA-ergic nerve endings of the inhibitory interneurons of the hippocampus that can also be prevented by *H. perforatum* [140–142]. Neuroprotective effects of *H. perforatum* (especially flavonoid fraction), seen in vitro, almost completely (over 70 %) blocked the NMDA-induced calcium influx, an effect was not confirmed in the in vivo models [141].

High amounts of corticosterone enhance action of NA via beta-adrenoreceptors and increase DA turnover in the prefrontal cortex that is accompanied by the decreased spatial memory performance [143]. Eight-week administration of hypericin led to a significant down-regulation of beta-adrenergic receptors in the frontal cortex of rats [144] that might positively affect associated memory processes based on this structure.

Cognitive improvement was paralleled by restoring proteins engaged in synaptic plasticity to control levels in the hippocampus as well as the PFC of the stressed and corticosterone-treated rats [129]. It was found that restraint stress reduced the expression of synaptophysin (SYP) protein (used as a specific protein marker for the presynaptic terminal) in the rat hippocampus by 50 % [145]. In has been demonstrated in patients with Alzheimer disease that SYP immunoreactivity is markedly reduced in the hippocampus and PFC and is associated with impaired cognitive functions [146]. Weak inter-neuronal connections and poor learning and memory abilities were also demonstrated in rats with the lowest SYP expression. *H. perforatum* protected neuronal synaptic structures and increased SYP level that may be one possible mechanism by which the plant improves learning and memory in stressed rats.

The above data indicate that extracts of *H. perforatum* may be a good alternative in the treatment of stress- and corticosteroid-induced cognitive deterioration.

Fish Oil

The next candidate to enter the group of natural medicines for cognitive impairment induced by exposure to chronic stress is cod liver oil and its active components (mainly docosahexaenoic acid, DHA). It is known that oral administration of cod liver oil, a principal dietary source of DHA and eicosapentaenoic acid could also be effective in preventing the consequences of prolonged stress and prolonged GC therapy on cognitive functioning [147, 148]. DHA (22:6n-3) has been found beneficial for several brain processes involved in cognitive functioning. DHA comprises more than 20 % of the membrane phospholipids [149] in the brain and plays a crucial role in maintaining structural and functional integrity of biological membranes [150–152], as well as being essential for normal neurologic status [153]. It was found that deficiency of brain DHA is associated with reduced learning ability and memory in rats [153] and with memory loss in patients with Alzheimer disease [154]. In studies employing DHA supplementation in a mouse model of Alzheimer disease or in aged animals, significant improvement of learning and memory processes was observed [151, 155, 156]. Fish oil administration to fatty acids-deficient rats caused recovery of brain DHA levels and significant improvement of both spatial reference and working memory. They also showed nootropic properties of DHA, which were confirmed by other authors [148, 157, 158]. In addition, Wu et al. [159] revealed that DHA supplementation increases levels of m-BDNF and pro-BDNF in the hippocampus. Data in the literature indicate that m-BDNF may modulate synaptic plasticity [160] and that hippocampal m-BDNF is important for cognitive functions [161] as well as for induction of LTP [162], which is a physiological correlate of learning. DHA may increase BDNF in the brain by multiple mechanisms: conversion of DHA to its derivative—neuroprotectin D1—increase levels of BDNF [163]; the action of DHA on plasma membranes may activate Akt signaling mechanisms that can result in increase

BDNF level [164]; and finally, DHA may help glucose transport across the blood–brain barrier to provide an energy source for the growth of neurons [165]. These do not exhaust all possible mechanisms of the positive effect of polyunsaturated omega-3 fatty acids on cognitive processes because it was shown that DHA-enriched diet modulates hippocampal CaMKII activation, another signaling system whose action is critical for learning and memory [166] that apparently plays a role in the effect of DHA on hippocampal-dependent cognitive processes [167]. Stress exposure increased p-CaMKII in the BLA and decreased p-CaMKII in the mPFC [168]. DHA acts synergistically with the effect of exercise on synaptic plasticity and water-maze learning by increasing levels of CaMKII, cAMP-response element binding protein (CREB) and synapsin 1 in the hippocampus of the adult rats [159]. These authors suggest that n-3 fatty acids increase CaMKII and CREB levels and enhance LTP resulting in dendritic spine formation, BDNF secretion, and increase of the number of c-Fos-positive neurons. Furthermore, the beneficial effect of DHA (300 mg/kg per day for 12 weeks) on learning and memory measured in the radial maze was related to the increase of Fos expression in the hippocampus [158]. It was recently described that dietary supplementation with DHA and EPA in aged rats results in almost complete reversal of the age-related decline of AMPA, GluR2, and NMDA NR2B glutamate receptor subunits. These effects were observed in the cerebral cortex, hippocampus, and striatum [169].

Moreover, it appears that a dietary supplement such as fish oil, both natural and DHA-enriched, can be equally effective in relieving stress-induced memory impairment. Additionally, the fish oil has cardioprotective and immunomodulatory properties that are particularly useful in the elderly.

Curcuma Longa

Another raw material of natural origin, which is also a dietary supplement, is curcumin, the yellow pigment used as a spice, extracted from the rhizomes of *Curcuma longa*. It has been extensively studied for its beneficial therapeutic properties, such as antioxidant [170, 171], neuroprotective [171, 172], anti-inflammatory [172], antidepressant [173], and cognition-improving activities in various pathologies [174]. Curcumin has been reported to possess an inhibitory effect on acetylcholinesterase and is active against scopolamine-induced amnesia [175] and streptozotocin-induced memory impairment [176]. It was also reported to ease cognitive impairment caused by chronic use of phenobarbitone and carbamazepine [177]. There are studies showing that curcumin inhibits its MAO activity in different brain regions of mice [173] that can explain its previously observed antidepressant effect. Moreover, it has been shown that use of curcumin in the diet reduces oxidative damage, normalizes levels of BDNF, synapsin I, and CREB and counteracts cognitive impairments [174]. Curcumin is also reported to reduce serum corticosterone levels and hippocampal dendritic remodeling in restraint stress-induced memory dysfunction in rats [174]. The same authors showed that administration of curcurmin caused blocking of stress-induced phosphorylation of CaMKII along with the concurrent up-regulation of the NMDAR2B glutamate receptor. This subunit activates different postsynaptic proteins important for memory formation [178]. All the above data clearly suggest a beneficial role of curcumin against stress-induced cognitive impairment. However, a significant disadvantage of this preparation is poor oral bioavailability of curcumin that limits its therapeutic efficacy.

Crocus Sativus

Crocus sativus L., another nutraceutical product commonly known as saffron seems to be interesting in the context of cognitive impairment relieving actions. *C. sativus* causes a number of medicinally important activities such as antihypertensive, free radical scavenging, anticonvulsant, anti-genotoxic and anti-cytotoxic effects as well as anxiolytic, antidepressant, antinociceptive, anti-inflammatory, and relaxant ones [179].

Administration of *C. sativus* extract abolished ethanol-induced impairments of learning behaviors in rodents, prevented ethanol-induced inhibition of hippocampal LTP [180, 181] and prevented scopolamine-induced amnesia [182]. The spice was also active against cognitive decline evoked by chronic brain hypoperfusion [183] and had significant anxiolytic properties [184]. Its active constituent, crocin, showed positive effects on learning and memory and reduced excessive production of free radicals in the hippocampus caused by chronic stress [185]. Moreover, they found that crocin significantly decreased plasma levels of corticosterone, as measured after end of stress. There are also studies indicating that extract from *C. sativus* exerted a stimulatory effect on beta2 adrenoreceptors, and had an inhibitory effect on histamine H1 receptors [186], both mechanisms being relevant for memory impairment caused by stress. Although the potential use of saffron in the treatment of cognitive disorders caused by stress requires further detailed studies, this plant undoubtedly has a high potential to modify cognitive processes.

Cannabinoids

In the last decade the endocannabinoid system has emerged as a promising therapeutic target for the treatment of stress-related emotional and cognitive disorders [187–190]. It was found that chronic stress exposure affects endocannabinoid system regulation and activates 2-arachidonoylglycerol (2-AG). It is an endogenous agonist of the CB1 receptor mediating signaling in the hypothalamus, amygdala, hippocampus, and mPFC [188]. Similar stimulation of 2-AG in the amygdala was observed in rats after prolonged administration of exogenous corticosterone [191, 192]. It was shown that immobilization stress decreased *N*-arachidonoylethanolamine (anandamide) content in the mPFC and amygdala irrespective of the number of previous exposures [188, 193]. Exposure to repeated restraint/acoustic stress increased expression of CB1 receptor in the PFC of mice and daily pretreatment with the selective CB1 agonist (arachidonyl-2-chloroethylamide) prevented this stress-induced change [194]. Moreover, it was reported that activation of cannabinoid receptor in the BLA using the CB1/2 receptor agonist (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone (WIN55,212-2) successfully prevented the effects of acute stress on emotional learning [190, 195]. The same agonist also reversed stress-induced release of corticosterone and stress-induced alterations in HPA axis [195]. As can be seen from the above data, the interference with the cannabinoid system can effectively remove the adverse effects of stress on different levels, but modification of cognitive processes is uncertain. Chronic activation of CB1/2 receptor by administration of WIN55,212-2 significantly impaired hippocampal-dependent short-term memory measured in the object location task, an effect present even after 75 days [196]. Unfortunately, considerable evidence shows that cannabinoid system activation causes impairment of hippocampal-dependent learning (spatial) and memory processes (context-related) [197]. Moreover, there are findings that suggest that cannabinoids can impair emotional (aversive) as well as neutral (or rewarding) memory formation-related processes in a task-, brain region-, and memory stage- (acquisition, consolidation, retrieval) dependent manner [198].

Histamine H3 Receptor Antagonists

At the end of this review of the effective means of relieving stress-induced memory impairments, we return to the chemical agents (i.e., drugs). Fortunately, they can be extremely effective in alleviating the cognitive impairment induced by chronic stress as well as chronic corticosteroids. To this end, even a single administration of histamine H3 antagonist (ciproxifan) in animals previously exposed to chronic stress as well as GCs, abolished their negative effects on cognitive functions [199]. H3 receptor was first described as an autoreceptor inhibiting the release of histamine (HA) in the brain [200]. Subsequently, its

role as heteroreceptor involved in the release of other important neurotransmitters was postulated [201–204]. HA release is a sensitive indicator of stress response [205, 206], and stress is a powerful activator of HA neurons in the tuberomammillary nucleus located in the hypothalamus [207]. Neurons of the tuberomammillary nucleus are influenced by a number of neuroendocrine factors [208] and can integrate extero -and interoceptive stimuli in controlling arousal evoked by stress. HA mediates the activation of neuroendocrine stress response and hence release of several pituitary hormones (e.g., ACTH, β-endorphin, vasopressin) [209] and controls the stress-evoked release of monoamines (5-HT, NA, DA) and ACh [204]. Brain H3-receptors are directly involved in cognitive processes and their blockade in some structures and neural projections of these structures leads to cognitive modifications. The structures in which there are H3 receptors are the cerebral cortex, hippocampus, and hypothalamus [210]. Application of the selective antagonists results in increased release and synthesis of HA, and also release of other neurotransmitters such as ACh, DA, NA and 5-HT, which play an important role in cognitive processes and whose levels are perturbed by exposure to chronic stress. Specifically, H3 receptor antagonists have been shown to increase levels of ACh and DA in the prefrontal cortex [211, 212]; ACh, DA, and NA in the anterior cingulate cortex [213]; and ACh in the hippocampus [211]. It is known that chronic stress induces reduction of dopaminergic transmission in the PFC [132] and that the dopaminergic system in the PFC plays an important role in working memory performance. Chronic stress-induced working memory impairment is closely related to the reduced dopaminergic transmission in this brain structure [214]. It seems that the increase in DA turnover by interacting with H3 receptors and HA transmission may be responsible for improving working memory induced by exposure to chronic stress.

HA may also indirectly influence NMDA receptors [215, 216] because activation of H3 receptors causes reduction of glutamatergic neurotransmission in the dentate gyrus of the hippocampus and cortico-striatal pathways [203, 210, 217]. This may be important particularly in the context of preventing excitotoxicity occurring as part of a cascade of negative events associated with exposure to chronic stress or chronic GC, and resulting cognitive impairment. The histaminergic system interferes with neurons releasing vasopressin and CRH in the PVN of the hypothalamus and amygdala [218], and then controlling sympathetic-adrenal response, cardiovascular response, and complex behavior (flight-fight, grooming). It was shown that administration of HA into the PVN activates the HPA axis for the release of CRH, which in turn leads to excessive flux of GCs [209]. Thus, inhibiting HA neurotransmission in the brain by affecting the autoreceptor H3, we can expect an effect restoring normocortisolemia and decrease of excessive response of the HPA axis observed in stress.

Currently, selective antagonists of the H3 receptors are also considered as a promising group of new potential class of drugs for the treatment of cognitive deficits observed in attention deficit hyperactivity disorder, Alzheimer disease, and schizophrenia.

Summary

Exposure to excessive stress becomes commonplace. Persistent stress is our enemy, whom we know best, and the attempt to find the effective strategy to defend ourselves against it is our intention. As can be seen from the above, the search efforts for effective and safe means of preventing cognitive disorders caused by exposure to stress, or the chronic administration of glucocorticoids, take several directions. Screening includes both synthetic compounds and several natural products. There are some considerable successes. The remarkable diversity of these would-be drugs is advantageous because it allows individualized therapeutic options according to the needs of a patient. Almost each of the above-mentioned substances or complex preparations has its own therapeutic indication and the interactions with cognitive processes are their additional newly discovered feature. Taking into account a multitude of individual substances, their final

clinical effect, as well as the scope and nature of their adverse effects, make it possible to adjust the treatment in order to bring the patient a benefit in the treatment of the primary disease and also to improve cognitive functions.

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Circadian Synchronization of Cognitive Functions

11

Ana Cecilia Anzulovich-Miranda

The Circadian System

The time of day is an aspect of the physical environment that can be learned much in the same way that an organism can learn spatial parameters [1]. Thus, animals can adapt their behavior to (predictable) temporal fluctuations in the environment (such as day and night alternation, food and water availability, risk of predation, or social contact) through learning and memory processes and an endogenous biological clock. The day/night cycle is the major synchronizer or *zeitgeber* (from German, *zeit*: time and *geber*: giver, thus a 'time-giver' or synchronizing agent) for human circadian rhythms, being even more powerful than social *zeitgebers*, such as an alarm clock or a work schedule [2]. Before continuing with this chapter description, it is important to define the term 'circadian', which comes from the Italian *circa*: close, near, and *diem*: day, thus, 'circadian' refers to the periodicity of an event or parameter which is repeated every 24 ± 2 h.

Adaptation to environmental temporal cues becomes manifested in well-known behavioral, physiological, and biochemical circadian rhythms such as the sleep–wake cycle, feeding schedule, body temperature, hormonal levels, and key enzymes activity oscillations. However, a review performed by Carrier and Monk showed that cognitive and memory performances also vary over the 24 h cycle [3]. All these rhythms are crucial for the good health of an individual and rely on the integrity and functioning of the circadian system.

The mammalian circadian system is a set of related neural structures whose function is to provide a temporal organization to the rest of the brain, peripheral organs and tissues, and consequently, set the timing of physiological processes and behavior, synchronizing them to the environment. The system is composed of three major components: (1) entrainment pathways, which receive environmental cues and translate them into neural signals; (2) a main pacemaker or central clock in the suprachiasmatic nucleus (SCN), which is entrained by those environmental signals and transmits the external periodicity to downstream targets; and (3) output pathways, which couple the main pacemaker to the effectors: organs or tissues outside the SCN that express circadian functioning. Table 11.1 summarizes those components and sequential facts.

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Table 11.1 Summary of environmental cues, circadian system components, and circadian rhythms in humans

Environmental cues	Entrainment pathways	Pacemarker or master clock	Output pathways	Effectors	Circadian rhythms
Light/dark cycle Social and physical activities Feeding	RHT (glutamate, PACAP) Pineal gland-SCN (melatonin) Intergeniculate leaflet-SCN (neuropeptide Y)	SCN	SCN-PVN SCN-LC SCN-MPO SCN-ARC SCN-DMH SCN-DMV SCN-MBH SCN-SC	Pineal gland Hippocampus Brain cortex HPA axis Heart Liver Other	Melatonin secretion Sleep-wake cycle Body temperature Endocrine cycles Attention Synaptic plasticity

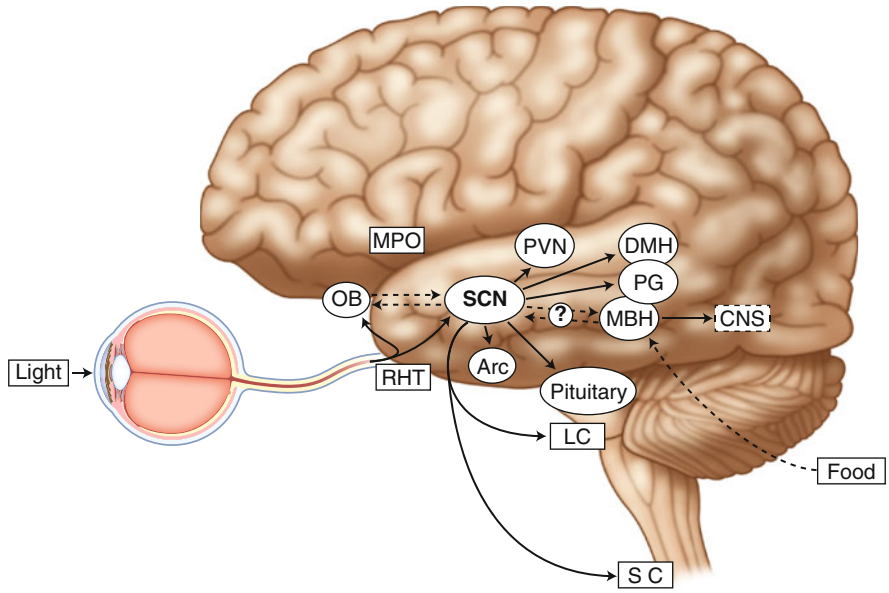

Fig. 11.1 Schematic representation of some SCN input and output pathways (modified from Kwom and collaborators [13]. *RHT* retino hypothalamic tract, *OB* olfactory bulb, *SCN* suprachiasmatic nucleus, *PVN* paraventricular nucleus, *MBH* mediobasal hypothalamus, *DMH* dorsomedial hypothalamus, *PG* pineal gland, *Arc* arcuate nucleus, *LC* locus ceruleus, *SC* spinal cord, *CNS* central nervous system

The day/night cycle is the stronger environmental *zeitgeber* and light excites specialized melanopsin-containing ganglion cells in the retina. From there, the retinohypothalamic tract (RHT) transmits a "daytime" signal toward the master clock in the SCN, thus the RHT, via glutamate, constitutes the entrainment pathway in mammals, humans among them. The SCN comprises individual neuronal oscillators coupled into a neural network. First it was thought that the SCN was composed of identical resettable oscillators, however, more recent investigations have shown that circadian rhythmicity in the SCN is the product of a highly organized network of heterogeneous cells [4].

The SCN, located in the anterior hypothalamus, maintains a near-24-h rhythm of electrical activity, even in the absence of environmental cues. This circadian rhythm is generated by intrinsic molecular mechanisms in the SCN neurons. However, the circadian clock is modulated by a wide variety of influences, including glutamate and pituitary adenylate cyclase-activating peptide (PACAP) from the RHT, melatonin from the pineal gland, and neuropeptide Y from the intergeniculate (Table 11.1 and Fig. 11.1). By virtue of these and other inputs, the SCN responds to environmental cues such as light, social, and physical activities. In turn, the SCN controls or influences a wide variety of

physiologic and behavioral functions, including attention, endocrine cycles, body temperature, melatonin secretion, and the sleep–wake cycle [5]. Thus, projections from the SCN toward the paraventricular nucleus (PVN), the locus coeruleus, the medial preoptic area (MPO), the arcuate nucleus (ARC), the dorsomedial hypothalamus (DMH), the mediobasal hypothalamus (MBH), the dorsal motor nucleus of the vagus (DMV), and the spinal cord translate oscillating neural signals from the master clock to more widespread and multiple neural and humoral signals that reach the rest of the tissues and organs in the body (Table 11.1 and Fig. 11.1). For example, neural circadian signals from the PVN reach the pineal gland and regulate the secretion of nocturnal melatonin. Melatonin secretion is inhibited by the SCN during the light phase but, in turn, the SCN contains melatonin receptors that inhibit SCN firing, thereby creating a negative feedback loop [6].

In external 24-h light–dark cycles, the circadian system synchronizes daily rhythms in the body to the changing environment. However, in the absence of *zeitgebers* (i.e., in constant external conditions) for example, the rhythms are endogenously driven and show a free running pattern of about 24 h in constant darkness [7].

A vasopressinergic projection from the PVN also reaches the CA2 area in the hippocampus. In turn, CA2 forms disynaptic connections with the entorhinal cortex to influence dynamic memory processing. Thus, the pathway SCN-PVN-CA2-entorhinal cortex would explain a circadian control of cognitive functions. Additionally, dorsal CA2 neurons send bilateral projections to the medial and lateral septal nuclei, vertical and horizontal limbs of the diagonal band of Broca, and supramamillary nuclei (SUM). Novel connections from the PVN and to the SUM suggest important regulatory roles for CA2 in mediating social and emotional input for memory processing [8].

The Molecular Clock Machinery

In addition to the clock in the SCN, peripheral oscillators have their own cellular and molecular clock machinery. In mammals, it consists of a network of interlocking transcriptional-translational feedback loops that drive the rhythmic expression of core clock components as well as of clock-controlled genes [9]. The molecular clock components are transcription factors that constitute the mechanism for generation and maintenance of circadian rhythms within individual cells throughout the body. The heterodimeric basic helix-loop-helix-Per Arnt Sim transcription factor, Brain and Muscle Aryl hydrocarbon receptor nuclear translocator-*Like* 1:Circadian Locomotor Output Cycles Kaput (BMAL1:CLOCK) is the main participant in the clock's positive feedback loop by binding to the E-box (CACGTG) *cis-regulatory* enhancer elements, in their target genes. Such genes include Period (Per1, Per2, and Per3) and Cryptochrome (Cry1 and Cry2), as well as clock-controlled genes. A negative feedback loop is achieved when Per and Cry proteins form heterocomplexes that translocate back to the nucleus and inhibit their own and other clock-controlled gene transcription. In addition to the primary feedback loops, an auxiliary regulatory loop is achieved by the nuclear receptor subfamily 1 group D member 2 (also known as REV-ERB) and the Retinoic acid related orphan receptor (ROR). Circadian transcription of these nuclear receptors is also driven by the BMAL1:CLOCK heterodimer. In the nucleus of neural cells, REV-ERBα competes with RORα for binding to the ROR-responsive element in the BMAL1 gene promoter. Whereas RORα activates transcription of Bmal1, REV-ERBα represses it. Consequently, the rhythmic expression of Bmal1 is achieved by both positive (RORα) and negative (REV-ERBα) regulation [9, 10].

If transcriptional activation were only followed by feedback repression, a molecular feedback loop would take just a few hours to run a cycle. However, epigenetic and post-translational modifications are also involved in the normal functioning of the circadian (~24 h) clockwork. Therefore, if not for the significant delay mediated by such modifications between transcriptional activation and repression, 24-h circadian periodicity would not be achieved [10]. For instance, studies on molecular circadian clock machinery in different species disclose the participation of several protein kinases in circa-

dian regulation. Thus, mammalian casein kinase members (CK1δ and CK1ε) are considered as part of the cellular clock. This affirmation comes from the fact that a mutation in the CK1ε gene results in a shorter free-running period of hamsters [11]. Studies made in humans by Toh and collaborators have also showed that families with familial advanced sleep-phase syndrome have mutations in the CK1δ and CK1ε phosphorylation sites in the Per2 gene [12, 13].

Phosphorylation is also needed for the recruitment of ubiquitin ligases and the subsequent degradation of Per. Experiments where CK1δ or CK1ε are overexpressed show moderately shortened Per1 and Per2 proteins half-lives. Phosphorylation of Per creates binding sites for β-transducin repeat-containing protein, an F-box-containing E3 ubiquitin ligase. Another F-box protein, Fbxl3, is a Cry E3 ligase. Mutation in Fbxl3 results in impaired ubiquitination and subsequent degradation of Cry. Consequently, prolonged stability of the Cry proteins leads to an extended negative phase and period lengthening [13]. Furthermore, the cAMP-dependent protein kinase and mitogen-activated protein kinase (MAPK) pathways are also implicated in the cAMP response element (CRE) mediated induction of Per1. In addition to the CRE binding protein-mediated induction, the phosphorylation of CLOCK by Ca^{2+}-dependent Protein kinase C would be involved in the phase resetting of the mammalian circadian clock [13].

Post-translational modifications of BMAL1 include sumoylation, acetylation, and phosphorylation. Sumoylation is a post-translational modification involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through the cell cycle. Small ubiquitin-like modifier proteins are a family of small proteins that are covalently attached to and detached from other proteins in cells to modify their function [14]. BMAL1 is rhythmically sumoylated in vivo, and the interaction with CLOCK is necessary for proper sumoylation. Lee and colleagues showed that sumoylation promotes BMAL1 transactivation and ubiquitin-dependent degradation [15]. Additionally, BMAL1 acetylation and phosphorylation by CK2α, are essential for the maintenance of its circadian rhythmicity, nuclear accumulation, and circadian clock function [13].

Circadian clock work is also influenced by the cellular redox state. Studies from Rutter and colleagues have shown that DNA-binding activity of BMAL1:CLOCK and BMAL1:NPAS2 heterodimers is regulated by the redox state of nicotinamide adenine dinucleotide (NAD) cofactors in a purified system. The reduced forms of the redox cofactors, NAD(H) and NAD phosphate(H), strongly enhance DNA binding of the BMAL1:CLOCK and NPAS2:BMAL1 heterodimers to the E-boxes, whereas the oxidized forms inhibit it [16]. It is known that the cellular redox state is determined by the levels of several redox couples such as 2GSH/GSSG, NADH/NAD, and NADPH/NADP, which are the product of a coordinated balance in metabolic pathways and antioxidant systems. All the above observations raise the possibility that oxidative stress and unbalance of the cellular redox state observed, for example, in older individuals, might have an effect on the clock activity and circadian expression of putative target genes, such as brain-derived neurotrophic factor (BDNF), RC3, APP, or PSEN, by modulation of BMAL:CLOCK DNA-binding activity in memory-and-learning-related areas of the brain.

Following the core clockwork, circadian clock output is mediated by cAMP/MAPK/CREB, some of the same molecular signaling cascades that regulate memory formation [17].

Circadian Chronotype

Based on their individual disposition to sleep and wakefulness, humans can be categorized as early chronotype (EC), late chronotype (LC), or intermediate chronotype. While ECs prefer to wake up early in the morning and find it hard to remain awake beyond their usual bedtime, LCs go to bed late and have difficulties getting up in the early morning. Beyond sleep-wake timings, chronotypes show distinct patterns of cognitive performance, gene expression, endocrinology, and lifestyle [18].

A variable number tandem repeat polymorphism in the clock gene Per3 would be a genetic

determinant of those individual differences because Per3(5/5) individuals are more likely to show morning preference, whereas homozygosity for the four-repeat allele, Per3(4/4), associates with evening preferences. The association between sleep timing and the circadian rhythms of melatonin and Per3 mRNA in leukocytes is stronger in Per3(5/5) than in Per3(4/4). Other differential characteristics include: electroencephalographic alpha activity in rapid eye movement sleep, theta/alpha activity during wakefulness and slow wave activity in nonrapid eye movement sleep, which are elevated in Per3(5/5). It has also been observed that sleep deprivation leads to a greater cognitive decline, and a greater reduction in functional magnetic resonance imaging-assessed brain responses to an executive task, in Per3(5/5) individuals [19].

Genetic variations in other clock genes are also related to different sleep and circadian phenotypes [19]. For example, variants of the human CLOCK gene have been associated with diurnal preference, sleep duration and modulation of differential sleep disturbance patterns in mood disorders. Worthy of mentioning is a non-circadian role for clock genes in sleep homeostasis. Many studies cited in a comprehensive review made by Maire and colleagues found that the expression of a number of clock genes at the cortical level is affected by the sleep–wake history and that the homeostatic sleep regulation is altered in mice that are mutant for one or more clock genes [20]. Intriguingly, circadian clock genes can also act on homeostatic markers in humans [21].

The Temporal Organization of Cognitive Functions at the Molecular, Biochemical, and Behavioral Levels and Their Clock-Mediated Regulation

Early findings by Holloway and Wansley demonstrated that memory performances for associative learning oscillate in a circadian manner across time, with high memory retention at multiples of 24 h following learning [22]. Later, Stephan and Kovacevic reported that SCN lesions impair hippocampus-dependent long-term memory in rodents [23]. Hoffmann and Balschun demonstrated that mice trained on an alternating T-maze produced fewer errors and faster rates of acquisition when training takes place during the dark-(active) phase [24]. Similarly, habituation to spatial novelty is more robust during the mouse's endogenous active phase [25]. More recently, time-of-day effects on learning and memory have also been observed in human primates, non-human primates, and rats [26–28]. For example, several forms of cognition such as working memory, associative learning, declarative memory, motor skill learning, and fear conditioning vary on a circadian basis in both humans and rodents [29–33].

The molecular basis of those and other behavioral rhythms are constituted by differences between day and night in the expression of gene transcripts in the hippocampus, cerebral cortex, and cerebellum. Katoh-Semba and colleagues reported significant diurnal variations of the BDNF levels in those brain areas as well as of both BDNF and neurotrophin 3 in the visual cortex [34]. The authors found the highest protein levels occur during sleep in both the neocortex and cerebellum. As expected, BDNF protein peak follows mRNA maximum which occurs during wakefulness (the dark phase) in the same brain areas [35]. All those observations would indicate that neurons in the neocortex and cerebellum are actively working during sleep. That was also supported by reports showing that cortical neurons still work during sleep to restructure new memory representations and to facilitate fixation of memory [36, 37]. Thus, given BDNF is a memory-related molecule, it is likely to play roles in forming, rearranging, and fixing memory during sleep. Furthermore, the patterns of diurnal variations in BDNF levels are characteristic for individual brain regions, different from the SCN [34].

Golini and colleagues found that not only BDNF, but also neurogranin (RC3), the postsynaptic substrate of protein kinase C, display rhythmic expression patterns in the rat hippocampus. Maximal RC3 expression occurs at the end of the night preceding BDNF mRNA peak, as in the

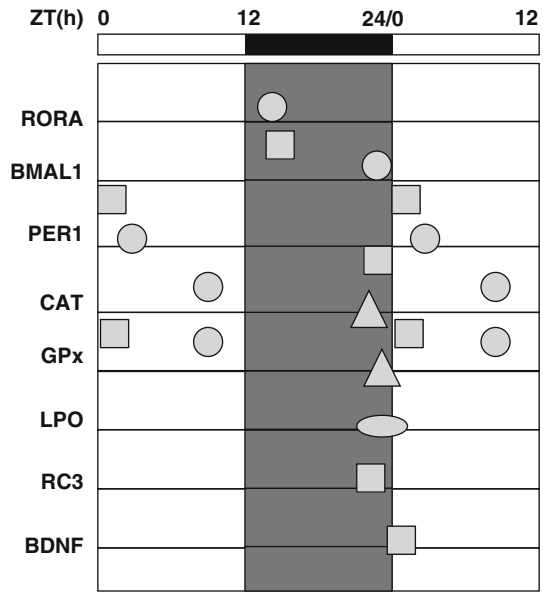

Fig. 11.2 Temporal organization in the hippocampus. Schematic phase map of daily rhythmic parameters that interact harmonically to determine the best time for cognitive performance in the hippocampus. Graph shows calculated phases of abundance for cycling mRNA (*squares*), protein (*circles*), enzymatic activity (*triangles*) and lipoperoxides (*oval*) throughout a 24-h period, 12-h day (*white panels*) and 12-h night (*grey panel*). *ZT zeitgeber* time (with ZT0 when light is on), *RORA* retinoic acid related orphan receptor, *CAT* catalase, *GPx* glutathione peroxidase, *LPO* lipoperoxidation, *RC3* neurogranin, *BDNF* brain-derived neurotrophic factor

cellular events which occurs for postsynaptic activation [38]. As expected, BDNF and RC3 rhythms are in phase with the circadian clock, BMAL1 and Per1, protein levels as well as with the maximal antioxidant enzyme activity shown by Fonzo and colleagues [39]. In turn, the nocturnal peaks of catalase (CAT) and glutathione peroxidase (GPx) activity seen in the rat hippocampus would be in phase with the best time for performing learning and memory tests, as shown in young rats by Winocur and Hasher [27, 39].

All the above-mentioned observations allow us to build an illustrative phase map (Fig. 11.2) and to propose the existence of a temporally well-orchestrated RC3 and BDNF cycle underlying temporal patterns of synaptic plasticity in the hippocampus [38].

Cognitive performance is under the combined influence of circadian processes and homeostatic sleep pressure throughout the day. Homeostatic sleep pressure can be considered a sleep promoting process that continuously accumulates with increasing time spent awake, concomitantly with a decrease in waking cognitive performance [20]. As homeostatic sleep pressure increases, activity in the suprachiasmatic area decreases, indicating a direct influence of the homeostatic and circadian interaction on the neural activity underlying human behavior [40].

There exist substantial interindividual differences in the way an individual reacts to an imbalance between circadian and homeostatic processes, a situation provoked by, for example, total or partial sleep deprivation or by performing night or rotating shift work [20]. As mentioned previously, EC individuals perform best in the morning (beginning of the activity period), whereas LC individuals are more alert in the evening (end of the activity phase). Chronotypes provide a unique way to study the effects of sleep–wake regulation on the cerebral mechanisms supporting cognition. Using functional magnetic resonance imaging in extreme chronotypes, Schmidt and collaborators found that main-

taining attention in the evening is associated with higher activity in a region of the locus coeruleus and in the suprachiasmatic area, including the circadian master clock in the SCN, in LC individuals than in EC individuals [40].

Increasing evidence suggests a genetic contribution on the individual's vulnerability to cognitively perform under sleep homeostatic challenges or at an adverse circadian phase. In addition to age and chronotype, other reported variables potentially contributing to cognitive vulnerability are gene polymorphisms such as the adenosine deaminase, adenosine receptor A2A, BDNF, catechol-O-methyltransferase, and Per3 genes [20, 41].

A variety of neuroanatomical, neurophysiological, molecular, and neurochemical mechanisms have been revealed. The neuroanatomical circuit mediating circadian regulation of arousal is a multisynaptic pathway between the SCN and the noradrenergic neurons of the locus coeruleus. The behavioral state of arousal and wakefulness is induced by stimulation of the frontal cortex by noradrenergic neurotransmission arising from the locus coeruleus [42]. Particularly, circadian influence on learning may be exerted via cyclic gamma-aminobutyric acid output from the SCN to target sites involved in learning such as the septum and the hippocampus [32].

Circadian Hormonal Regulation: Role of Glucocorticoids in the Temporal Regulation of Memory and Learning

Learning-dependent remodeling of synaptic connections is important in learning and memory. For example, motor learning induces the formation of persistent postsynaptic dendritic spines, and the survival of these spines is strongly correlated with behavioral performance after learning. Recent studies indicate that glucocorticoids (GC) have rapid effects on dendritic spine development and plasticity in the mouse somatosensory cortex.

GC refers to a class of multifunctional adrenal steroid hormones with activity on glucose metabolism. The main GC, cortisol in humans and corticosterone in rats and mice, are synthesized by the *zona fasciculata* cells in the adrenal cortex. GC secretion varies phasically with stressful environmental triggers and tonically with the circadian rhythm [43]. GC levels display a robust oscillating pattern, with a peak occurring at the onset of the daily activity phase; thus, early in the morning in humans, and the beginning of the night in rats and mice [44].

Evidence strongly supports the notion that the periodicity of GC involves the integrated activity of multiple regulatory mechanisms related to circadian timing system along with the classical hypothalamus/pituitary/adrenal (HPA) neuroendocrine regulation. In the case of the adrenal gland, the SCN activates rhythmic release of corticotrophin-releasing hormone from the PVN that evokes circadian adrenocorticotropin hormone (ACTH) release from hypophysial adrenocorticotrophs. In turn, ACTH regulates circadian corticoid release from the zona glomerulosa and the zona fasciculata of the adrenal cortex. In addition, neuronal signals generated by the SCN propagate through the autonomic nervous system to the adrenal cortex to contribute to the circadian regulation of GC production. The adrenal-intrinsic oscillator as well as the central pacemaker in the SCN plays a pivotal role in GC rhythmicity [44, 45].

GC influences numerous biological processes such as metabolic, cardiovascular, immune, and even higher brain functions; however, it also acts as a resetting signal for the ubiquitous peripheral clocks, suggesting its importance in harmonizing circadian physiology and behavior [46]. Glucocorticoids influence synaptic glutamate release and receptor trafficking through nontranscriptional mechanisms and rapidly modulate the function of inhibitory interneurons in the prefrontal cortex through nontranscriptional regulation of endocannabinoid signaling [43].

An interesting finding is that training increases spine formation when it concurs with the circadian GC peak. Elevated GC secretion during the circadian peak facilitates the formation of stable new spines after learning, and thus, it would enhance long-term memory retention [43].

Learning-related new spines are initially highly unstable: most new spines will be pruned within days after their formation, but a subset will be

selectively stabilized over time, and most of those that survive will contain functional synapses. GC selectively stabilizes a subset of learning-related spines during the circadian trough while pruning a corresponding set of preexisting synapses. Disruption of the circadian trough during a critical period after learning interferes with this stabilization and pruning process. Thus, circadian GC peak is important for forming new spines after training, whereas trough is required for stabilizing a subset of new spines during a critical time after their formation. Liston and collaborators investigated nontranscriptional mechanisms of GC action. To do that, they obtained mouse cortical biopsies 20–25 min after direct cortical application of corticosterone and examined changes in protein expression [43]. Cofilin is a known regulator of actin filament dynamics. Lin11, Isl-1 and Mec-3 (LIM) protein-kinase 1 (LIMK-1), a serine/threonine kinase containing LIM and postsynaptic density protein 95/disc large/zonula occludens (PDZ) domains, is able to phosphorylate cofilin at SER 3 [47]. Liston's group results showed rapid increases in the expression of phosphorylated forms of LIMK-1 and its substrate cofilin. These results correlate with phospho-glucocorticoid receptor (GR) expression levels, a marker of GR activity. They observed comparable effects after coadministration of corticosterone and actinomycin D, an interferent of DNA transcription, indicating that the effect of glucocorticoids on spine formation is likely mediated through a nontranscriptional LIMK1-cofilin pathway. These signaling mechanisms may generate new spines through direct effects on the dendritic cytoskeleton by modulating neuronal network activity, or by some combination of both mechanisms [43].

Factors that Could Disrupt the Normal Functioning of the Circadian System and Contribute to the Etiology of Cognitive Disorders

Cognitive disorders associated with circadian dysfunction can have their origin at every level of the circadian synchronization pathway. Thus, interferences in the input pathways to the SCN, uncoupling of autonomous oscillators in the SCN, disruptions in the output signaling from the SCN to other parts of the brain [42, 48], or alterations in local circadian clocks in learning and memory-related areas may constitute the circadian basis of cognitive disorders [38, 49].

Normal functioning of the circadian system guarantees sleep, wakefulness, and activity as well as peaks of neural and hormonal rhythms occur at an appropriate endogenous biological time. Thus, the circadian system benefits cognitive functions throughout the lifespan. Yet, when circadian rhythms are phase shifted and, for example, wakefulness occurs at inappropriate biological times because of environmental pressures (e.g., early school start times, long work hours that include work at night, shift work, jet lag) or because of circadian rhythm sleep disorders, the resulting misalignment between circadian and wakefulness-sleep physiology leads to impaired cognitive performance, learning, emotion, and safety [50].

Light reaches the ganglion photoreceptors in the retina from where the signal is transmitted throughout the RHT which releases neurotransmitters such as glutamate and the PACAP to the photic-receptor cells in the ventrolateral SCN. In certain cases, such as transmeridian flights, shift work, and night work, the light–dark cycle is badly perceived leading to rhythm desynchronization. It has been observed that chronic jet lag produces atrophy of the temporal cortex and neuronal degeneration in the human brain [51].

Rhythm desynchronization occurs when the clock is no longer in phase (harmony) with the environment, resulting in a phase shift (phase advance or phase delay) which can produce fatigue, sleep and mood disorders, impaired mental and physical performance, and severely compromise long-term health. Clock desynchronization is related to a loss of adaptation between the SCN and the environmental synchronizers, to an inability for the SCN to be entrained, or to a dysfunction of the master clock itself [52]. Circadian disruption is more severe during adaptation to advances in local time because the circadian clock takes much longer to phase advance than delay [53].

In in vivo experiments, Reddy and collaborators demonstrated that the mouse Period (mPer) circadian expression in the SCN responds faster

than the mouse Cryptochrome (mCry) mRNA circadian rhythm to an advance in the lighting schedule. Rhythmic mCry1 expression advances more slowly, in parallel to the gradual resetting of the activity–rest cycle. On the contrary, the speed of mPer and mCry response is faster during a delay in local time. Per and Cry expressions complete the phase shift together by the second cycle, in parallel with the activity–rest cycle.[53] In the authors' words, these results reveal the potential for dissociation of mPer and mCry expression within the central oscillator during circadian resetting and a differential molecular response of the clock during advance and delay resetting. Uncoupling of autonomous oscillators in the SCN and disruptions in the output signaling from the SCN to other parts of the brain have been described by Yang and coworkers in patients with bipolar disorder [48].

There is evidence of alterations in local circadian clocks which affect learning and memory in related brain areas. For example, in a model of hamsters made arrhythmic by an experimental lighting protocol, Ruby and collaborators report impaired spatial memory and long-term object recognition. Taking into account that both the novel object recognition as well as the spontaneous alternation in the T-maze test require normally functioning hippocampal or septal-hippocampal circuits, the authors propose that memory impairments caused by circadian arrhythmia might derive from changes in the excitability of these circuits or conceivably in others that comprise the medial temporal lobe [32].

Circadian rhythms in clock gene expression are observed in many brain regions including those with roles in motivational and emotional state, learning, hormone release, and feeding [54]. Meal time, which can be experimentally modulated by restricting feeding to a few hours within the individual's rest phase, is a potent synchronizer for peripheral oscillators with no clear synchronizing influence on the SCN clock [55]. In particular, restricted feeding (RF) schedules, which limit food availability to a single meal each day, lead to the induction and entrainment of circadian rhythms and food-anticipatory activities in rodents. Food-anticipatory activities include increases in core body temperature, activity, and hormone release in the hours before the predictable mealtime. RF schedules and the accompanying food-anticipatory activities are also associated with shifts in the daily oscillation of clock gene expression in diverse brain areas involved in feeding, energy balance, learning and memory, and motivation [54]. Thus, for instance, RF-induced anticipatory activity rhythm is associated with a phase-shift, from night or subjective night to day hours, of the circadian mPer1 and mPer2 mRNA peaks, in the cerebral cortex and hippocampus of mice [56].

Analysis of clock mutant mice has highlighted the relevance of some, but not all, of the clock genes for food-entrainable clockwork. Npas2-mutant or Cry1- and Cry2-deficient mice show more or less altered responses to restricted feeding conditions. Moreover, a lack of food anticipation is specifically associated with a mutation of Per2, demonstrating the critical involvement of this gene in the anticipation of meal time [55].

The statement that feeding is a synchronizer as powerful as light/dark cycles relies on many empirical observations on laboratory rodents or from studies on consumers [57, 58]. Feeding can exert its entrainment activity on peripheral clocks either through temporal windows of food access (in or out of phase with the nocturnal or diurnal nature of the species including or not a fasting period) either through the meal nutrient composition. Examples of the latter include the effects of hipocaloric diets, intake of D-glucose, fasting, insulin injection, and feeding vitamin-free diets on the circadian regulation of clock and clock-controlled genes in peripheral oscillators [38, 39, 58, 59].

Nutritional Deficiencies

Nutritional deficiencies, particularly vitamin deficiencies such as B1 (thiamine), B9 (folic acid), B12 (cobalamin), and A (retinoids) as well as iron and magnesium deficits, have been related to changes in circadian expression in memory- and learning-related peripheral oscillators.

It is common knowledge that an inadequate supply of thiamine to the brain leads to an acute neuropsychiatric disorder called Wernicke's encephalopathy. Bennett and Schwartz found that about a month before the expected onset of overt neurological illness, locomotor rhythmicity in thiamine-deficient animals exhibited a shortened free-running period, which was fully reversible by thiamine administration [60]. Thus, it is possible that circadian dysfunction precedes and contributes to altered physiological responses in Wernicke's encephalopathy.

Folic acid or vitamin B9 has been described as a cofactor of clock Cry factors [61]. Folic acid deficiency provokes circadian disruptions at different levels of the circadian system. For example, dampened rhythms of Per2 and vasopressin (AVP) proteins in the SCN, shortened free running period, and decreased rhythms of melatonin secretion have been found in folate-deficient animals [62, 63]. These alterations have also been associated with aging-related cognitive disorders such as Alzheimer disease [63, 64].

In our group experience, feeding a vitamin A-free diet leads to oxidative stress, as a consequence of altered daily patterns of antioxidant enzymes such as CAT and GPx, as well as cycling glutathione (GSH) levels in the hippocampus. Particularly, the hippocampal formation is essential to several types of memory and learning (working memory, short-term memory, memory consolidation, declarative memory, spatial learning) while the prefrontal cortex is fundamental for working memory or long-term memory [65–67]. Interestingly, the nocturnal peaks of CAT and GPx antioxidant activity seen in the hippocampus of our control rats is in phase with the best time for performing learning and memory tests seen by Winocur and Hasher in young rats [27]. Vitamin A deficiency abolishes rhythmic CAT expression and activity, while it phase-shifts GPx oscillating protein and activity in the hippocampus. Consequently, the lower level of GSH concurs with the lipoperoxidation peak at the end of the day–beginning of the night, increasing oxidative stress at that particular time of the day. Taking into account that the cellular clock function depends on the local redox state, temporal disorganization of the antioxidant defense system may underlie disruption of temporal patterns of clock and/or synaptic plasticity-related factors expression. Thus, the lower amplitude of BMAL1 and Per1 circadian rhythms as well as the phase shift observed in the daily BDNF and RC3 expression in the hippocampus of vitamin A-deficient rats, might be explained by alterations in the circadian activity of antioxidant enzymes [38, 39, 59].

Chronopathological forms of magnesium depletion are related either to hypofunction or to hyperfunction of the biological clock [68].

It has been observed that in the case of brain iron deficiency, it affects the striatal dopaminergic-opiate system, resulting in alterations in circadian behaviors, cognitive impairment, and neurochemical changes closely associated with them [69].

Aging

Aging is a multifactorial process determined by genetic and epigenetic factors resulting in a broad functional decline including endocrine, immunological, and cognitive functions. Most aging individuals show gradual impairment of cognitive capabilities which are associated with hippocampal and cortical alterations, the two brain regions involved in learning and memory processes. Circadian hormonal and neurochemical rhythms are also frequently dysregulated in aging [70]. Characteristic perturbations of daily patterns in aged individuals include: phase advances, reduced endogenous period, lower amplitude, increased intra-daily variability, and decreased inter-daily stability of the rhythms [71].

The participation of the cellular clock in aging and the associated deterioration may be assessed in animal models lacking one or more clock genes. For instance, deletion of the core clock gene, Bmal1, ablates circadian rhythms and Bmal1-deficient mice have a reduced lifespan and various symptoms of premature aging, such as increased levels of reactive oxygen species, cognitive deficits, and tissue atrophy [72, 73]. Manipulation of circadian gene expression or

maintaining animals under constant lighting also produce impairment of hippocampal plasticity. Thus, animals lacking Per1 expression which display altered activity rhythm, also show impaired spatial learning and long term memory [70]. Aging has a differential effect on circadian rhythmicity in different brain areas. On one hand, it dampens clock gene rhythmic expression in the central pacemaker (SCN), and on the other hand, phase shifts CLOCK and BMAL1 protein rhythms in the hippocampus, shifting the maximal protein level toward the dark phase [70, 74]. The changes in molecular rhythm could underlie behavioral alterations in the elderly, such as decreased sleep quality and fragmentation of the activity pattern, both associated with impaired cognitive functions. The links between molecular changes in the SCN and/or the hippocampus and the deteriorated behaviors is given in a first instance by the oscillating expression of clock-controlled genes such as those that codify for daily hormonal and neurotransmitters synthesis and release. In a second instance, the altered circadian signaling involves humoral and neural pathways from the SCN to other areas in the central nervous system, including memory and learning-related areas, and the rest of the body such as the HPA axis. A question that arises from a recent review is whether altered patterns of clock gene expressions themselves contribute to the cognitive deficits associated with aging, or whether altered sleep patterns mediate memory deficits, or both [70].

Consequences of altered sleep-wake cycling in aged individuals are manifested in different cellular processes. One of them is the neurogenesis, a form of hippocampal structural plasticity, which is attenuated following sleep deprivation either in a glucocorticoid-dependent, either—independent, manner [75, 76]. In addition to the suppression of hippocampal cell proliferation, sleep deprivation or sleep fragmentation also reduces the number of cortical and hippocampal dendritic spines [70, 77].

In Stranahan's words, the effect of aging on circadian rhythms is a double-edged sword; on one hand, poor sleep quality compromises neuronal structure and function in regions that support cognition, and on the other hand, perturbation of central and peripheral oscillators changes the hormonal milieu, with consequences for neuroplasticity [70].

Alzheimer Disease

Alzheimer disease (AD) is one of the most devastating psychiatric disorders associated with aging. From the biochemical and molecular points of view, AD is characterized by extracellular amyloid plaques and intraneuronal neurofibrillary tangles containing hyperphosphorilated Tau protein [78]. The amyloid beta (Aβ) peptide is produced by the abnormal cleavage of the amyloid precursor protein (APP) by β- and γ-secretases, and participates in the formation of the senile plaques. Following the amyloid hypothesis, the AD is the result of an unbalance between the Aβ synthesis and its clearance [79]. The increased deposit of Aβ peptide is associated with the loss of neurons and synapses and to alterations of neuronal functions [80]. Jiang and colleagues demonstrated that ApoE plays a direct role in the normal clearance of the Aβ peptide, by promoting its intra- and extracellular proteolytic degradation [81].

ApoE expression is regulated at the transcriptional level by the peroxisome proliferator-activated receptor gamma (PPARγ). Recent clinical assays have demonstrated that synthetic agonists of PPARγ have some effects on alterations associated with AD [82].

PPARγ heterodimerize with the retinoid X receptor (PPARγ:RXR) to activate transcription [83]. It is known that vitamin A and its derivatives, the retinoids (all-trans-retinoic acid; 9-*cis*-retinoic acid, 9cisRA; and 13cisRA) regulate a large number of biological processes through their nuclear receptors, RAR (α, β y γ) and RXR (α, β, y γ) activation. There is evidence that RA regulates the expression of genes related to the processing of the APP, inhibits and reverses the Aβ peptide accumulation as well as the tau hyperphosphorilation, and rescues memory deficits in a transgenic mouse model of AD [84, 85],

In addition to the cognitive deterioration, AD is characterized by disturbances of the circadian

rhythms [86], alteration of the sleep-wake cycle [87, 88], and behavioral disorders [89]. Recent investigations have shown that alterations of the circadian rhythms accelerate aging and favor aging-related pathologies and psychiatric and neurodegenerative disorders [72, 90, 91]. Clinical findings have demonstrated that some rhythms, such as body temperature cycles and GC circulating levels, are modified in patients with AD [86, 92].

Parkinson Disease

Parkinson disease (PD) is characterized not only by the hallmark motor disorders, but also by a variety of cognitive, autonomic, sensory, neuropsychiatric, and circadian disorders. A recent review by Rutten and collaborators discusses, among others, factors that lead to the biological clock desynchronization in patients with PD. There is a reciprocal regulation between dopamine and the circadian clock. For example, Per2 regulates dopamine metabolism while D2 receptor-mediated dopamine regulates the rhythms of Per1 and Per2 proteins in the striatum. Dopamine depletion associated with PD is one of the factors underlying disruption of sleep and circadian rhythms in patients with PD [6].

Taking into account that dopamine also regulates the rhythmic expression of melanopsin in retinal ganglion cells, it influences the light entrainment of the circadian system [93]. In addition to this, aged individuals receive less illumination in the retina resulting from pupillary miosis and reduced crystalline lens light transmission and usually are predisposed to stay indoors because of motor problems or a decreased postural balance and expose themselves less to environmental light and physical activities [94]. Thus, in patients with PD, it is expected that exposure and sensitivity to *zeitgebers* decreases, hampering the SCN input and contributing to desynchronization of the circadian rhythms. The amplitude of the circadian rhythm also decreases in patients with PD, as reflected by a decrease in sympathetic activity during the day, diminishing of the diurnal variation of cortisol secretion, and decreasing of the amplitude of the melatonin secretion rhythm. This flattening of circadian rhythms makes them more prone to desynchronization [6].

Additionally, patients with PD may experience periodic limb movement disorder, restless legs syndrome, REM sleep behavior disorder, and excessive daytime sleepiness, all contributing to a reduced quality and/or quantity of sleep and, consequently, a worsen performance of cognitive tasks [6].

Depression

Depression and sleep problems share a common cause, among others, a disturbed circadian rhythm [95, 96]. Some of the major neurotransmitters implicated in mood regulation, such as serotonin, norepinephrine, and dopamine, as well as their receptors, oscillate in a 24-h basis. Additionally, at the molecular level, various polymorphic variations of clock genes such as Timeless, Bmal1, and Per2 are associated with mood disorders [6, 95].

A phase advance in the circadian rhythms of melatonin and cortisol has been observed in some patients with a depressive disorder [96, 97]. As described above, these hormonal temporal patterns result of an interaction between circadian and homeostatic signals as well as the local molecular clock work in the pineal and adrenal gland, respectively. On the other hand, melatonin and cortisol regulate, among others, sleep and rest/activity cycles. Thereafter, as mentioned before, it is expected that dysfunction of the circadian clock can lead to sleep disturbances; nonetheless, the interaction between sleep and depression likely comprises more than a failure of the biological clock [6].

Huntington Disease

Huntington disease (HD) is a neurodegenerative condition characterized by progressive motor, psychological and cognitive decline, as well as circadian disturbances and sleep disorders. Alterations in intrinsic circadian rhythmicity

include a delayed-phase position of hormones such as melatonin and cortisol. A significant lower number of vasoactive intestinal peptide (VIP) and AVP in immunoreactive neurons in the SCN of patients with HD indicate the compromise of the central clock in this disease [98].

Additionally, R6/2, a transgenic mouse model of HD, also shows a complete disorganization of circadian behavior, with arrhythmic expression of two clock genes, mBmal1 and mPer2 and reduced levels of VIP and its receptor VPAC2 in the SCN [99, 100].

Williams Syndrome

Williams syndrome is a genetic neurodevelopmental disorder caused by the deletion of LIMK1 and other genes on the long arm of chromosome 7 [101, 102]. Liston and collaborators showed that GC-mediated nontranscriptional regulation of LIMK1 and cofilin is critical in generating new spines. Thus, these authors propose that LIMK1 and cofilin signaling pathways may likewise contribute to cortical spine abnormalities in stress-related neuropsychiatric diseases [43].

As mentioned previously in this chapter, GC rhythms are critical for cognitive functions. For example, as Liston and coworkers discuss in their recent work, learning-induced spine remodeling and memory are enhanced when learning occurs during the circadian peak or coincides with elevated GC secretion and when subsequent GC troughs remain intact. On the contrary, they observe that disruption of the GC rhythm reduces the survival of learning-associated new spines and impairs memory retention, indicating that loss of the glucocorticoid oscillation may contribute to cognitive deficits in neuropsychiatric diseases [43].

Regarding the mechanisms underlying GC regulation of spine turnover, the same authors found that corticosterone increases both spine formation and pruning in the mouse cortex, but effects on formation are faster than those on elimination. Corticosterone-mediated spine formation involves a rapid non-transcriptional mechanism while the delayed spine elimination entails mineralocorticoid receptor-dependent transcriptional mechanisms, which mediate the regulation of several genes expression, many of them involved in synaptic plasticity [43].

Smith-Magenis Syndrome

Smith-Magenis syndrome (SMS) is a disorder characterized by intellectual disability, multiple congenital anomalies, obesity, neurobehavioral abnormalities, and a disrupted circadian sleep–wake pattern. SMS results from an interstitial deletion of chromosomal region 17p11.2, including retinoic acid-induced 1 (RAI1), or heterozygous mutation of RAI1. Some of the pathological alterations can be explained by a phase shift of the melatonin rhythm (i.e., melatonin peaks during the day instead of at night) and associated sleep-phase disturbances in patients suffering SMS. Additionally, mice lacking RAI1 show altered expression of circadian Clock, Per2, Npas2, Nrfd2 genes in the hypothalamus during light and dark phases, abnormal circadian behavior, and a shortened endogenous period. All of the above observations support SMS as a circadian-rhythm-dysfunction disorder [103].

Disclosures/Conflicts None.

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The Role of Iron and Other Trace Elements on Mental Development and Cognitive Function

12

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Introduction

Cognition is defined as "the mental processes by which knowledge is acquired". These include perception, reasoning, acts of creativity, problem solving, and possibly intuition. Cognition is important for quality of life. The appropriate levels of micronutrients and trace elements are recognized as essential for maintaining cognitive functions [1]. Putting aside the problems of toxicity from excessive intake of trace elements, there are two periods where an unbalanced intake of trace elements can cause disorders with profound alterations of cognition throughout life. These are: (1) childhood development and (2) aging.

(1) Micronutrient malnutrition impairs cognitive performance and developmental potential in children [2]. In 2001, Benton [3] reviewed 13 studies that investigated the role of multiple micronutrients on cognition in children aged 6–16 years, of which most reported a positive effect of the micronutrient supplementation, mostly with nonverbal measures. The author theorized that performance on nonverbal tests results, at least in part from basic biological functions, could be influenced by diet. In contrast, verbal intelligence comprises the acquired knowledge that was thought not to be affected by nutrition in the shorter term. Furthermore, it remained unclear whether there are other specific cognitive domains beyond nonverbal intelligence that could be influenced by micronutrient supplementation and whether the effects would depend on other factors (i.e., age and nutritional and socioeconomic status). Since Benton's review, additional trials have been published in the literature, most of which were conducted in developing countries. Children in developing countries have a more monotonous diet in general, and may have a higher risk of micronutrient deficiencies. Hence, these children might ben-

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efit from micronutrient supplementation more than their peers in developed countries [4].

In 2010, in a posterior study, Eilander et al. [4 systematically reviewed the literature that was current to date and performed a meta-analysis to quantify the effect of multiplemicronutrient interventions on cognitive performance in children from infancy through late adolescence (i.e., 0–18 years old). Moreover, because they expected heterogeneity among the studies, they explored whether factors such as age, country, nutritional status, duration, and type of micronutrient supplementation would predict the effects of micronutrients on cognition. The meta-analysis suggested the possibility of a small positive effect of multiple micronutrient supplementation on fluid intelligence (reasoning ability), which was not statistically significant, and a positive effect on academic performance (based on a limited number of four trials) in children 5–16 years old. There were no effects on crystallized intelligence (acquired knowledge) and other cognitive domains.

On the other hand, iodine is required for the production of thyroid hormones, which are necessary for normal brain development and cognition. Globally, >1.9 billion people, including 285 million children, have an inadequate iodine intake [5]. This deficiency is a serious problem and therefore this topic is deeply studied in all internal medicine and endocrinology manuals, which is why this element is not analyzed in this chapter.

(2) Trace elements are key regulators of metabolic and physiological pathways known to be altered during the aging process and therefore have the capacity to modulate the rate of biological aging. Optimal intake is required to maintain homeostasis and to increase cell protection. Deficiencies are associated with specific illnesses. However, the contribution of commonly observed life-long suboptimal intake of trace elements to the development and severity of age-related chronic diseases is less appreciated. Additionally, reduced intake of several trace elements has been shown to be particularly challenging for elderly people [4]. Dementia is one of the most pressing public health problems with social and economic implication. The form called cognitive impairment non-dementia (CIND) represents a subclinical phase of dementia. Different studies have shown a possible effect of micro- and macro-nutrients on cognitive function. Because trace elements are involved in metabolic processes and redox reactions in the central nervous system (CNS), they could influence the cognitive functions. Smorgon et al. [6] evaluated the presence of an eventual correlation between serum trace element concentrations and cognitive function in a group of subjects with CIND and manifest dementia (Alzheimer dementia and vascular dementia), and compared them with a control group. In the study they found a positive correlation between cognitive function and selenium, chrome, cobalt, and iron serum levels, while a negative correlation was observed with copper and aluminum serum levels. Futhermore, some statistically significant differences in selenium, chrome, cobalt, copper, and aluminum concentrations were found among the groups. According to these results, the authors could suppose that selenium, chrome, and cobalt protect cognitive function, that copper influences the evolution of cognitive impairment, while aluminum contributes to the pathogenesis of AD (Fig. 12.1).

Consideration should be taken into account regarding the handling of trace elements. Because micronutrient deficiencies often coexist and synergistic effects of micronutrients on physical functions may indirectly affect cognition, supplementing children and elderly people with multiple micronutrients could have advantages over single micronutrient supplementation. In contrast, micronutrients might also have antagonistic effects, affecting their bioavailability and their functioning in physiologic processes which could lead to impaired cognitive functioning. Because iron and zinc and copper and manganese compete for intestinal uptake, a high dose of one of these minerals may limit the absorption of the others [7].

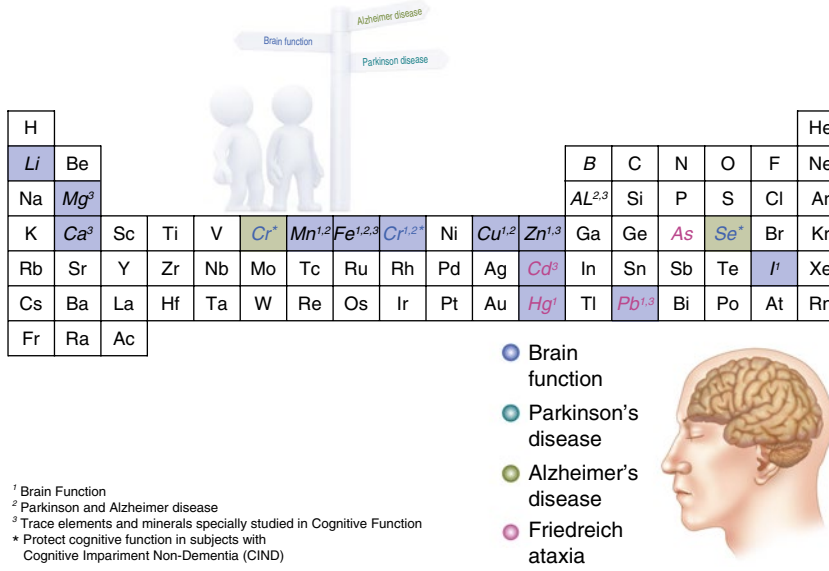

Fig. 12.1 Minerals and trace elements: relationship with cognition, brain function, Parkinson and Alzheimer disease and other diseases such as Friedreich ataxia

Iron

Observational studies have found relationships between iron-deficiency anemia in children and poor cognitive development, poor school achievement, and behavior problems. However, it is difficult to separate the effects of ID anemia (IDA) from other types of deprivation in such studies, and confounding factors may contribute to the association between ID and cognitive deficits [8].

Several possible mechanisms link IDA to altered cognition. Anemic children tend to move around and explore their environment less than children without anemia, which can lead to developmental delays [9].

Conduction of auditory and optic nerve impulses to the brain has been found to be slower in children with IDA. This effect could be associated with changes in nerve myelination, which have been observed in iron-deficient animals [10]. Neurotransmitter synthesis may also be sensitive to ID [11]. Impaired intellectual development in children can be prevented through the treatment or prevention of ID.

On the other hand, iron overload can be related to neurodegenerative disease. Several genetic disorders can lead to pathological accumulation of iron in the body; the body's tight control of intestinal iron absorption protects it from the adverse effects of iron overload [12]. Iron is required for normal brain and nerve function through its involvement in cellular metabolism and in the synthesis of neurotransmitters and myelin.

However, accumulation of iron excess can result in increased oxidative stress, and the brain is particularly susceptible to oxidative damage. Iron accumulation and oxidative injury are currently under consideration as potential contributors to a number of neurodegenerative diseases such as AD and PD [13, 14].

The abnormal accumulation of iron in the brain does not appear to be a result of increased dietary iron, but rather a disruption in the complex process of cellular iron regulation. Although the mechanisms for this disruption in iron regulation are not yet known, it is currently an active area of biomedical research.

Iron in the Brain

The iron needs of the brain vary with the stage of the life cycle and the cell types in the CNS. Iron is the key component of the many enzymes that involve essential oxidation/reduction reactions, synthesis of neurotransmitters, catabolism of neurotransmitters, and synthetic processes such as the production of myelin.

The highest levels of iron in the brain are found in the basal ganglia, but iron is also found throughout the brain, including the white matter [15]. Both biochemical and histochemical studies reveal white matter throughout the brain as a major site of iron concentration. Iron uptake into the brain is at a maximum during the period of rapid brain growth, coinciding with the peak of myelinogenesis.

Lack of iron in the diet for the first 2 years of life has an important effect on development because that is the time when the majority of brain growth occurs. Although the most rapid brain growth is seen in the months leading up to birth, at birth the brain has only reached 27 % of its adult size and it continues to grow for the next 2 years [16]. It has been shown that iron levels in the brain at birth are 10 % of eventual adult levels, with the remainder accumulating through childhood and young adulthood [15].

Research in humans was directed to impaired iron transport across the placenta in several prenatal conditions (e.g., diabetes mellitus, prenatal alcohol exposure, intrauterine growth retardation, maternal stress). There is direct evidence of decreased brain iron or ID in the offspring son and daughter [17].

Infants born of mothers with nutritional ID during pregnancy are rarely anemic, but they may have lower iron stores and ID sooner in the postnatal period. There is now solid evidence that brain ID can occur even with a normal hemoglobin level.

Brain iron accumulates from birth to early adulthood [15]. Perhaps for this reason, brain iron levels are affected more seriously by ID in the very young than in the adult animal. A brief period of severe ID in the young rat, but not in the adult, resulted in a deficit of brain iron which was not corrected by iron therapy although all signs of systemic ID were reversed [18].

This resistance contrasts with the rapid normalization of hepatic iron and hemoglobin (Hb) concentrations following iron repletion. The failure to reverse iron depletion in the brain with iron treatment seems to be the result of a slow rate of replacement of brain iron compounds [19].

The development of iron-deficient animal models has been an invaluable tool for looking at the consequences of ID. Most animal studies have involved rats because the distribution of iron in their brain is comparable with that of the human brain. Animal studies have shown that ID is associated with hypomyelination of neurons in the developing brain [20].

It must be recognized, however, that there are certain limitations in using animal models. Rats are less mature at birth, and humans and rats have different rates of neuronal development, both of which are particularly important when assessing the effect of early iron deprivation on mental function [21].

There has been considerable research on the possible mechanisms through which IDA affects cognitive function. Most emphasis has been placed on a direct neurochemical effect. ID causes low levels of brain iron, which leads to a reduction in neurotransmitter levels, impaired transmitter function, hypomyelination, and delayed neuromaturation. Another possibility is that the systemic effects of anemia lead to low oxygen delivery to the brain, directly affecting cognition [22].

When ID occurs early in the development of the rat, there are lasting deficits in brain iron, electrophysiological changes, a decrease in the number of dopamine D2 receptors, and alterations in neurotransmitter function, hypomyelination, and persisting behavioral changes that suggest an altered threshold of arousal [23].

The second hypothesis for the mechanism is that ID also has an indirect effect on behavior. IDA infants and children have been shown to be less attentive and less responsive. Lower developmental scores may reflect poorer mother/child interaction because of the child's reduced responsiveness, and this could result in less effective stimulation of the environment. The lower scores can also reflect poorer interaction with the developmental assessor [24].

To specifically understand the role of iron in neural circuits that underlie learning and memory development and function, in more recent models, iron uptake genes have been genetically manipulated in a tissue- and time-specific manner to generate a non-anemic model of hippocampal ID. The models are capable of isolating the role of iron independent of the potential widespread confounding effects of brain and body ID that accompany maternal dietary restriction (e.g., hypoxia, uptake of other divalent metals [25].

Optimal neurodevelopment is shaped by a variety of factors including growth factors, synaptic activity, and environment. Structures are most sensitive to these factors during rapid development [26].

As noted above, humans are most vulnerable to early ID from late gestation through 2–3 years of age, during the most rapid period of hippocampal structural maturation. Functionally, hippocampus-dependent memory appears and matures between 3 and 18 months of age [27].

This increased metabolic activity is coincident with extensive dendrite arborization, spine formation, and synaptogenesis [28] as well as the maturation of electrophysiological plasticity [29]. In conjunction, the timing and energy demands of hippocampal development with long-term deficits support the vulnerability of the structure to the metabolic consequences of early-life ID [30].

The effects of early-life ID on hippocampus-based learning and memory have been largely ascribed to primary abnormalities in iron-containing proteins, although many effects can be attributed to iron-containing proteins (e.g., reduced neuronal energy capacity).

Iron is necessary for energy production and cellular metabolism because it is essential for many mitochondrial enzymes integral for oxidative phosphorylation and adenosine triphosphate (ATP) production, including cytochromes, Nicotinamide adenine dinucleotide phosphate, and flavoproteins [31]. Adequate energy availability is necessary to support neuronal development and synaptic activity.

At birth, the brain comprises 50 % of resting metabolic energy [32, 33]. Approximately one half of the energy consumption is used to maintain Na^+, K^+, and Ca^{2+} gradients necessary for generating membrane potentials required for synaptic transmission. In addition, the generation and maintenance of the complex neuronal structure requires large amounts of energy.

Another important cellular process dependent on iron availability is nucleic acid metabolism. Iron-containing enzymes such as ribonucleotide reductase, deoxyribonucleic acid (DNA) helicase elongation protein 3, and BACH1 are integral for deoxynucleotide triphosphates (dNTP) synthesis, DNA transcription, elongation, and repair, and histone modification [34, 35].

The exact mechanism by which ID induces these acute and persistent gene expression changes is not clear because the experiments utilized maternal dietary restriction models of early IDA and the alterations may be due in part to the contribution of hypoxia. However, the evidence suggests that early life ID affects the regulation of gene expression throughout life in the hippocampus.

Another set of important signaling pathways that are likely affected by ID are found in mitochondria. The cellular functions of mitochondria reach beyond ATP synthesis and include maturation of Fe–S proteins that are crucial for cell function [36, 37]. As part of their function, mitochondria are an important factor in the regulation of intracellular Ca^{2+} levels. This function is crucial for many aspects of neuronal function, including secondary signaling cascades, neurotransmitter release, and apoptosis [38].

Iron Deficiency and Neural Functioning in Humans

Impaired Intellectual Development in Children

Infancy is considered the age range of highest vulnerability for the CNS because it corresponds with the brain growth spurt and the unfolding fundamental mental and motor processes. Altered behavior and development are among the greatest concerns regarding ID in infancy, especially because the nutrient deficiency is most prevalent in the period between 6 and 24 months of age.

Because this age range coincides with a period of maximal brain growth and the unfolding of many neuron developmental processes, several investigators have focused on the question of CNS effects on ID [39].

In observational studies, anemia and ID are associated with cognitive deficits, suggesting that iron supplementation may improve cognitive function and the studies show cross-sectional associations between IDA and poor cognitive function, motor development, behaviour, or school achievement levels [9]. However, it is difficult to separate the effects of IDA from other types of deprivation in such studies, and confounding factors may contribute to the association between ID and cognitive deficits [40].

In observational studies, anemia and ID are associated with cognitive deficits, suggesting that iron supplementation may improve cognitive function. The effect of iron supplementation on a range of health outcomes in infants and young children has been well documented. It is estimated that 47 % of preschool children worldwide have anemia, the highest prevalence of any population group [41].

Longitudinal studies show that ID in infancy is related to poorer cognition in childhood [9]. One systematic review that included seven randomized controlled trials on the effects of supplementary iron in young children with anemia or ID found no evidence of an effect of iron supplementation on psychomotor development [42], while another included 17 randomized controlled trials in children of any age and with any initial iron status, found that iron supplementation was not associated with improved mental development scores in children younger than 5 years [43], or with improved physical growth [44].

A more recent systematic review addressed a range of health risks and benefits of iron supplementation in infants and children 5 years old or younger [45], finding that supplementation led to improvements in cognition and motor development in children with anemia and ID, but was associated with increased risk of death in areas with endemic malaria.

As previously explained deficiency of enzymes involved in the development of parts of the brain is important for cognitive functions such as memory (e.g. the hippocampus). Deficiency and supplementation may have different effects on infants and young children than in other population groups.

Older Children, Adolescents, and Adults

Older children and adolescents are less at risk of anemia than preschool children, but global statistics indicate that approximately 25 % of older children have anemia, as do 30 % of non-pregnant women and 42 % of pregnant women, and 17 % of elderly people (rising to 40–50 % of those admitted to hospital or living in nursing homes), demonstrating that it is a very large and important health problem [41, 46].

A meta-analysis has been published to assess whether iron supplementation improved cognitive domains: concentration, intelligence, memory, psychomotor skills, and scholastic achievement in adults. Evidence was found that iron supplementation improved attention and concentration in adolescents and women, regardless of baseline level of iron status. Iron supplementation also improved performance in intelligence quotient (IQ) tests in adults and children who were anemic at baseline, but had no effect in other groups or on other cognitive domains [47].

The prevalence of depleted iron stores is substantially greater in pre- or perimenopausal women than in postmenopausal women or in men. Poor iron status affects premenopausal women more often than men because of the combination of low dietary iron intake, menstruation, and gestational requirements. Physical performance is affected by poor iron status, including decreases in work productivity, voluntary activity, and athletic performance. Cognitive, affective, behavioral, and neurophysiologic decreases have been associated with poor iron status in premenopausal women [48].

It is important to consider that ID can often be present without anemia [41]. A requisite for successful treatment is the correct diagnosis of depletion and to assess the causes of ID [49].

Iron supplementation may be less effective when there are a number of nutritional problems at baseline (all of which may be contributing to

cognitive limitations) than when patients are nutritionally replete except for variations in iron status. Iron and zinc deficiencies often occur together, and zinc deficiency can be exacerbated with a high dose of iron supplements [50]. Zinc may also play a role in cognitive function, therefore iron supplementation could exacerbate cognitive deficits [51].

Although it is not surprising that the brain functions poorly when iron is deficient, the long-term deficits, despite iron repletion, remain mechanistically enigmatic and a fruitful area of research. Furthermore, this research may contribute to defining the time point at which iron repletion can no longer reverse the behavioral phenotype. It would be critical in determining the optimal timing of iron treatment regimens.

Because iron is not only a critical nutrient for brain development but also a potentially toxic element, further research is also necessary to determine optimal iron doses. Arguably, an iron-deficient developing brain that has responded to ID by prematurely expressing large amounts of iron transporters [52] can be at risk for iron overload and generation of reactive oxygen species if large amounts of medicinal iron are suddenly delivered to this "activated system".

Iron Overload and the Central Nervous System

Several proteins implicated in brain iron homeostasis are involved in disorders associated with abnormal iron metabolism. A basic understanding of mechanisms of iron homeostasis has a clinical relevance, as either accumulation or depletion of intracellular iron may impair normal function and promote cell death.

Iron accumulates in selective brain regions during aging, in acquired neurodegenerative disorders such as AD and PD, and in genetic disorders such as neurodegeneration with brain iron accumulation (NBIA). Dysregulation of iron homeostasis is also a critical feature of FA [53].

Iron is known to catalyze the formation of reactive oxygen species (ROS), such as hydroxyl radical, and initiation or enhancement of lipid peroxidation by reacting with hydrogen peroxide (H_2O_2) via the Fenton reaction [54]. ROS are highly reactive oxygen-containing radicals that can easily react with other molecules such as protein, DNA, lipids, and antioxidants [55, 56].

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + \bullet O_2^-$$

$$2\bullet O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow \bullet OH + OH^- + Fe^{3+}$$

Iron can also react with lipid peroxides in a way similar to its reaction with H_2O_2 and produce alkoxy (R-O·) and peroxy radicals (R-O_2·). The resulting peroxy radical leads to propagation of lipid peroxidation [57].

Lipid peroxidation can proceed until the lipid radicals interact with one another and/or a "chain breaker," such as vitamin E, forming a no-radical species.

$$Fe^{2+} + R - OOH \rightarrow Fe^{3+} + R - O^{\cdot\cdot}$$

$$Fe^{3+} + R - OOH \rightarrow Fe^{2+} + H + R - O_2^{\cdot\cdot}$$

$$R - O^{\cdot\cdot} + L - H, (L - OOH) \rightarrow ROH + L^{\cdot}\left(LOO^{\cdot}\right)$$

Iron compounds such as free Hb can also catalyze peroxidation of purified arachidonic acid and other polyunsaturated fatty acids within normal cell membranes in the presence of H_2O_2 and $\bullet O_2-$ [58].

The CNS is separated from the systemic circulation by the blood–brain barrier (BBB), a tight epithelial barrier analogous to that of the mammalian duodenum, which is the site of absorption of dietary iron. After absorption, iron in its oxidized (ferric) form binds to serum transferrin and is distributed throughout the general circulation. The presence of a BBB explains the relative independence of the brain iron from the total body iron content [59].

Incorporation and Transport of Iron in the Nervous System

Iron is transported to the brain via the BBB which is composed of endothelial cells in small vessels throughout the brain that contain tight junctions which regulate the brain iron levels.

Iron incorporation and transport in the brain depends on interactions between the endothelial cells and astrocytes. Brain endothelial cells express the transferrin receptor 1 (Tf R1) in their luminal membrane. This receptor binds iron-loaded transferrin and internalizes this complex in endosomes.

Within the endosomes, the acid environment facilitates the release of ferric iron from transferrin and this is followed by reduction of ferric to ferrous iron by action of endosomal reductases. The mechanism by which iron is released from transferrin is transported from the interior of the brain endothelial cells to the interstitial fluid is a matter of controversy [60, 61].

One possibility is that ferrous iron is transported from the endosome to the cytosol by the divalent metal transporter-1 (DMT1) and then exported into the extracellular fluid by action of ferroportin [60].

However, there is some disagreement as to the degree of expression of DMT1 and ferroportin and their contribution to iron transport across the brain endothelial cells. Alternatively, it has been proposed that the transferrin-Tf R1 receptor complex is transported within the endosomes from the luminal to the abluminal surface where endosomes release iron at the interface between the endothelial cells and astrocyte end-foot processes [61].

The end-foot processes express ceruloplasmin, which acts as a ferroxidase that oxidizes newly released ferrous iron to ferric iron and binds to the transferrin in the brain interstitial fluid [60–62]. Transferrin is the main source of iron for neurons, which express high levels of Tf R1. Whereas transferrin is synthesized by oligodendrocytes, the primary source of transferrin in the brain interstitium is its diffusion from the ventricles.

It has been shown that transferrin uptake and the ratio of iron to transferrin uptake by the brain decrease with age, and the transferrin recycling time increases with age [63].

Neuronal Iron Homeostasis

There is a tight regulation of the cytosolic iron pool in brain cells, and is critical for two reasons: (1) iron is an important source for numerous cytosolic, mitochondrial, and nuclear ferroproteins; and (2) excessive accumulation of free cytosolic ferrous iron predisposes to oxidative stress and cytotoxicity.

Iron regulatory proteins sense cytosolic iron levels and interact with iron-responsive elements and regulate translation of mRNA encoding for proteins involved in iron uptake, storage, and mobilization, including Tf R1, ferritin, and ferroportin.

In the cytosol, the storage protein ferritin sequesters and reduces levels of free iron. Ferritin consists of a heavy [ferritin heavy chain (FTH1)] subunit that catalyzes the rapid oxidation of ferrous to ferric iron and a light (FTL) sub-unit that may be involved in the nucleation of the iron core within the protein shell. Thus, ferritin has a dual function of iron detoxification and iron reserve [64].

Ferritin is also present in axons and may transport iron to the synapse. Ferroportin, present in synaptic vesicles, may allow release of ferrous iron at the synapses [60, 61]. Neuromelanin is an insoluble pigment produced from oxidation of excess cytosolic catechols and is present in granules in dopaminergic neurons of the substantia nigra and in noradrenergic neurons of the locus ceruleus. Neuromelanin binds iron avidly forming stable complexes and sequesters large amounts of iron in those cells [65].

Mitochondrial iron is required for heme biosynthesis and for the generation of iron/sulfur (Fe/S) clusters in many essential enzymes. Iron is transported into the mitochondria by mitoferrin, a transporter expressed in the inner mitochondrial membrane.

Frataxin is a mitochondrial iron chaperone that is involved in the biosynthesis of Fe/S clusters by interactions with critical assembly proteins. [62, 66–68] The Fe/S clusters form the prosthetic group of many enzymes of the respiratory chain and also constitute the main mechanism for iron exit from the mitochondria.

The major route of iron export out of the brain is via the cerebral spinal fluid (CSF) and its reabsorption into the blood from the subarachnoid space. The concentration of transferrin in CSF is very low and its capacity to export iron is limited. Lactoferrin, ferritin, and non-protein-bound iron

are also present in the CSF and may contribute to iron export. In normal conditions, iron concentration is very low but can increase considerably under pathologic circumstances as discussed below. Microglia and other phagocytic cells are additional important mediators of iron export after cell death and intracerebral hemorrhage.

Iron accumulates in the brain as a function of age, primarily in the form of ferritin, particularly in the microglia and astrocytes, but also in the oligodendrocytes. The brain areas richest in iron are the globus pallidus, substantia nigra, putamen, caudate nucleus, dentate nucleus, and frontal cortex [69].

Impaired regulation of iron homeostasis in those cells may lead to either excessive accumulation of free cytosolic iron or decreased iron availability for critical enzymes. Free cytosolic ferrous iron reacts with endogenously generated hydrogen peroxide to yield hydroxyl radicals, which damage cell membranes. A recent study identified a signaling cascade that links the activation of the N-Methyl-D-aspartate (NMDA) receptors, which mediate glutamate triggered excitotoxicity, with iron homeostasis. This cascade involves activation of nitric oxide synthase and adaptor proteins that interact with ferroportin [70].

Iron deposition or dysregulation occurs in several neurodegenerative disorders, including sporadic AD, PD, FA, and NBIA.

Brain Neurodegenerative Disorders and Iron

Dysregulation of iron homeostasis is also a critical feature of AD, PD, NBIA and FA. Although these diseases have their own distinctive features, they all have one thing in common: accumulating iron in the brain. Increased iron in the brain, rich in oxygen and fatty acids, provides an ideal environment for oxidative stress and possible irreparable tissue damage.

The link between iron and neurodegenerative disease provides potential therapeutic targets for these disorders. If, indeed, iron and/or oxidative processes are involved in the pathogenesis of neurodegenerative disorders, approaches such as iron chelation therapy and antioxidant supplements might help to slow the degenerative processes or ameliorate brain tissue injury.

Alzheimer Disease

AD is a progressive degenerative disease with a gradual deterioration in memory, cognition, behavior, and the ability to perform activities of daily living. Evidence of increased brain metal levels such as iron and copper has been associated with AD [71].

The amyloid-beta (Aβ) plaques in the brain are the hallmark pathologic features of AD and are derived from the cleavage of amyloid precursor protein (APP). Deposition of fibrillar aggregates of Aβ in the brain parenchyma, which is caused by Aβ overproduction, impaired clearance, or both, has been hypothesized to explain the cause of AD [72].

APP has binding sites in its amino-terminal domain and in the Aβ domain for copper and iron. Iron is primarily complexed with ferritin and concentrated in the neuritic processes associated with amyloid plaques [69, 73]. Iron might have a direct impact on plaque formation through its effects on APP processing by alfa-secretase, deposition of Aβ, and oxidative stress [63, 73].

High levels of iron may interact with the Aβ peptide, leading to the reduction of molecular oxygen to superoxide and eventually to H_2O_2 by reducing iron. It has also been demonstrated that overexpression of the carboxyl terminal fragment of APP (Aβ) significantly reduces the level of copper and iron in the transgenic mouse brain. This suggests a role for APP and Aβ in physiologic metal regulation in AD [74].

Additionally, overexpression of melanotransferrin has been reported in AD. Because purified melanotransferrin can bind iron, it has been proposed as another protein that also might be involved in iron transportation [75].

The role of iron in the pathogenesis of AD is thought to be related to enhance oxidative stress mediated by free iron. Transferrin, ferritin, and iron regulatory protein 2 also have been associated with neurodegeneration in AD. The latter might be responsible for the disturbance in brain iron homeostasis and the overall decompartmentalization of

iron and the resulting oxidative processes suggestive of AD [74].

Genetic alterations specific to iron-management proteins, including HEF1 mutations (associated with congenital hemochromatosis) or the transferrin subtype C2, may increase the risk of AD [76].

Friedreich Ataxia

FA is an autosomal recessive neurodegenerative disease characterized by degenerative atrophy of the posterior columns of the spinal cord followed by the spinocerebellar tracts and corticospinal motor tracts, leading to progressive ataxia, sensory loss, and muscle weakness. It is also characterized by degeneration of large sensory neurons of the dorsal root ganglion, cerebellum (particularly the iron-rich dentate nucleus), and cardiomyocytes.

FA is an autosomal recessive disorder resulting from a large guanine adenine adenine (GAA) triplet-repeat expansion in the first intron of the Friedreich ataxia (FRDA) gene, resulting in a reduction in expression of the encoded protein, frataxin [66–68, 77].

Frataxin is a mitochondrial protein and is suggested to have a role in mitochondrial iron transport or in iron-sulfur assembly and transport. High levels of iron in the mitochondria can react with superoxide ($\bullet O_2-$) and H_2O_2 to produce the hydroxyl radical (•OH), which can oxidize cellular components, damage respiratory chain complexes, and result in cellular injury and eventually cell death.

Whereas excess mitochondrial iron is detected in neurons and cardiomyocytes from affected patients, indicating a predisposition to oxidative stress, there is evidence that impairment of heme and Fe/S cluster biosynthesis is the most likely proximate cause of neurodegeneration in this disorder [62, 66–68, 77].

Brain and heart cells depend highly on aerobic metabolism and are more susceptible to free radical generation in mitochondria [78, 79]. The effects of treatment with the antioxidants coenzyme Q and vitamin E in patients with FA are being studied, and preliminary results seem to be promising [80].

Parkinson Disease

PD is a progressive disorder manifesting as tremor at rest, bradykinesia, gait abnormalities, rigidity, postural dysfunction, and loss of balance. Iron has been suggested to be responsible for nigrostriatal dopamine neuron degeneration in PD owing to its ability to produce toxic ROS and cause lipid peroxidation [81].

PD is characterized by iron accumulation in dopaminergic neurons of the substantia nigra. Free cytosolic iron may trigger oxidative stress and promote alfa-synuclein aggregation with deposition of Lewy bodies [69].

Lewy bodies can have deleterious effects on the extrapyramidal system and on psychomotor function [82, 83]. The presence of the pigment neuromelanin in the substantia nigra in PD also might result in iron accumulation because neuromelanin can function like ferritin and store iron [84].

Over expression of lactoferrin (a protein that reversibly binds iron) receptors on neurons and microvessels in regions of neuronal degeneration in PD-affected brain tissue suggests a possible link to iron overload in affected brain regions [82].

All these mechanisms suggest that disturbances in iron homeostasis and metabolism in PD occur at several levels, such as iron uptake, storage, intracellular metabolism, release, and posttranscriptional control [85].

As indicated in the preceding text, a disturbance in iron homeostasis can provide a favorable condition in which free iron, via generation of ROS, causes permanent tissue damage. Neuromelanin may exert neuroprotective action at early stages of PD because it prevents free iron accumulation and thus hydroxyl radical production and formation of neurotoxic dopamine quinones [65].

However, in advanced stages, extravasation of neuromelanin granules from dying neurons may attract and activate microglia, causing release of neurotoxic molecules leading to cell injury [65]. Both iron chelation and over expression of iron-sequestering ferritin have been shown to be protective in animal models of PD [69].

Type I Neurodegeneration with Brain Iron Accumulation

Type I NBIA (NBIA-1) was formerly known as Hallervorden-Spatz syndrome or pantothenate kinase-associated neurodegeneration.

NBIA-1 is a rare, genetically determined neurodegenerative disorder characterized by extrapyramidal dysfunction and mental deterioration. Iron accumulates mainly in the globus pallidus and the pars reticularis of the substantia nigra, and presents as brown-pigmented iron deposits [86].

NBIA comprises a clinically and genetically heterogeneous group of disorders that include pantothenate kinase–associated neurodegeneration (PKAN), infantile neuroaxonal dystrophy, neuroferritinopathy, and hereditary aceruloplasminemia [87]. These disorders are characterized by iron accumulation and cell loss affecting primarily the globus pallidus and, in many cases, retinal photoreceptors [87].

PKAN is caused by a mutation of the gene encoding pantothenate kinase 2 (PANK2), the key enzyme in the synthesis of mitochondrial coenzyme A. The mutation in a novel pantothenate kinase gene, PANK2, is predicted to cause the accumulation of cysteine, which binds iron and causes oxidative stress in the iron-rich globus pallidus [88].

Neuroferritinopathy is a dominantly inherited, adult-onset disorder caused by mutations in the ferritin light chain (FTL1) gene [89–91]. It results in accumulation of ferritin-iron aggregates in neurons and glial cells of the globus pallidus, substantia nigra, striatum, and cerebellum. Vacuolated glial and neuronal nuclei may be characteristic of the disease [90]. Focal onset limb dystonia or chorea, orolingual dyskinesia, dysarthria, aphonia, and dysphagia are prominent features.

Hereditary aceruloplasminemia is a rare autosomal recessive disorder caused by a mutation of the ceruloplasmin gene, leading to iron overload in the brain and reticuloendothelial system. It manifests with anemia, diabetes, retinal degeneration, and progressive neurologic disorder [92, 93]. A characteristic neuropathologic finding is the presence of enlarged deformed astrocytes and accumulation of spheroid-like, grumose foamy deposits in the astrocytic foot processes [94].

Application of high-resolution imaging technologies such as electron energy-loss spectroscopy and electron tomography may allow early identification of the cell type and intracellular location of iron deposits in patients with neurodegenerative disorders.

If, indeed, iron and/or oxidative processes are involved in the pathogenesis of neurodegenerative disorders, approaches such as iron chelation therapy and antioxidant supplements might help to slow the degenerative processes or to ameliorate brain tissue injury. Several chelators are currently under investigation for treatment of these and other disorders associated with abnormal brain iron homeostasis.

The neuroprotection that is provided by iron chelators in animal models indicates that iron-chelation therapy could be a viable neuroprotective approach for treatment of disorders such as PD or AD.

Manganese

Manganese is an essential nutrient that is common in the environment. It is the fifth most abundant metal and the twelfth most abundant trace element in the earth's crust [95]. It is necessary for the adequate functioning of the human CNS, but it also has the potential to produce neurotoxic effects when, depending on the route and dose of exposure, it accumulates in an organism (particularly in the brain) exceeding the homeostatic range [96]. It is released into the environment as a product of industrial activities, the use of the manganese-containing pesticides such as Maneb® and Mancozed®, and through the use of methyl-cyclopentadienyl manganese tricarbonyl, as a gasoline antiknock agent (Agency for Toxic Substances and Disease Registry, ATSDR, 2000).

The vast majority of studies on neurotoxic effects of manganese were conducted in occupational settings where exposure occurs mainly through inhalation of airborne particulates (ferroalloy smelting, welding, mining, battery assembly, etc.). Several studies have demonstrated the impact of manganese toxicity in adults, resulting

in cognitive, neurological, motor, and psychological impairment [97–102].

In the past years, many studies have investigated possible overexposure of children to manganese and subsequently the neuropsychological effects produced. It is generally accepted that children are at greater risk than adults exposed to the same contaminants from the environment [103].

In general, with normal dietary consumption, systemic homeostasis of manganese is maintained. Although very low levels of manganese in air, soil, water, and food are normal, nevertheless, excess exposure can occur by inhalation in areas where there is high manganese concentration in air and dust, or by drinking water that has had long contact with bedrock enriched in manganese, or by consuming high amounts of food sources rich in the trace element. The highest concentrations are found in nuts, legumes, and blueberries teas [104]. Other authors have hypothesized the possibility of overexposure to manganese through ingestion of infant milk formula [105], or even by iatrogenic manganese exposure than occurs in individuals receiving total parenteral nutrition resulting in increased concentrations of manganese in the brain [106]. Furthermore, exposure to at-risk populations with compromised or immature BBBs or underdeveloped excretory pathways (i.e., children) can also result in increased brain manganese levels [107].

Several factors could predispose children to manganese overexposure and subsequent toxic effects. Exposure to manganese by ingestion or inhalation can have different consequences in children than in adults and through different mechanisms [108]. First, children are exposed to a larger amount of manganese from inhalation because of their higher breathing rates (the ratio of inhaled air/weight is much higher in children because of the lower body mass), and greater intestinal absorption rate [103, 109]. Absorption can be as high as 80 % in neonates compared with 1–5 % in adults [11]. Second, high demand for iron linked to growth could further enhance the absorption of ingested manganese [110]. Third, a low excretion rate was observed in infants because of their poorly developed biliary excretion mechanism [111]. In fact, exposure during this period may result in increased delivery of manganese to the brain and other tissues.

Neurotoxic effects resulting from excessive manganese exposure were first described in 1837 by Couper in Scottish laborers who were grinding manganese black oxide in the chemical industry [112]. Neurological symptoms of *manganism* include decreased memory and concentration, fatigue, headache, vertigo, equilibrium loss, insomnia, tinnitus, trembling of fingers, muscle cramps, rigidity, alteration of libido, and sweating [113]. Many reports of neurotoxic effects in manganese-exposed workers were later published [100], and the definition of manganese intoxication has evolved to include subclinical signs of intoxication indicated by alterations of neurobehavioral functions [114].

Manganese plays a role in immune response, blood sugar homeostasis, ATP regulation, reproduction, digestion, and bone growth [115]. It is a necessary component of metalloenzymes such as manganese superoxide dismutase, arginase, phosphoenol–pyruvate decarboxylase, and glutamine synthetase. This glutamine synthetase enzymeconverts glutamate into glutamine. Glutamine synthetase [116].

Manganese shares several characteristics with iron; both are transition metals with valences of 2^+ and 3^+ in physiological conditions and proximate ionic radius. In addition, as manganese and iron both strongly bind to transferrin and accumulate in the mitochondria, low iron stores are associated with increased manganese uptake and retention in the blood [117], and increment of the accumulation in CNS. More than 2,000 million people on our pleanet, mainly children and pregnant women (and/or in fertile age), manifest ferropenic anemia after inadequate iron absorption. The potential effects associated to the accumulation in CNS of manganese in these populations represent a sanitary challenge of great magnitude. Manganese can accumulate in the CNS, particularly the basal ganglia but also the cortex. Exposure to manganese has been shown to interfere with several neurotransmitter systems, especially in the dopaminergic system in areas of the brain responsible for motor coordination,

attention, and cognition [117, 118]. Manganese is a potent dopamine oxidant, which could explain the toxic lesions in certain dopaminergic brain regions [119]. Excessive exposure could result in dopamine receptor loss or inactivation through damage to the membrane mediated by free radicals or cytotoxic quinones generated by the manganese catalyzing effect on autooxidation of this neurotransmitter [120]. The correlation between manganese and hyperactive behavior is probably a result of the dopaminergic and gamma-aminobutyric acidergic systems, which play a role in hyperactivity in children [121, 122].

One hypothesis for the toxic mechanism of manganese is the production of excess free radicals in the nerve cell, potentiating lipid peroxidation, and resulting in tissue destruction [122, 123]. Manganese neurotoxicity has been extensively studied and a lot has been learned about its mechanism of action at the cellular and molecular levels and the detection of subclinical effects at low exposures. In the last few years, several literature reviews have been published on aspects such as neurotoxic effects on exposed laborers [118, 124], the application of magnetic resonance imaging [125], neuropsychological testing for the assessment of manganese neurotoxicity, manganese neurotoxicity focused on neonates [126], neurotoxicology of chronic manganese exposure in nonhuman primates [127], and manganese exposure and neuropsychological effect on children and adolescents [108, 128].

In most studies, authors observed that manganese exposure was associated with poorer cognitive functions and hyperactive behavior. Many have suggested that manganese exposure is related to cognitive, motor, and behavior deficits in children. Some of them found an adverse effect of manganese on cognitive function, and overall an inverse association between manganese exposure and IQ [129–131]. Others studies have focused on motor effects of manganese, finding a positive association. Very few studies focused on the effects of manganese on children's motor skills, although data on motor effects in adults, occupationally or environmentally exposed to manganese, have been reported [132, 133].

The majority of the studies published have several limitations including sample size, research design, the lack of a validated biomarker of exposure or exposure index for manganese, and the lack of attention to mixed exposure. In this sense, most studies focused on a single agent of exposure and did not measure or adjust for potential effects of other chemicals.

Finally, despite these limitations, it is believed that adverse effects of manganese exposure in children is well demonstrated through different studies. Nevertheless, further investigations should promote preventive strategies to reduce manganese exposure.

Cadmium

Cadmium is a heavy metal found in the earth's crust that is released to the environment both by natural processes and by human activities such as fossil fuel burning, waste incineration, smelting procedures, mining, from factories of industrial production, mires of residual waters, and the use of phosphate fertilizers [134]. It is used in many industrial processes which include silver plating, paint, plastic stabilizers and nickel-cadmium batteries. The highest exposure to cadmium in humans is dietary. High levels are found in shellfish, liver, and kidney [135]. Soil cadmium is absorbed easily by plants. In general, leafy vegetables such as lettuce and spinach, potatoes and grains, peanuts, soybeans, and sunflower seeds contain high levels of cadmium. Cadmium also tends to concentrate in shellfish from polluted coastal waters. Another important route of exposure is through tobacco smoke (tobacco leafs accumulate high levels of cadmium from the soil). Cadmium blood levels in smokers are approximately twice as high as those of nonsmokers. Finally, occupational exposure is another important source to take into account.

Cadmium is toxic to the CNS of fetuses and infants. During pregnancy it interferes with the placental function, alters various enzymes, and modifies the availability of nutrients and essential elements in the CNS [136].

Neonatal exposure alters the levels of neurotransmitters such as norepinephrine, dopamine, serotonin, and acetylcholine. Cadmium exposure is also associated with increased free radical production in tissues causing damage to the cell membrane and changes in a variety of other physiological functions. Its fetal neurotoxic effects are the indirect result of impaired placental function, enzymatic dysfunctions, and metabolic alteration of essential trace elements for the CNS. Additionally, cadmium has been found to stimulate DNA synthesis and cell multiplication at low levels but increase apoptosis and chromosomal aberrations at high levels, suggesting different effects at low and high levels.

In prior risk assessments, kidney damage had been considered the most sensitive endpoint of cadmium toxicity, and reference levels for urinary cadmium have been established to protect against this effect because cadmium can be ingested or inhaled and can enter into the bloodstream and be stored in the liver or kidneys (EFSA 2009: 1 μg Cadmium/g creatinine, WHO/FAO 2011: 5.24 μg Cd/g creatinine [137]).

Chronic exposure to cadmium induces the production of the metallothionein protein that binds the metal and reduces its toxic effects. However, acute intermittent exposures may elude this mechanism and induce severe toxic responses.

Cadmium is a metal that has no essential biological function and may interfere with normal neurological development via different mechanisms [138]. Many studies have examined the neurological consequences of early exposure to cadmium.

In a recent study, the urinary levels of cadmium in 1,305 Bangladeshi women in the early stages of pregnancy and the levels of their children at 5 years of age were assessed. Both the maternal urinary cadmium levels and the concurrent urinary cadmium levels in their children were inversely associated with the intelligence of the children at 5 years of age [139]. A recent analysis of subsets of children from the National Health and Nutrition Examination Survey (NHANES) (1999–2004) suggested that children who have higher urinary cadmium concentrations may be at increased risk for learning disorders and are more likely to require special needs education [140].

Elevated cadmium exposure in adults has been linked to a variety of neuropsychological deficiencies such as reading difficulties, behavioral problems [141], poor visual-motor performance, complaints of decreased concentration [142], reduced attention, psychomotor speed, and memory [143] as well as lower cognitive scores among elderly adults with [144] or without concomitantly elevated zinc exposure [145].

Antother recent study evaluated the associations between neurocognitive exam scores and a biomarker of cumulative cadmium exposure among adults in the NHANES III. The results provide support for the evidence suggesting that cadmium exposure may be associated with diminished neurocognitive performance in adults. The relationships observed in this study were detected at cadmium exposure levels that are typical of US adults and are below the current WHO/FAO reference level [146].

On the other hand, the usual overlapping exposures to lead and cadmium make difficult the relative contribution of each metal on the observed effects. Lead and cadmium both cross the immature BBB and accumulate in the developing brain [147]. A significant correlation between high levels of cadmium and lead in hair and hyperactivity has been shown in children, with decreased verbal development and lower IQ. Lead and cadmium probably affect different aspects of intelligence. Lead levels are associated with a reduction in IQ, whereas increasing cadmium levels correlate with decreased verbal capacity [148]. At cadmium levels below the median, there was a significant interaction between the two metals that was antagonistic during the early pregnancy period. However, this antagonistic interaction occurred at a very low level for both cadmium and lead [149]. These findings suggest that there may be a dose-dependent interaction between prenatal lead and cadmium with respect to the effects of these heavy metals on neurodevelopment. They also demonstrate the biological complexities of examining the neurodevelopmental effects of co-exposure to multiple toxicants. For this reason, further research is necessary in this area.

Copper and Zinc: Cognition Loss

Serum Free Levels and Cognitive Function

Free copper appears to be a player in cognitive decline. Association of lower cognitive function with increased free copper may imply intoxication from this element. Perhaps when its deregulation is induced by deficiency, the free fraction increases even as total copper in the body decreases, and higher values of free copper were associated with lower cognitive function, which is of pathophysiological importance in cognitive decline [150]. Free copper may be a risk factor in the development of impaired cognition. It has been hypothesized in some studies [151] that there is a relationship between the physiological levels of copper in cognitively normal individuals and their cognitive performance. They showed a significant inverse correlation of the serum levels of free copper with both Mini Mental State Examination and attention-related neuropsychological tests scores [151]. Free copper may modulate attention skills via a disturbing action on the neurons of the locus coeruleus. A hint connecting copper metabolism to neuronal viability of the brain structures involved in attention comes from the fact that in the normal brain, copper deposition is higher in the prefrontal cortex, nucleus caudatus, substantia nigra ,and locus coeruleus, all structures that have been associated with attention [151].

Copper Toxicity and Cognition Loss in Alzheimer Disease

There is a strong temporal association with the use of copper plumbing in developed countries and the epidemic of AD. But association does not prove causation, many other things are also associated with development. It has been postulated that the environmental factor is beef eating and AD is a prion disease. Beef eating is certainly associated with development, but there is no supporting evidence that AD is a prion disease [152, 153]. Organic copper is absorbed into the bloodstream and taken up by the liver, which then funnels it into safe channels. The inorganic copper that is absorbed directly into the blood is the part of the free pool which is the readily available and potentially toxic copper of the blood. Some authors have described that the blood free copper pool is increased in AD. Evidence sustains that the higher level correlates negatively with cognition in AD [154]. It has been postulated that inorganic copper ingestion from drinking water and copper supplements is a major factor in triggering the epidemic of AD [152, 154]. Toxicity of this element may be causing a decline in cognition in the aging general population [154].

There are other risk factors for AD fitting with the idea of a toxic role for copper: (a) *Age*, (b) *Apolipoprotein E4 (ApoE4) genotype*, (c) *High fat diet*, (d) *elevated homocysteine levels*, (e) *Certain "iron management" genes alleles* (certain hemochromatosis and transferring alleles), and (f) *Certain Wilson disease (WD) gene (ATP7b) alleles:* [152]:

(a) *Age:* Age fits with any risk factor because it simply increases the amount of exposure [155].
(b) *Apolipoprotein E4 genotype:* It is believed that copper binding ApoE alleles help remove copper from the brain. ApoE4 confers risk (because it has no copper binding cysteine), while ApoE2 is protective (ApoE2 has two copper binding cysteines) and ApoE3 is slightly protective (ApoE3 has one copper binding cysteine).
(c) *High fat diet:* A high fat diet causes risk of AD. There is a correlation between fat intake and prevalence of AD across countries. It seems to be a risk factor for AD and cognition loss, and appears to work in conjunction with inorganic copper ingestion.
(d) *Iron management genes alleles:* Elevated homocysteine levels are a risk factor for AD as well as for artheroscherosis. Homocysteine binds copper, and then oxidizes cholesterol to intermediates toxic neurons. Hemochromatosis and transferring alleles convey an increased risk of AD. Iron and copper cause toxicity in the same way, by increasing oxidant damage.

(e) *WD gene alleles:* Single nucleotide polymorphisms associated with the gene ATP7b, the WD gene, affects risk of AD. Certain alleles of ATP7b cause higher copper levels in WD and these higher levels increase risk of AD.

Copper Toxicity and Mild Cognitive Impairment Subjects

In patients affected by AD, serum copper not bound to ceruloplasmin ("free" copper) appears elevated, slightly but significantly enough to distinguish AD subjects from healthy elderly subjects. Free copper can help in discriminating mild cognitive impairment (MCI) subjects from healthy subjects, but not on an individual basis [156]. The clinical condition of MCI is characterized by memory impairments and is verifiable via objective measures. It precedes the clinical definition of dementia in severity. The importance of an accurate diagnosis of MCI lies in the fact that, despite the mildness of the condition, MCI is normally considered a a precursor of AD. AD is an irreversible, progressive neurodegenerative disorder, characterized by a gradual appearance of cognitive deficits, leading to full dementia. It is a genetically heterogeneous syndrome which includes a broad spectrum of phenotypes. Overt pathological alterations in the AD brain are diverse and include neuron loss, synapse loss, amyloid plaques, neurofibrillary tangles, and microgliosis, as well as functional changes, included metal imbalance, oxidative stress, and changes in cell cycle mediators [156]. Free copper also seems to disturb cognitive performances in healthy subjects. Ceruloplasmin and free copper levels increase in inflammatory conditions. However, copper increase in general circulation can also be explained in terms of its release consequent to neuronal death, and this could be the reason why it is not structural to ceruloplasmin. Free copper is also among the WD diagnostic tools which loses balance in the presence of defects in the ATPase 7B, the protein responsible for the correct copper incorporation into nascent ceruloplasmin. Defects in copper assemblage into ceruloplasmin, because of a minor ATP7B genetic defect such as heterozygosis for WD mutations must be taken into consideration also for AD [156]. Free copper is a fraction of copper loosely bound to and exchanged among albumin and micronutrients such as amino acids (histidine) and peptides. Free copper can easily cross the BBB, probably due to its binding to amino acids in processes mediated by some amino acid transport systems. Metal-related abnormalities (mainly copper, iron, zinc) have been shown to be related to Aß and tau toxicity, leading to AD pathology. CSF Aß, total, and hyperphosphorylated tau proteins are core CSF markers of AD dynamics and have been recently tested as markers for MCI, particularly in relation to brain imaging alterations or clinical symptoms.

Free copper could be a predictor for those patients with a more severe decline [156] and altered serum copper homeostasis predicts cognitive decline in MCI [157]. In one study [157] a significant elevation was observed in the ratio of copper to iron in serum in MCI subjects who subsequently progressed to dementia. This elevation appears to be transient as subjects with early AD were nearly identical to controls and stable MCI subjects and longitudinal data show progressive MCI cases trend downward over time. Altered serum copper homeostasis may serve as a biomarker to identify subjects with subject memory complaints who are at risk of developing further cognitive decline [157].

Zinc Deficiency Associated with Cognition Loss and Alzheimer Disease

Patients with AD are zinc deficient. Zinc has many protective roles in neurons, and zinc deficiency may play a causal role in AD. Zinc therapy appears to at least prevent some cognition decline. In addition to restoring normal levels, it reduces serum free copper levels in AD [158]. Serum zinc declines with age in people for unknown reasons. The neurons of many parts of the brain have high zinc levels, and it is clear that this element plays many critical roles in neurons. In some neurons, high concentrations of zinc are

secreted along with glutamate into the synapse. Glutamate initiates firing and zinc quenches, or shuts down, the firing. With inadequate zinc, glutamate-induced firing persists and can damage the neuron [158].

Serum zinc levels decline with aging, but patients with AD present lower levels of serum zinc [159], therefore, patients with AD are zinc deficient by serum status. ZnT_3 is the pump that loads synaptical vesicles with the metal. The content of these vesicles is secreted into the synapse and the released zinc plays many important neuronal functions [153].

Extracellular amyloid plaques in the AD brain are avid zinc binders, further depleting available zinc for neurons. One of its important neuronal functions is to limit glutamate neuronal firing. Glutamate excitotoxicity damages neurons and may be a problem in many neurodegenerative diseases. Excess glutamatergic excitotoxicity is believed to be a common occurrence in many neurodegenerative disorders, including AD [158].

Another possible mechanism by which low availability of zinc in the brain can have harmful effects is through failure of adequate inhibition of calcineurin. Increased neuronal calcineurin activity as a causative factor in AD is postulated, because it is increased in AD brain. It affects many downstream biochemical functions adversely. Calcineurin activity is increased by exposure to β-amyloid and inhibited by zinc. It seems increasingly likely that neuronal deficiency is playing an important, perhaps a key, role in decreasing neuronal function and increasing damage leading to cognition loss in AD. The zinc depletion of aging, exaggerated in AD, and the loss of ZnT_3 function with aging, exaggerated in AD, leads to severe neuronal deficiency and neuronal damage. Increased zinc in the brain may allow it to displace copper from sites where copper is generating oxidant radicals, and thus reduce the damage from copper.

Zinc therapy significantly slows cognition loss in AD, significantly lowering blood-free copper in patients with AD, which may occur in the brain as well thus limiting copper toxicity in this manner. Zinc could be mediating these effects, interacting with copper, while also independently stabilizing neuronal health [153].

To add to the problem created by systemic zinc deficiency there is another mechanism in the AD brain that depletes neurons of much-needed zinc. The beta amyloid plaques, which build up in the AD brain, are avid binders of this metal. Thus, it seems likely that the neurons of the AD brain are seriously lacking in available zinc and many are probably injured and die as a result [158].

Patients with AD are more zinc deficient than age-matched controls, and that deficiency by amyloid plaques further depletes the neurons of zinc. We pointed out how important adequate zinc is for neuronal health [158], and ingestion of inorganic copper in drinking water and zinc deficiency both contribute to cognition loss [153].

Other Trace Elements

Aging is associated with neurobehavioral deficits. Certain brain areas are more vulnerable to neuronal degeneration than others, reflecting an altered resistance to stress of the tissue itself and/or the lack of adequate immunological defense mechanisms in these regions. Calcium and iron are mediators of the aging process in the normal brain. Enhanced calcium levels are related to apoptosis. Excess concentrations of a number of elements in the brain are capable of producing harmful effects by displacing some essential elements, while in turn, numerous toxic and essential elements have been reported to be imbalanced in AD.

AD is the most common cause of dementia among older people. Trace elements may be important in the pathogenesis of AD. Metal ions are concentrated in senile plaques, neurofibrillary tangles, and CSF. These findings support this notion. Several metals have been proposed as pathogenic cofactors in AD, but various toxic heavy metals (i.e., cadmium, lead, and mercury) are especially prevalent in nature because of their high industrial use. These metals serve no biological function and their presence in tissues reflects contact between the organism and its environment. Metals could be connected to the

risk factors for dementia or the pathophysiology of dementia. Although toxicity and the resulting threat to human health are a function of the concentration of a contaminant, chronic exposure to arsenic, cadmium, mercury, and lead at relatively low levels can cause adverse effects [160].

Arsenic exposure induces changes that coincide with most of the developmental, biochemical, pathological, and clinical features of AD and associated disorders [160, 161]. Inorganic arsenic at high doses is a known neurotoxin with both neurodevelopmental and neurocognitive consequences. From a neuropathological standpoint, arsenic exposure has been associated with an increase in the production of β amyloid, hyperphosphorylation of tau protein, oxidative stress, inflammation, endothelial cell dysfunction and angiogenesis, all of which have been linked to cognitive dysfunction and are proposed mechanisms underlying AD [160].

Lead is the most historically pervasive and well-established neurotoxic pollutant. Lead can cause white matter damage, cell death, and changes in cellular architecture. It is believed to interfere with functions essential for neuronal homeostais, such as inhibiting glycolytic enzymes in neurotransmitter metabolism [160]. Workers with high blood lead concentrations present an association among mild impairment of attention, verbal memory, and linguistic processing. Lead affects specific areas in the brain such as the hippocampus and frontal cortex. Neural systems subserving language functions are more sensitive than other cognitive functions to perturbations from the effects of lead. Nervous system symptoms such as irritability, poor attention and concentration, forgetfulness, depressed effect, and sleep disturbance are common after lower doses. Even at very low levels, lead is associated with impaired cognitive function in children. Chronic, low-dose exposure to lead may adversely affect cognitive function in older age in several ways. Several possible mechanisms that could result in structural changes in the brain support the hypothesis that there is a relationship between lead and cognitive decline. Lead could increase apoptosis. It causes changes in cellular architecture, increases oxidative stress, or enhances vascular or inflammatory mechanisms. Lead alters the permeability of the BBB and is accumulated within the astroglia that is an essential element for the maintenance of the neuronal environment. Exposure to lead interferes with several calcium-dependent processes and activates protein kinase C, which has been implicated in neurotoxicity. Exposure to lead in a non-occupational setting is associated with accelerated decline in cognition [160].

Oxidative stress appears to play a major role in chronic cadmium-induced hepatic and renal toxicity. The lateral choroids plexus sequesters mercury, cadmium, arsenic, and lead. This is probably an important mechanism to protect the CSF and the brain from fluxes of heavy metals in the blood. However, mercury and cadmium can directly damage the choroid plexus [160]. Blood cadmium concentrations lower than the level known to produce acute toxicity do not affect cognitive functions [160].

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New Insights in Glutamate-Mediated Mechanisms Underlying Benzodiazepines Dependence and Cocaine Vulnerability

13

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Benzodiazepines and Cocaine: Pharmacological Actions

The pharmacological actions of benzodiazepines (BZD) are mediated by a high-affinity site on GABA-A receptor, widely distributed on neurons into the central nervous system (CNS). The binding of BZD agonists results in facilitation of GABA-operated chloride channels, that is, enhancement of the inhibitory actions by GABA [1–4]. BZD are prescribed for reduction of anxiety and aggression, sedation, induction of sleep, reduction of muscle tone, and anticonvulsant effect usually requiring long-term treatment. It is generally understood that the neurophysiological activity of the mammalian brain is maintained by the balance between inhibitory (i.e., GABA) and excitatory (i.e., glutamate) neurotransmission. Indeed, there is close interaction between GABA-A receptors and the *N*-methyl-D-aspartate (NMDA) receptors in the CNS [5, 6]. For example, kindling induced by the GABA-A receptor channel blocker pentylenetetrazol was prevented by NMDA receptor antagonist treatment [7, 8]. A protective effect of muscimol against NMDA-induced neuronal injury has also been reported [9]. Furthermore, it has been demonstrated that pentylenetetrazol and BDZ inverse-agonist induced seizures are suppressed by the noncompetitive NMDA receptor antagonist dizocilpine [10, 11]. Previous studies on rodents have shown that chronic exposure to BDZ produces tolerance and physical dependence [12, 13]. Clinical studies have also demonstrated that the long-term use of BZD often results in tolerance to and dependence on many of the therapeutic actions of BZD [14–16]. Additionally, severe withdrawal syndrome has been reported with seizures and even death in humans [17, 18]. Indeed, the mechanisms by which BZD tolerance and dependence are mediated have been the focus of much interest.

Another drug commonly used with nontherapeutic purposes is cocaine (COC). Despite its known harmful effects, abuse of this illegal drug is one of the most important problems in public health worldwide [19]. COC is an alkaloid obtained from Erithroxylon coca leaves, an ancestral plant originating from Bolivia, Perú, Colombia, and Ecuador. COC was chemically isolated in 1859 and therapeutically used as local anesthetic in 1890 because it blocks voltage gated sodium channels [20–22]. COC also impairs the

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reuptake of different monoamines such as noradrenaline, dopamine (DA), and serotonin in the presynaptic terminal in the CNS. The sustained monoamine increases on the synaptic gap, resulting from the repeated exposure to COC and other psychostimulants, can influence the synaptic plasticity in different brain areas [23–25], synaptic remodeling on the postsynaptic membrane, and the anatomical structure of the dendrites spines [26]. Pharmacological effects of COC include increase in energy, improved mood, alertness, reduced appetite, and fatigue. COC produces a general increase of psychomotor activity and improves output of repetitive and simple tasks, causing a euphoric state. Once this state disappears, a dysphoric sensation is experienced causing the desire for a new drug experience [27].

Effects of Psychoactive Drugs in the Brain: Dependence and Addiction

Drug addiction is defined as the loss of control over the intense urges to take drugs even at the expense of adverse consequences. Such loss of control may develop as a result of deregulation of the dopaminergic reward system in the brain [28]. Drug dependence is defined as neural adaptations in several brain areas, including those related to the reward system, which is associated with severe withdrawal symptoms when drug use is discontinued. These phenomena are particularly persistent because even after withdrawal from use, patients remain vulnerable to cravings, and relapse can be elicited by reexposure to stimuli previously associated with drug use [29]. Then the transition from use to abuse is in accordance with neuroplasticity, considered as the CNS ability to modify its responses to certain stimuli depending on previous experience. In this context, addiction could be considered as an exceptional potent and persistent form of plasticity because cravings can persist for years in humans [30], but in animals, conditioned responses to stimuli associated with drug administration are resistant to extinction [31].

The ventral tegmental area (VTA) and the nucleus accumbens septi (NAS) are part of the mesolimbic system, which is an integral component of a brain reward system. DA neurons in the VTA provide one of the major sources of DA to the limbic structures, including the NAS. DA has been implicated in the encoding of reinforcement and in learning [32]. The striatum is the major entry into the basal ganglia, receiving inputs from all areas of cortex as well as afferents from the thalamus and limbic structures such us the HP and amygdala [33]. The NAS is considered a limbic–motor interface to which relevant stimuli are processed to influence initiation of behavior [34–36] and thus plays a key role in generating motivated behaviors related to natural rewards as well as drugs of abuse [37]. Descending projections from the frontal cortex to the NAS and other brain regions exert inhibitory control over reward-seeking behaviors. Interconnections between these areas constitute the main neuronal circuitry involved in the neurobiology of addiction.

Drug use and abuse has been a problem faced by mankind for centuries, yet little progress has been made toward developing effective treatment strategies because the fact that focusing on DA reward mechanisms has not yielded an effective therapy for addiction. New treatment strategies must then consider chronic drug use-induced neuroplasticity that underline synaptic and circuit efficiency, and fundamental adaptive behavioral processes such as learning [38].

Several animal models have been used to mimic addiction in humans such as conditioned place preference, drug self-administration, and sensitization, among others. Sensitization is defined as an increased drug response by repeated exposure [39–41]. Sensitization to psychostimulants such as COC is characterized by progressive increases in locomotor activity and produces persistent functional brain alterations [42, 43]. Because sensitization can be context dependent, long lasting, and can increase subsequent drug self-administration [44], it is a useful model of relapse in humans [45, 46]. Furthermore, drug sensitization has been observed in humans and can contribute to enhancement of psychoses with repeated psychostimulant exposure.

It has been shown that after chronic COC administration, only a portion of animals exposed to the drug exhibit sensitization [47–50], and it was associated with alterations in DA and glutamate release [39, 49, 51] and increased α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor surface expression in the NAS [47], and impairment of the inhibitory control mechanisms from the frontal cortex [52, 53]. Furthermore, we have described that passive and repeated COC administration to rats was able to cause development of sensitization to the locomotive effects of COC, in a range of 50–60 % of the animals, independent of the administration protocol and the dose used [50].

On the other hand, as was mentioned previously, repeated BZD administration induced development of tolerance and dependence to many BZD pharmacological properties in both animals and humans [12–15, 54, 55]. In fact, acute exposure to BDZ increases firing of DA neurons of the VTA by stimulation of presynaptic GABA-A receptors in nearby interneurons. The resulting disinhibition can activate plasticity mechanisms within excitatory inputs to VTA contributing to the reinforcement effects of BZD [56]. An anxiety-like behavior in rats is expressed as a mild withdrawal symptom of chronic DZ administration, which was assessed by the activity of rats in an elevated plus maze [12, 55]. Using this test, many studies have described that chronic DZ administration induces dependence in around 60 % of rats exposed to the treatment [57, 58].

Maladaptative Learning and Memory Processes During Addiction: Role of Hippocampus and Medial Prefrontal Cortex

Pavlovian conditioning is a form of associative learning related to the contingency between two stimulus events. Repeated administration of drugs often results in the conditioning of physiological responses. These conditioned responses can be distinguished from other direct and indirect drug effects by the fact that, under appropriate circumstances, they can be elicited without drug administration. This form of classical conditioning is important because it may occur whenever drugs are chronically administered. The drug administration process may act as a conditioned stimulus that will eventually elicit a conditioned response. These conditioned responses have been suggested as playing a role in drug tolerance, dependence, and sensitization [57, 59–61]. A large body of evidence demonstrates that a context previously associated with drug experience increases the risk of relapse, even after years of withdrawal [62–64]. Furthermore, development and persistent expression of addictive behaviors occur through the usurpation of natural learning and memory mechanisms within the limbic system, and long-lasting neuroadaptations resulting from repeated drug exposure involve an associative learning process [42, 57, 58, 65]. These learning and cognitive aspects of addiction suggest the existence of common neurobiological mechanisms mediating drug addiction and memory [37, 42, 66, 67]. Both phenomena are accompanied by alterations in synaptic plasticity at glutamate synapses in the reward pathway, involving the VTA, NAS, and frontal cortex [67, 68].

In addition to the relevance of the reward circuitry in the development of addiction, other brain areas such as the HP have been implicated [69] because HP sends projections to NAS through the ventral subiculum. Furthermore, it is known that contextual memories recruit the HP, a structure that plays a large role in processing the associations between the environmental context and unconditioned stimuli, such as drug sensitization and abuse [70, 71]. A major form of synaptic plasticity in HP is long-term potentiation (LTP) characterized by an enduring increase in the efficacy of glutamatergic synaptic transmission. This phenomenon is accepted as a molecular mechanism for learning and memory in the brain in which contextual cues are relevant [72, 73].We have demonstrated a marked enhancement in the HP dentate gyrus synaptic transmission after both sensitization to the locomotive effects of COC [50, 74] and dependence to DZ [55, 57, 58]. Moreover, a modulatory effect on CA1 HP synaptic plasticity was observed after chronic COC treatments [75].

The mPFC is a brain structure implicated in executive function and emotional regulation [76–79]. Physiological DA modulation in the mPFC is required for several cognitive processes such as decision making, working memory, and goal planning, impairments of which contribute to addiction and other diseases such as schizophrenia. The mPFC is involved in several aspects of drug addiction, including the primary rewarding effects of COC and mechanisms underlying addiction and craving [80]. In humans, the mPFC is activated during COC withdrawal [81] and also by cue-induced COC craving [82]. In rodents, the mPFC is necessary for the development of behavioral sensitization induced by COC and other psychostimulants. Lesions of the mPFC prevent the development of COC sensitization as well as neuroadaptations in VTA and NAS [83], indicating that intact glutamatergic outputs from the mPFC are necessary for the enduring neuroadaptations related to COC addiction and psychostimulant-induced behavioral sensitization [84, 85].

Adaptive changes observed during repeated COC administration modify the normal DA modulation in mPFC, contributing to the reinforcing effects of COC, development of COC sensitization, drug-elicited conditioned place preference, COC self-administration, and reinstatement of COC seeking and relapse [80]. Furthermore, membrane excitability of pyramidal neurons within the mPFC is increased and the glutamatergic output from the mPFC is facilitated following chronic treatment with psychostimulants. In fact, the inhibitory effects of dopamine on mPFC neuronal activity are found to be attenuated, whereas glutamate-induced excitation is enhanced following repeated COC or amphetamine administration [51, 86]. Moreover, the density of voltage-gated outward potassium currents is also significantly decreased in a voltage-dependent manner, and repetitive firing evoked by depolarizing current pulses is facilitated in COC pretreated mPFC neurons [87], indicating that the glutamatergic outputs from the mPFC are facilitated, particularly in the NAS of rats that had developed behavioral sensitization to COC [49]. Repeated COC administration alters membrane properties and ion channel function of rat mPFC pyramidal neurons after withdrawal, increasing the number of spikes evoked by depolarization [88], reducing activity of whole-cell voltage-gated potassium channels [87] and inward rectifiers potassium channels [88], resulting in a persistent increase of membrane excitability in mPFC. All these data suggest that glutamatergic inputs from the mPFC play a critical role in COC-induced neuroadaptations in the reward system.

Nitric Oxide Participation in the Mechanisms Underlying Addiction

The diffusible neuromodulator NO is synthesized in the brain mainly by the neuronal NO synthase (nNOS) enzyme from L-arginine, and signals in multiple ways. NO activates soluble guanylyl cyclase (sGC) stimulating the production of cyclic guanosine monophosphate (cGMP) [89, 90]; it also may act through cyclic adenosine monophosphate formation, and it can react directly with proteins interacting with sulfhydryl groups of cysteine, a phenomenon called protein s-nitrosylation [91]. Neuronal excitability and synaptic plasticity are modulated by NO in different brain structures including the HP [92]. Furthermore, NO plays a major role in initiating and maintaining the behavioral effects of the psychostimulants [93, 94]. In fact, pharmacological or genetic nNOS activity disruption attenuates the development of sensitization, conditioned place preference, and self-administration of psychostimulants [43, 95]. In addition, nNOS inhibition during repeated COC administration prevented the persistent increase in membrane excitability of mPFC pyramidal neurons observed after a short-term withdrawal from COC [96]. Additionally, it has been demonstrated that NO could be involved in some of the acute effects of BZD, such as their antinociceptive [97], anticonvulsant [98], and hypnotic activities [99] and also in the development of tolerance after chronic treatment with DZ [100]. Furthermore, nNOS inhibition significantly attenuated the withdrawal syndrome (i.e., pentetrazole-induced seizures) in DZ-dependent mice [101].

Table 13.1 Distribution of sensitized and nonsensitized animals exposed to different treatments

Treatment	% Sensitized	% Nonsensitized
VEH+COC	49.02	50.98
7-NI+COC	31.04*	68.96
ODQ+COC	27.78**	72.22
SILD+COC	67.44***	32.56

Percentages of animals distributed in sensitized or nonsensitized groups in response to daily cocaine (COC) administration during 5 days preceded by vehicle (VEH), or the neuronal nitric oxide synthase (nNOS) inhibitor 7-nitroindazole (7-NI), or the soluble guanylyl cyclase (sGC) inhibitor 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one(ODQ) or the phosphodiesterase 5 inhibitor(PDE5i) sildenafil (SILD). Asterisks indicate differences from VEH+COC (Multiple 2 × 2 Chi-Square test: $*X^2_{(0.95)} = 3.67$; df =1; $**X^2_{(0.95)} = 3.24$; df = 1; $***X^2_{(0.95)} = 2.44$; df = 1) (adapted with permission from Springer Science+Business Media: Psychopharmacology, Involvement of nNOS/NO/sGC/cGMP signaling pathway in cocaine sensitization and in the associated hippocampal alterations: does phosphodiesterase 5 inhibition help to drug vulnerability? Table 1 of [74])

Although the mechanisms by which NO participates in behavioral sensitization and in the enhanced HP excitability after repeated COC administration are not well understood, a pharmacological approach was used to interfere or activate the nNOS/NO/sGC/cGMP signaling pathway. With this approach we clearly demonstrate that the proportion of sensitized animals increases from 50 % to approximately 70 % with activation of nNOS/NO/sGC/cGMP pathway, whereas blockade of this pathway reduced this proportion from 50 % to 30 % (see Table 13.1). A further observation supporting the participation of nNOS/NO/sGC/cGMP pathway is that sGC inhibition by 1*H*-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one (ODQ) prevented COC sensitization (Table 13.1) without affecting the hyperlocomotor effect, while upstream inhibition of this pathway (nNOS inhibition using 7-Nitroindazole [7-NI]) not only prevented COC sensitization but also reduced the hyperlocomotor effect. Oppositely, the enhanced cGMP availability by sildenafil (SILD), a phosphodiesterase 5 inhibitor (PDE5i), increased the proportion of sensitized animals (Table 13.1) [74]. These results support the possible involvement of nNOS/NO/sGC/cGMP pathway in the vulnerability to develop COC sensitization.

The NAS works as an interface between limbic and motor systems [102] and drug seeking behavior depends on glutamate transmission in this structure [103]. HP is one of the glutamate projecting afferents to NAS [104], and synaptic changes occurring in this structure can affect the level of neuronal activity within the NAS. HP LTP has long been postulated to underlie learning and memory processes and may play a role in the complex associative learning that contributes to drug-seeking behavior and relapse [42]. In fact, repeated COC administration enhances HP LTP [105], and theta burst stimulation of HP ventral subiculum reinstates COC-seeking behavior in rats [52]. We reported that COC sensitization induced an increased HP synaptic transmission, observed as a reduction in the threshold to generate LTP [50], and this enhancement was prevented by nNOS inhibition. Similar results were observed when sGC was inhibited by ODQ administration during repeated COC. On the other hand, when cGMP availability was increased using SILD, the COC-facilitated synaptic transmission was maintained [74]. This facilitation could indicate an increased susceptibility to stimuli able to strength glutamate synapses. Then, activation of nNOS/NO/sGC/cGMP pathway by repeated COC may facilitate the LTP generation in HP, activating glutamate containing pathways to NAS, finally responsible for motor execution of goal-directed behavior such as sensitization. These results are in accordance with the effects of cGMP up regulation on cortico-striatal synaptic transmission in vivo [106]. Surprisingly we further observed that the acute or repeated SILD treatment facilitated synaptic transmission in HP dentate gyrus, indicating a possible enhancement in the strength of glutamate synapses by sildenafil exposure [74], agreeing with previous reports showing that SILD induces long-term memory retention and reconsolidation [107] and rescues synaptic plasticity in an Alzheimer disease mouse model [108].

It has been demonstrated that a single COC exposure increases NO release in mPFC, HP, and striatum [109, 110] and repeated administration enhances nNOS activity and NO production in

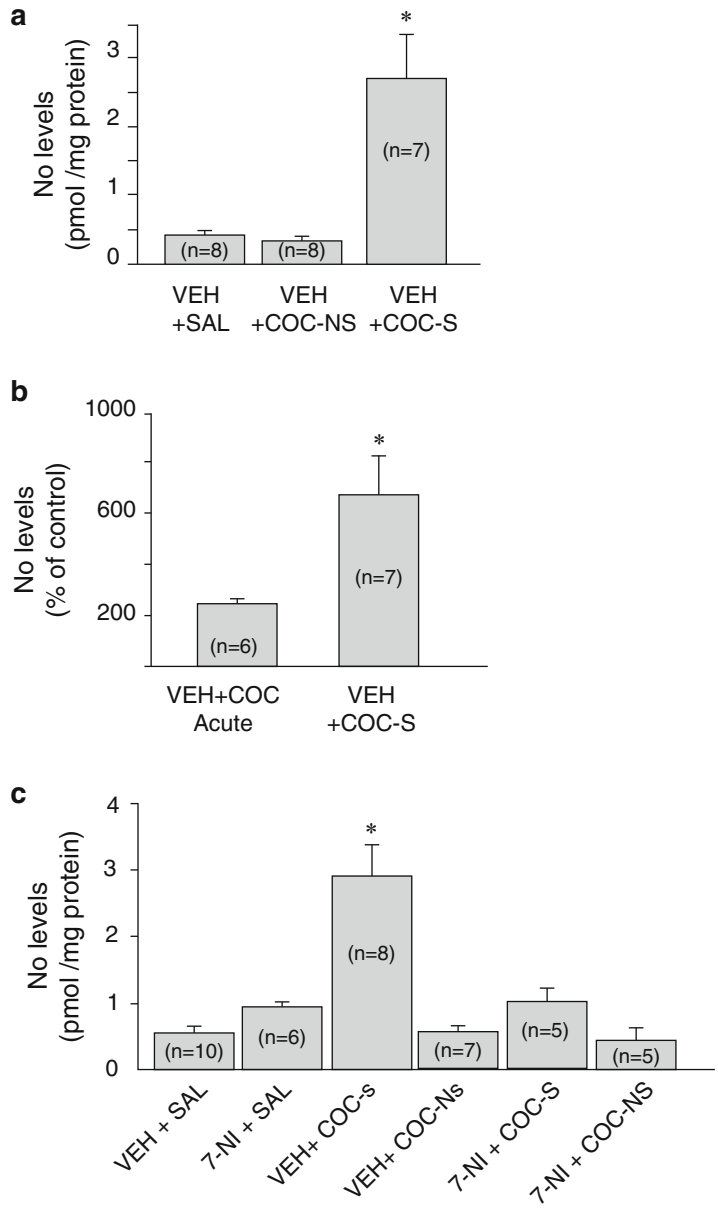

Fig. 13.1 Cocaine sensitization increases neuronal nitric oxide synthase (nNOS) activity in the hippocampus. Bars graph indicating nitric oxide (NO) levels in: (**a**) sensitized and nonsensitized groups with or without nNOS inhibition and their controls, $*p<0.05$ compared with other groups. Results are expressed as means of NO pmol/mg protein ± standard error (S.E.); (**b**) acute treated animals [vehicle (VEH)+cocaine (COC) Acute] and sensitized rats [VEH+cocaine-sensitized (COC-S)], $*p<0.05$. Results are expressed as means of percentage of their respective controls [VEH+saline (SAL) acute and VEH+SAL] ± S.E. Number of animals are indicated in *parenthesis* (adapted with permission from Springer Science+Business Media: Psychopharmacology, Involvement of nNOS/NO/sGC/cGMP signaling pathway in cocaine sensitization and in the associated hippocampal alterations: does phosphodiesterase 5 inhibition help to drug vulnerability? Fig. 3 of [74])

many brain regions [111]. We have observed that nNOS activity was increased in HP only in sensitized animals (VEH+COC-S) (Fig. 13.1). Furthermore, the percentage of nNOS activity increase in this group was considerably greater than in the acute COC group. These results may indicate that nNOS enzyme evidenced a neuroadaptative process of "sensitization" after repeated administration, which is associated with behavioral sensitization and elevated efficiency of hippocampal synaptic transmission [50]. We also showed that inhibition of nNOS during repeated COC administration (7-NI+COC-S) prevented the "sensitization" of the enzyme activity observed in sensitized animals (VEH+COC-S) (Fig. 13.1). These results are further supported by the correlation analysis between locomotor activity on day 5 and NO levels in both sensitized and nonsensitized groups in which high locomotor activity is positively correlated with elevated NO

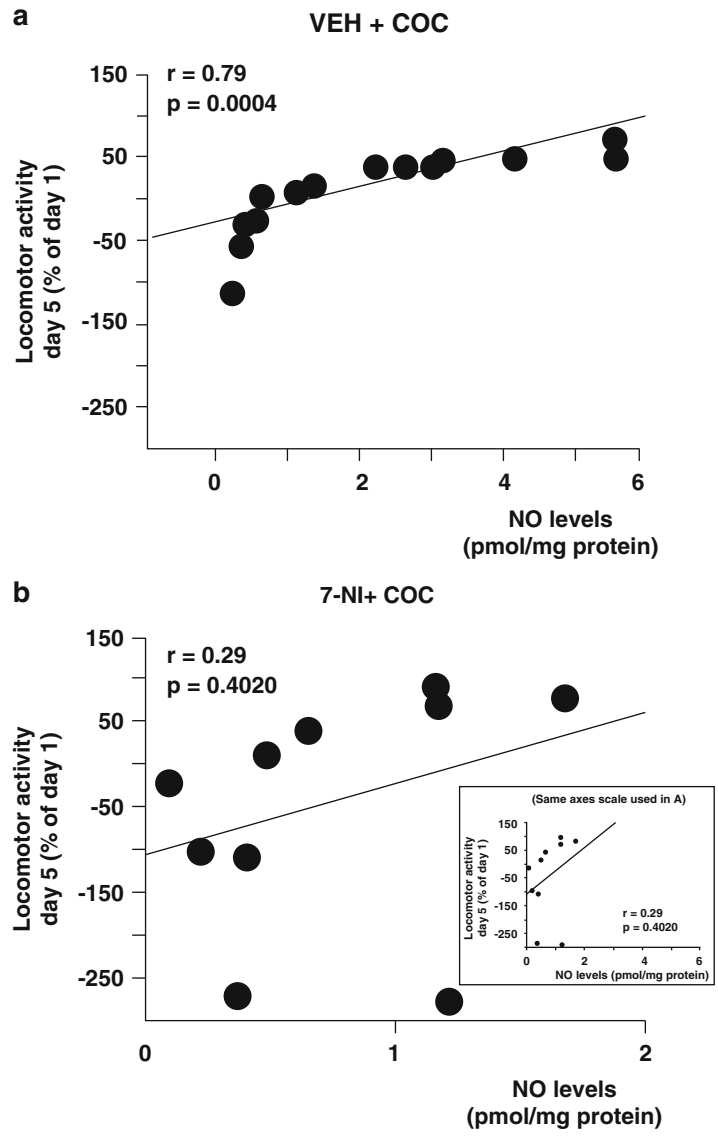

Fig. 13.2 Development of sensitization correlates with high levels of nitric oxide production in the hippocampus. Graphs showing correlation between the percent of increase in locomotor activity (day 5 respect to day 1) and the threshold to generate long-term potentiation (LTP) in: (**a**) vehicle (VEH)+cocaine (COC) (sensitized and nonsensitized) treated rats and (**b**) 7-nitroindazole (7-NI)+COC (sensitized and nonsensitized) treated rats. The correlation coefficients (*r*) are indicated in *bold* (adapted with permission from Springer Science+Business Media: Psychopharmacology, Involvement of nNOS/NO/sGC/cGMP signaling pathway in cocaine sensitization and in the associated hippocampal alterations: does phosphodiesterase 5 inhibition help to drug vulnerability? Fig. 4 of [74])

levels. However, correlation was weakened when nNOS inhibitor was administered concomitantly to COC (Fig. 13.2) [74]. Systemic administration of different nNOS/NO/sGC/cGMP modulators such as 7-NI, ODQ, or SILD, affects NO or cGMP availability in the whole CNS. Thus, changes observed in the HP can be the result of a primary effect of such inhibitors in brain areas related to the reward circuit that project to the HP, such as the VTA, modulating neuronal activity. A local effect in the HP cannot be ruled out because single or repeated COC administration induces increments in nNOS activity in this area.

Reversion of Diazepam Dependence: Behavioral and Pharmacological Strategies

It has been demonstrated that the environmental context of an experience induced by a drugs of abuse is crucial for drug-seeking behavior and relapse in human addicts [64]. Furthermore, learning and memory, particularly contextual memories, are important for the establishment of conditioned responses to drugs of abuse [64, 65]. In our work we described that environmental cues related to drug experience have a direct

impact on retrieval of the memory acquired when the DZ withdrawal signs were associated with the context of withdrawal because alteration in the cues presented 24 h after last administration prevented the expression of the anxiety-like behavior when animals were reexposed, 15 days after last administration, to the withdrawal environment. Alterations of contextual cues consisted of a new operator, modified location of the animals in the plus maze test room from the original assessment, and the animals were held with a piece of fabric. These changes constitute a significant alteration in the context of withdrawal and are able to modify conditioned responses previously observed. It is interesting to point out that manipulation of environmental cues during reexposure prevented the expression of anxiety-like behavior in the majority of animals considered to be DZ dependent. Only a small portion of them expressed anxiety during reexposure to the withdrawal environment in spite of the altered contextual cues [112]. This differential susceptibility could be attributed to the fact that the associative component can be particularly strengthened in these animals, and the environmental changes are not enough to disrupt the acquired memory. Moreover, because of the experimental design in which the experimental groups were defined by classification, we cannot rule out the possibility that other selected characteristics, unrelated to anxiety or dependence, may have caused the observed effects.

We have recently demonstrated that DZ dependence is associated with an increased HP synaptic plasticity in both short- [57] and long-term withdrawal [58]. Furthermore, alteration in contextual cues associated with the withdrawal experience previously described, not only impaired the expression of the anxiety-like behavior, but also disrupted the facilitated HP LTP in most of the DZ-dependent animals reexposed to the altered withdrawal environment. Interestingly, the animals that expressed anxiety-like behavior in spite of the altered contextual cues preserved the facilitation in HP LTP [112]. These persistent changes in synaptic strength during synaptic plasticity require new gene expression and protein synthesis [58, 113, 114]. The immediate-early gene activity-regulated cytoskeleton-associated protein (Arc) has been proposed as a marker of neuronal activity linked to synaptic plasticity and to consolidation of spatial memory in the HP, acquiring a key role in the organization of information storage in the CNS [115, 116]. We have reported increased HP Arc protein levels related to the facilitated LTP in DZ-dependent animals during short-term withdrawal [58]. Similar to the observed electrophysiological changes, we observed that the increased HP Arc protein levels were reversed in DZ-dependent animals reexposed to the plus maze test with contextual cue changes. However, only the small proportion of animals that expressed anxiety in spite of the altered contextual cues maintained increased Arc protein levels, indicating that Arc could be responsible, at least in part, for the maintenance of the facilitated HP LTP during this period of withdrawal. These results further support the hypothesis that the HP is a brain structure involved in the associative memory formation and retrieval when contextual cues associated with drug exposure and withdrawal are presented to a dependent subject [58]. Moreover, it has been suggested that Arc promotes LTP consolidation through regulation of actin dynamics [117]. In addition to the maintenance of synaptic potentiation during late-LTP, long-term memories are dependent on the persistent phosphorylation and sustained activation of the brain-specific atypical protein kinase M zeta (PKMζ) [118]. It is also known that actin polymerization is critical for PKMζ synthesis, leading authors to suggest the possibility of a sequential mechanism of LTP maintenance in which Arc-dependent stabilization of F-actin promotes PKMζ synthesis and expression of enduring LTP [117]. We have described that inhibition of PKMζ using the zeta pseudosubstrate inhibitory peptide (ZIP, selective PKMζ inhibitor) prior to reexposure to the withdrawal environment with preserved contextual cues prevented the anxiety expression. Furthermore, LTP facilitation in all DZ-dependent animals was reversed, suggesting that plasticity in the HP has a crucial role in maintenance of these memories that were vulnerable to the amnesic effects of ZIP [112, 118].

These results are in accordance with the hypothesis that the persistently active PKMζ might perpetuate information both at synapses and during long-term memory [119]. Furthermore, LTP was generated in DZ-dependent animals intra-HP infused with ZIP at a similar frequency to control animals, whereas vehicle infusion in DZ-dependent animals did not affect the lower LTP threshold associated with exposure to the contextual cues related to DZ withdrawal experience as previously described. Therefore, inhibition of PKMζ in long-term withdrawal reversed the facilitated LTP generated during DZ chronic administration and maintained through withdrawal, without affecting the mechanisms involved in vitro LTP generation [112].

Final Remarks

Abuse of psychostimulants such as cocaine and amphetamines, and misuse of prescribed drugs with or without a prescription represent a significant health and social problem worldwide. A major problem in the treatment of drug addiction is relapse to drug abuse. Then, highlighting common mechanisms underlying persistence of abuse to different drugs could be interesting for the development of strategies of effective treatments. Our work is focused on the study of common mechanisms underlying COC sensitization, as a model of craving and relapse, and DZ dependence. We hypothesize that up regulation of NOS/NO/sGC/cGMP signaling pathway in different brain areas could initiate, contribute, or exacerbate addictive behaviors in humans. These results are of significant importance considering that patients participating in drug detoxification programs or addicts in withdrawal may use other drugs to mitigate withdrawal symptoms or side effects of the primary drug used. In fact, the misuse and recreational use of PDE5i have been described in different human populations [120] and linked to the illicit use of drugs of choice for abuse [121]. Taking our results into consideration, we can speculate that PDE5i may increase vulnerability to drug abuse.

Moreover, our results demonstrate that memories related to drug experience and withdrawal are relevant to the expression of anxiety-like behavior when DZ-dependent animals are reexposed to the contextual cues associated with the withdrawal experience. We further characterize the HP as a pivotal brain structure involved in engagement of learning processes related to drug dependence and withdrawal. These findings contribute to the establishment of the mechanisms by which drugs of choice for abuse can alter brain function that can be used as treatment or prevention of drug dependence and abuse.

Disclosures/Conflicts None.

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Soy and Psychotropic Effects: A Brief Overview

14

Rose E. Nina Estrella

Introduction

Phytoestrogens are produced by many plants and best investigated are isoflavones that are widely present in soy (Glycine max) and red clover (Trifolium pratense) [1]. During the past 20 years, a remarkable number of research lines about the health effects of soy consumption have been conducted, which in large part can be attributed to the presence of isoflavones in the soybean [2].

Isoflavones first came to the attention of the scientific community in the 1940s based on the fertility problems observed in sheep grazing on an isoflavone-rich clover. In the 1950s, as a result of their estrogenic effects in rodents, isoflavones were studied as possible growth promoters for use by the animal feed industry, although shortly thereafter it was shown that isoflavones could also function as antiestrogens. Despite this early work, it was not until the 1990s, largely because of research sponsored by the U.S. National Cancer Institute that the role of soy foods in disease prevention began to receive widespread attention. Subsequently, isoflavones and soy foods were being studied for their ability to alleviate hot flashes and inhibit bone loss in postmenopausal women. In 1995, soy protein attracted worldwide attention for its ability to lower cholesterol. At the same time, isoflavones began to be widely discussed as potential alternative to conventional hormone therapy. In 2002, it was hypothesized that individuals possessing the intestinal bacteria capable of converting the soybean isoflavone daidzein into the isoflavan equol were more likely to benefit from soy intake. More recently, in vitro and animal research has raised questions about the safety of isoflavone exposure for certain subsets of the population, although the human data are largely inconsistent with these concerns [2].

Isoflavones, which have been known for over 100 years to exist in plants, have a relatively limited distribution in nature. In the commonly consumed human foods, they are present in physiologically relevant amounts only in soybeans and foods derived from this legume [3], although a variety of plants (e.g., red clover) [4] are also rich sources.

Asian populations have consumed foods made from soybeans for centuries, whereas in the West, certain subpopulations, namely Seventh-day Adventists and vegetarians, have used soy foods for ~100 years although the quintessential soy food tofu was first introduced on a large scale to the general U.S. population beginning only in the early 1970s. Health-conscious and ecologically

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minded consumers were particularly attracted to soy at that time because it was perceived as being a source of high-quality protein low in saturated fat that was more efficiently produced than animal sources of protein [2].

The mechanism of action of the phytoestrogens are a partial estrogenic receptor agonists; anti-estrogenic effects in premenopausal women, but weak estrogenic effects in postmenopausal women. They also may stimulate osteoblastic activity, increase sex hormone-binding globulin levels, and antioxidant activity. The suggested dosing in symptoms of menopause is between 34 and 120 mg daily [5].

Soy isoflavones are diphenolic compounds that are frequently used for alternative treatment of symptoms of aging in both genders [6].

The impact of soy food intake on risk of breast cancer has been investigated extensively. Much of this focus can be attributed to the soybean being a dietary source that is uniquely rich in isoflavones. The chemical structure of isoflavones is similar to that of estrogen, and isoflavones bind to both estrogen receptors (ER-α and ER-β) (although they preferentially bind to and activate ER-β) and exert estrogen-like effects under some experimental conditions. Isoflavones also possess nonhormonal properties that are associated with the inhibition of cancer cell growth. Thus, there are several possible mechanisms by which soy can reduce the risk of breast cancer. However, the role of isoflavones in breast cancer has become controversial because, in contrast to the possible beneficial effects, some data from in vitro and animal studies suggest that isoflavones, in particular genistein, the aglycone of the main soybean isoflavone genistin, may stimulate the growth of estrogen-sensitive tumors. Limited human data directly address the tumor-promoting effects of isoflavones and soy. Because the use of soy foods and isoflavone supplements is increasing, it is important from a public health perspective to understand the impact of these products on breast cancer risk in women at high risk of the disease and on the survival of breast cancer patients [7]. While moderate isoflavone consumption seems to be safe in the majority of the population, women with breast cancer should avoid long-term use of soy products or isolated isoflavones [8]. Isoflavones protect against mammary cancer, but only when taken peripubertally when the mammary gland develops [1].

Soy isoflavones may prevent postmenopausal osteoporosis and improve bone strength thus decreasing risk of fracture in menopausal women by increasing lumbar spine in the bone mineral density (BMD) and decreasing bone resorption marker urine deoxypyridinoline. Further studies are needed to address factors affecting the magnitude of the beneficial effects of soy isoflavones and to assess the possible interactions between soy isoflavones and anti-osteoporosis drugs, and to verify effects on BMD of other skeletal sites and other bone turnover markers [9].

Isoflavones have been investigated in detail for their role in the prevention and therapy of prostate cancer. This is primarily because of the overwhelming data connecting high dietary isoflavone intake with reduced risk of developing prostate cancer. A number of investigations have evaluated the mechanism(s) of anticancer action of isoflavones (e.g., genistein, daidzein, biochanin A, equol) in various prostate cancer models, both in vitro and in vivo. Genistein quickly jumped to the forefront of isoflavone cancer research, but the initial enthusiasm was followed by reports on its contradictory prometastatic and tumor-promoting effects. Recent research indicates a novel role of genistein and other isoflavones in the potentiation of radiation therapy, epigenetic regulation of key tumor suppressors and oncogenes, and the modulation of mRNA, epithelial-to-mesenchymal transition, and cancer stem cells, which has renewed the interest of cancer researchers in this class of anticancer compounds [10].

The most promising news for soy may be its positive effect on lipid profiles. Soy protein has also been the subject of considerable investigation, especially in regard to its hypocholesterolemic effects [9]; recent scientific interest in soy largely parallels the interest in isoflavones. Of the ~2,000 soy-related papers currently published annually, more than one half are related to isoflavones [2].

A meta-analysis of 38 controlled human studies of soy consumption provides compelling

evidence for its positive effect on improved lipid profiles including reduction in low-density lipid and triglycerides and an increase in high-density lipid levels [11, 12].

The U.S. Food and Drug Administration has approved a health claim for isoflavone-rich soy protein to reduce cholesterol with 25 g of soy protein consumption daily [13]. However, it is important to note that it appears to require that soy isoflavones are consumed intact in soy protein [14].

There are other aspects to investigate regarding soy isoflavones which have been less studied. These are the psychotropic effects in anxiety, depression, and cognitive symptoms in the menopausal women. The following is a brief overview based on evidence of the past decade about the use of soy isoflavones in the treatment of these menopausal psychological symptoms.

Symptoms Associated with Menopause

Transitioning into menopause is a natural part of life. All women, if they live long enough, will experience menopause. A variety of symptoms are reported frequently associated with menopause. These include hot flashes, night sweats, menstrual irregularities, vaginal dryness, depression, nervous tension, palpitations, headaches, insomnia, lack of energy, difficulty concentrating, and dizzy spells. How a woman experiences or reports symptomatology is greatly influenced by a multitude of variables including race, ethnicity, and other psychosocial factors. Her ability to manage symptoms associated with this life transition affects her quality of life and imposes physical, psychological, and economic burdens [15, 16].

The menopausal transition can be categorized into several stages. In the Study of Women's Health Across the Nation (SWAN) and the Four Major Ethnic Groups (FMEG) study, the menopausal status was categorized into pre-, early peri-, late peri-, and postmenopausal [17, 16]. Both studies examined the prevalence of various symptoms by menopausal status in a multiethnic sample of women transitioning to menopause states. The study comprised two stages. The first was a cross-sectional telephone or in-home survey conducted between November 1995 and October 1997. The second was a longitudinal investigation to track changes in women's physical and mental health as they age and traverse the menopausal transition. FMEG used an Internet survey and qualitative online forums [16].

Literature reviews consistently report a relationship between culture and menopause. The sociocultural organization of one's life course in specific geographical locations profoundly affects the menopausal experience for women. Many factors are hypothesized to influence the menopause experience (e.g., diet, smoking, exercise, attitude, expectation, marital status, socioeconomic status, etc.) [4, 17].

Avis et al., [18] from SWAN reported that two consistent clusters of clearly symptoms of menopause emerged from the data. The first was vasomotor symptoms such as hot flashes and night sweats, and the second consisted of psychological and psychosomatic symptoms. There were racial/ethnic differences in symptom reporting as well as differences of menopausal status. Controlling for the following variables (e.g., age, education, health, and economic strain), Caucasian women reported significantly more psychosomatic symptoms than other racial/ethnic groups. African American women reported significantly more vasomotor symptoms. Tension feelings, depression, irritability, headaches, and stiffness were all frequently reported symptoms. In some studies, fatigue, muscle/joint pain, back/neck aches, and headaches exceeded reports of hot flashes [12, 18]. In general, Japanese and Chinese women were less likely than the other groups to report any symptoms.

Im et al., from the FMEG study, also reported statistically significant ethnic differences in the total number of symptoms experienced during the menopausal transition [19].

Hispanics reported significantly larger numbers of total symptoms, physical symptoms, and psychosomatic symptoms than Asians. Caucasians reported significantly larger numbers of total symptoms, physical symptoms, psychological

symptoms, and psychosomatic symptoms than Asians. African Americans reported a significantly larger number of psychosomatic symptoms than Asians. There were statistically significant ethnic differences in the frequencies of 41 individual symptoms [20].

Both studies reported racial and ethnic differences in symptoms experienced in menopause transition in different groups of women. The racial and ethnic differences are consistent and serve as evidence against a universal menopausal syndrome [18]. It must be pointed out that both studies had limitations related to the selection of participants [20]. Thus, the generalizability of the study findings is limited. Nonetheless, these studies support racial and ethnic differences in how women experience menopause [20].

Soy Treatment in the Symptoms of Menopause

All women reach menopause and approximately two thirds of women develop symptoms of menopause, primarily hot flashes. Hormone therapy was long considered the first line of treatment for vasomotor symptoms. However, given the results of the Women's Health Initiative (WHI), many women are reluctant to use exogenous hormones for symptomatic treatment and are turning to botanicals and dietary supplement (BDS) products for relief. Despite the fact that there is limited scientific evidence describing efficacy and long-term safety of such products, many women find these "natural treatments" appealing. Peri- and postmenopausal women are among the highest users of these products, but 70 % of women do not tell their healthcare providers about their use. Compounding this issue is the fact that few clinicians ask their patients about use of BDS, largely because they have not been exposed to alternative medical practices in their training and are unfamiliar with these products [21].

In a National Health and Nutrition Examination Survey, researchers confirmed ethnic differences in the use of hormone replacement therapy (HRT); white women were more likely to use HRT than non-Hispanic, African American, or Mexican American women [22].

This finding is consistent with trends noted by other investigators. In 2001, Avis et al., from SWAN reported that hormone use was highest among Caucasian women and lowest among African American and Hispanic women [18]. Multiple investigators cited similar findings indicating white women were more likely to use HRT than non-Hispanic, African American, or Mexican American women. Additionally, white women were prescribed or offered HRT more often [15, 23]. This trend is interesting given the SWAN data, which reported white women having fewer vasomotor complaints when compared with African American and Hispanic women. In addition, post WHI, there was a significant decline in hormone prescribing across all ethnic groups [24].

Over the years, the number of prescriptions for hormone therapy has reflected scientific findings. In the 1970s, the number of prescriptions increased to approximately 30 million per year. This practice was likely because of data describing the cardioprotective effects of hormone therapy [25].

In the 1980s, reports of increased rates of endometrial cancer with unopposed estrogen led to a decrease in annual prescriptions to about 15 million. Then, the addition of progesterone for endometrial protection renewed interest in hormone therapy, and prescriptions again increased [25].

Between 1995 and 2002, annual prescriptions peaked at about 91 million. Termination of the estrogen-progestin arm of the WHI in July 2002 and release of the Heart and Estrogen/Progestin Replacement Study data received considerable media attention and raised serious questions about the safety of hormone therapy in postmenopausal women. Many women stopped taking hormones and began to seek out alternative therapies. Prescriptions for hormone treatment immediately decreased [25].

The use of complementary and alternative medicine (CAM) is very common. In 1999, the National Health Interview Survey data estimated approximately 40 % of women in the United States used some form of CAM in the past 12 months. More than $600 million was spent on CAM for treatment of menopause [11].

Women commonly use soy products, herbs, and other CAM therapies for symptoms of

menopause. Randomized, controlled trials have evaluated the efficacy and short-term safety of these therapies [9].

In this study, some sources were consulted. The MEDLINE searches were directed to articles published from January 1966 through March 2002. The Alternative and Complementary Database (AMED) of the British Library was used to search for articles published from January 1985 through December 2000. The authors' own extensive files were also used. The chosen research terms were hot flash/flush, menopause, and climacteric, combined with phytoestrogens, alternative medicine, herbal medicine, traditional medicine, Traditional Chinese Medicine, Ayurveda, naturopathy, chiropractic, osteopathy, massage, yoga, relaxation therapy, homeopathy, aromatherapy, and therapeutic touch [9].

A number of 29 randomized, controlled clinical trials of CAM therapies for hot flashes and other symptoms of menopause were selected. A group of 12 trials dealt with soy or soy extracts, 10 with herbs, and 7 with other CAM therapies. In the data synthesis, soy seemed to have modest benefit for hot flashes, but studies were not conclusive. Isoflavone preparations seemed to be less effective than soy foods. Black cohosh may be effective for symptoms of menopause, especially hot flashes, but the lack of adequate long-term safety data (mainly on estrogenic stimulation of the breast or endometrium) precludes recommending long-term use. Single clinical trials have found that dong quai, evening primrose oil, a Chinese herb mixture, vitamin E, and acupuncture do not affect hot flashes; two trials have shown that red clover has no benefit for treating hot flashes. It was concluded that black cohosh and foods that contain phytoestrogens show promissory properties for the treatment of symptoms of menopause. Clinical trials do not support the use of other herbs or CAM therapies. Long-term safety data on individual isoflavones or isoflavone concentrates are not available [9].

The use of botanical and dietary supplements (BDS) among menopausal women has increased in recent years in the US, with the largest increase in the use of so-called "natural hormonal agents" [26, 27]. Most women report use of these treatments because they find these alternatives to traditional medicine more congruent with their values, beliefs, and lifestyles [28, 29].

A recent survey of 500 peri- and postmenopausal women conducted at the University of Illinois Medical Center found that 70 % of women between the ages of 40 and 60 years reported using BDS to treat symptoms or diseases; however, fewer than 10 % of users could actually verbalize the health benefits of these supplements [4].

Asian diets are high in soy-based foods (40–80 mg per day of isoflavones in Asian diets as compared with <3 mg per day in American diets), and many women in these countries express few menopausal complaints [30]. It is unknown if the lower prevalence of hot flashes and other symptoms of menopause are due to dietary make-up, cultural factors, or a combination of both [21].

Historically, investigators have noted differences in use of CAM. Hirata et al. reported Asian women, especially Chinese women, were more likely to use acupuncture and dong quai, a Chinese herb traditionally prescribed as a tonic for women [25]. Korean women commonly use red ginger, which reportedly improves fatigue and depression [31, 32]. Asian women were more likely to use soy products [31]. Of note, however, is that a major predictor of subsequent CAM use in menopausal transition is the use of CAM before menopausal transition [24].

Chemically, isoflavones belong to the group of polyphenols. The most important food source is soy, which contains mainly genistein and daidzein in the form of glycosides. The absorption rate ranges from 20 to 55 %. Isoflavones are selectively incorporated in certain tissues such as the breasts and ovaries. They are able to bind to the alpha (ER-α) and beta (ER-β) estrogen receptors. However, the binding affinity for genistein to ER-α is only 4 %, and the affinity to ER-β is 87 % compared with 17 β-estradiol. Thus, depending on the estradiol concentration, they exhibit weak estrogenic or antiestrogenic activity. Isoflavones can influence transcription and cell proliferation. They modulate enzyme activities as well as signal transduction, and have antioxidant properties. Epidemiological studies have shown that the prevalence of hot flashes is lower in women from countries with high dietary

isoflavone intake such as Japan than in Western nations with low isoflavone intake. Results of clinical studies on the effects of soy products or isolated isoflavones on vasomotor symptoms are contradictory. Due to a strong placebo effect and a time-dependent reduction of hot flashes, phytoestrogens were seen to have no significant effect in most studies. However, the use of soy isoflavones could be considered for women with intense disorders [8].

Soy Treatment in the Menopausal Affective and Cognitive Symptoms

Affective Symptoms and Anxiety

Menopausal women suffer a wide variety of symptoms, including hot flashes and night sweats, which can affect quality of life. Although hormone therapy has been considered the correct choice for the treatment designated to alleviate these symptoms, it has been associated with increased breast cancer risk. It led many women to search for natural, efficacious, and safe alternatives such as botanical supplements. Data from clinical trials suggesting that botanicals have efficacy for menopausal symptom relief have been controversial, and several mechanisms of action have been proposed including estrogenic, progesteronic, and serotonergic pathways [25].

As previously stated, depressive disorders in menopause have been largely clinically observed [33], and hormone decrease induces important symptoms [34]. Symptoms of menopause improve after hormone replacement, with the risk of relapse after cessation of hormone replacement therapy [35, 36]. It has been reported clinically that antidepressants alone do not ensure success in treatment of depressive disorders in menopausal women [35]. Estradiol alone (transdermal estradiol replacement) has a significant antidepressant effect in perimenopausal depression [5]. Recently, the effect of the addition of raloxifene, a selective estrogen receptor modulator (SERM) to selective serotonin reuptake inhibitors gave satisfactory effects inducing complete remission in a postmenopausal depressive disorder [6].

The use of soy-derived isoflavones has been proposed as a protective factor against depression starting from basic translational approaches [37]. A relevant stimulatory effect of phytoestrogens on noradrenaline and serotonin transporter activity has been reported [3]. The effect of glutamatergic stimulation on sexual behaviors in rats appears to be driven by an induction of central adrenergic receptor prevalence modifications exerted by sexual hormones [33, 38] similar to those induced by antidepressants. In fact, it has been reported that ovarian steroids induce modifications in noradrenergic and serotoninergic receptors in the rat brain [39, 14]. Furthermore, estradiol has shown a synergistic antidepressant effect with fluoxetine in animal studies of experimental depression [40].

De Sousa-Muñoz and Filizola performed a placebo-controlled double-blind randomized study with 84 climacteric outpatients in the Lauro Wanderley University Hospital in Joao Pessoa, Brazil. The objectives were to evaluate the efficacy of soy isoflavones extract (SIE) in the treatment of depressive symptoms in women with climacteric syndrome [41].

In the assessment of the depressive symptoms, the Brazilian version of the Center of Epidemiologic Studies of Depression (CES-D) scale was used, on the pre-treatment visits (VT1), 8th (VT2) and 16th (VT3) weeks after treatment. The experimental group (EG) received the daily dose of 120 mg SIE and the control group (CG), placebo. The primary efficacy measure was the comparison of the percent reductions in the CES-D scores from VT1 to VT3 between EG and CG (*t*-test, $p < 0.05$). The security analysis consisted of laboratory and clinical evaluation of adverse events. *The treatment with soy isoflavones produces a reduction initially observed on the depressive symptoms of a predominantly affective nature evaluated in this study* [41].

On the other hand, a study conducted by Nina-Estrella et al., a pilot clinical trial where the effect of soybean, antidepressants, and the association of soybean with antidepressants was studied in 40 depressive menopausal women for 3 months. Patients were divided into four groups of 10 women: Fluoxetine (10 mg), soybean (100 mg),

sertraline (50 mg), and sertraline (50 mg) plus soybean (100 mg). The Hamilton and Zung Depression Scales were used to measure the treatment effects. Values at the beginning and at the end of the study were compared. In all cases a significant difference was observed when the treated groups were compared versus their untreated situation in both scales ($p<0.001$). When a comparison between pre- minus post-treatment Zung Scale scores was done, the effect induced by the association of sertraline and soybean was significantly higher than the other groups ($p<0.05$). These effects were also seen using the Hamilton Scale scores, showing significant differences between the association versus soybean ($p<0.05$) and setraline ($p<0.05$) groups, but not versus the fluoxetine group. We conclude that soybean has an antidepressant effect per se, and the association of soybean and antidepressants increase their effects [34].

The climacteric syndrome involves a variety of symptoms such as profuse sweating, insomnia, memory loss, decreased sexual drives, joint aches, and anxiety [42]. Three studies were found that explore a relation between anxiety symptoms and soy intake.

Melby conducted a study exploring whether Japanese kōnenki (climacteric) symptoms are unique to women or are experienced by men, to compare common symptom indices, and to explore the relationship between symptoms and soy intake. After a 2-week recall of 54 symptoms, an eight-item food frequency questionnaire, and views about kōnenki were collected from 60 individuals in Kanazawa, Japan.

Men had higher prevalence of stress, irritability, and nervousness ($p<0.05$). However, kōnenki was not reported by men. In women, four of eight symptom factors exhibited significant correlations with kōnenki status. All symptom index scores were lower in pre-kōnenki women than in peri-kōnenki women, but scores for men and women did not differ. Soy intake and some scores were negatively correlated among women. The conclusions where that several symptoms commonly associated with kōnenki in Japan are not unique to women and have higher prevalence in men [43].

Mucci et al. conducted a controlled, randomized, multicenter study in symptomatic menopausal women with sleep or mood alterations. They concluded that the results showed the efficacy of magnolia extract and magnesium on psycho-affective and sleep disturbances in menopause, in addition to the effects of isoflavones on vasomotor symptoms. It suggests that a global natural approach to menopause evidenced therapeutic usefulness and safety of natural products [44].

Ishiswata et al. conducted a randomized, double-blind, placebo-controlled trial with our new equol supplement for 12 weeks in 134 Japanese women (aged 40–59 years). They found that S-equol supplement improved mood-related symptoms in perimenopausal/postmenopausal equol nonproducers [45].

Cognitive Symptoms

Soy isoflavone intake is correlated with a reduction in arterial pressure, both in clinical trials [5, 27] and rat studies [4, 46] and is reported to have a positive effect on bone density [1, 47], endothelial function, cognitive performance, and mood [48].

The idea that soy isoflavones may influence cognition is based on the evidence that dietary phytoestrogens (especially those from soy) could decrease cardiovascular and neuronal damage in animal models of disease [10, 49]. The preventive action of soy isoflavones on cognition is indirect and based on its preventive effects on brain vasculature. There is clear evidence that hypertension impairs cognitive function, and that this relationship is positively correlated.

Animal models have been used in this field. Male spontaneous hypertensive rats (SHR) display an age-related decline in cognitive performance, beginning in the 10th–12th month of life. Although a similar fall in cognitive ability occurs in normotensive male rats, the onset is around 18 months of age. Female SHR also develop similar age-related deficits, albeit several months later than their male counterparts. Additionally, antihypertensive therapy attenuates the observed dec-

lination in cognition [50]. While studies in young animals are informative, experiments in middle-aged animals are likely more instructive regarding mechanisms that underlie beneficial effects of isoflavones in postmenopausal women [51].

Earlier studies indicated that the 83-fold binding selectivity for ER-β over ER-α. Thus we refer to it as a phytoestrogenic ER-β-selective modulator (SERM) formulation or phyto-β-SERM formulation. The phyto-β-SERM formulation is neuroprotective and promotes estrogenic mechanisms in the brain while devoid of feminizing activity in the periphery. Further investigation in a mouse model of human menopause indicated that chronic exposure to the phyto-β-SERM formulation at a clinically relevant dosage prevents or alleviates menopause-related climacteric symptoms. Zhao et al. conducted a study assessing the efficacy, in an early intervention paradigm, of the phyto-β-SERM formulation in the regulation of early stages of physical and neurological changes associated with Alzheimer disease (AD) in a female triple transgenic mouse model of AD. Results demonstrated that, when initiated prior to the appearance of AD pathology, a 9-month dietary supplementation with the phyto-β-SERM formulation promoted physical health, prolonged survival, improved spatial recognition memory, and attenuated amyloid-β deposition and plaque formation in the brains of treated AD mice [52]. In particular, the data suggest that there may be a crosstalk between ER-β and glycogen synthase kinase 3 signaling pathways that could play a role in conferring ER-β-mediated neuroprotection against AD. In conjunction, these results support the therapeutic potential of the phyto-β-SERM formulation for prevention and/or early intervention of AD, and warrants further investigations in human studies.

Clement et al. did a systematic review to evaluate the evidence regarding the efficacy of herbal and dietary supplements on cognition in menopause. Randomized clinical trials (RCTs) of herbal medicines and dietary supplements were identified using the MEDLINE, EMBASE, AMED, PsycINFO, CINAHL, and The Cochrane Library 2010 (Issue 2) electronic databases and by hand searches. Data were independently extracted and evaluated by two reviewers. Risk of bias was assessed by two independent reviewers using the Cochrane Collaboration tool. Twelve RCTs were included and five of these suggest that isoflavone, soy, and gingko biloba supplementation may improve cognition in postmenopausal women. However, most of the included studies had serious methodological flaws which demand a cautious interpretation of these findings. The conclusions based on evidence that herbal and dietary supplements might positively affect the cognitive decline during the menopause are not compelling [53].

Fournier et al. investigated whether soy (soy milk and supplement) could improve cognitive functioning in healthy, postmenopausal women. They concluded that soy isoflavones consumed as a food or supplement over a 16-week period did not improve or appreciably affect cognitive functioning in healthy, postmenopausal women [54].

On 2010, the North American Menopause Society organized the Utian Translational Science Symposium on Soy and Soy Isoflavones. The purpose was to clarify basic and clinical research findings as they relate to the risk and benefits of soy products for peri- and postmenopausal women. They concluded that additional clinical studies are needed with the goal of comparing outcomes among female intestinal bacterial concentrations. It was suggested that the ability to convert daidzein to equol (equol producers) with those that lack that ability (equol nonproducers) could derive in an idea about whether equol producers derive greater benefits from soy supplementation. Larger studies are needed in order to understand the modes of use of soy isoflavone supplements in women [55].

Soy and Adverse Effects

Some side effects have been reported related to soy administration, mainly thyroid dysfunctions such as goiter in infants [56]. However, in adult menopausal women, soybean administration seems not to affect thyroid function [56]. In adults, the most relevant problem is soy allergy [6].

Conclusion

Women commonly use soy products, herbs, and other CAM therapies for symptoms of menopause. Foods that contain phytoestrogens show promising possibilities for the treatment of symptoms of menopause. Isoflavones and soy foods have been studied because or their ability to alleviate hot flashes, inhibit bone loss, and lower cholesterol in menopausal women. Isoflavones have also been investigated in detail in men searching for their possible role in the prevention and therapy of prostate cancer [57]. There are few revisions based on evidence that study the psychotropic effects of soy in the depression, anxiety, and cognitive symptoms of the menopausal women.

Few studies that were found with antidepressants effects of soy, and they concluded that the administration of soybean could act as an interesting alternative to estrogens in the treatment of depressive disorders during menopause. There are very few studies of use of soy in menopausal anxiety symptoms and with soy and cognitive symptoms. They appear to have more beneficial effects in animal models. There is a need for additional studies in human models for the cognition in menopausal women.

In general, additional clinical studies are needed to search for the psychotropic effects of soy and soy isoflavones in depression, anxiety, and cognition symptoms in menopausal women.

Disclosures/Conflicts None.

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Use of Psychotropic Drugs: Between the Medicalization and Rationality

15

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Introduction

The transformation of social problems or simple circumstances of the life in diseases, syndromes or medical problems is constantly growing. Furthermore, it detects that medical interventions generate various iatrogenic and adverse events. For this reason, it is important to determine how health professionals participate in this complex process and how this situation can be reversed, considering our relationships with the pharmaceutical industry, and our performance through the prescription and dispensation stage.

The objectives of this review are to achieve greater understanding and promote reflection on this important issue, which intends to improve the quality of life and patient care through the proper and safe use of psychoactive drugs. For this reason, different contributions related to psychotropic drugs and their use, mental illness, medicalization, the aggressive promotion of the pharmaceutical industry, the reckless use of psychotropic drugs with and without prescription, and the serious consequences that it can generate. Also, non-pharmacological interventions are mentioned stressing that drugs are not the only or the best solution for the problems mentioned. Finally, proposals and ideas that could contribute to better use of psychoactive drugs are presented.

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Mental Illness

Angell [1] questioned the theory that reduces the explanation of mental disorders to simple biochemical imbalances, and how the strong interests of the pharmaceutical industry perpetuate this model, extend the diagnostic categories of mental illness, leading to increased consumption of psychotropic drugs worldwide. His work was done on the basis of research conducted by Kirsch [2], Whitaker [3], and Carlat [4]. Kirsch [2] raises the question of whether antidepressants actually work and tests their effectiveness. Whitaker [3] put in doubt whether psychotropic drugs are better or result worse for general health. Carlat [4] highlights that the alliance between psychiatry and the pharmaceutical industry must be considered to determine mental illness.

These three authors warn that drug companies have not only begun to determine what can be considered mental illness and how it should be diagnosed and treated, but they have also launched measures of abusive and aggressive sale of psychoactive drugs, both legal and illegal [1].

The authors question the theory that mental illness is caused by a brain chemical imbalance

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that drugs can correct. According to Whitaker [3], patients diagnosed with schizophrenia, depression, or other psychiatric disorders do not have such chemical imbalances prior to drug treatment, but, once treatment is established, their brain begins to function abnormally. Meanwhile, Carlat [4] argues that this theory is a myth that does destigmatize mental illness. Kirsch [2], who conducted focused research on depression, concludes that the traditional account of depression in the brain is simply wrong [5].

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) was criticized by various authorities both before and after it was formally published. Critics assert that many DSM-5 revisions or additions lack empirical support; inter-rater reliability is low for many disorders; several sections contain poorly written, confusing, or contradictory information; and the psychiatric drug industry unduly influenced the manual's content. Various scientists have argued that the DSM-5 forces clinicians to make distinctions that are not supported by solid evidence, distinctions that have major treatment implications, including drug prescriptions and the availability of health insurance coverage. General criticism of the DSM-5 ultimately resulted in a petition signed by 13,000 psychiatrists, and sponsored by many mental health organizations, which called for outside review of the document [5, 6]. Subsequently, Cosgrove et al. [7] unveiled the existence of a relationship between the principal investigators of clinical trials, members of the panel for DSM-5 and the pharmaceutical companies, concluding that although there is an increase in transparency, through the registration of clinical trials and mandatory disclosure policy, it is not sufficient to prevent the biases in the process of revising the DSM, or clinical intervention decisions for DSM disorders.

The pharmaceutical industry has extended traditional diseases such as depression, and contributed to an expansion of mental health problems amenable to treatment, including neighboring situations to normality, such as shyness or hyperactive behavior in children [8].

In regard to children, before the DSM-5, Vasen [9] noted that an epidemic of improper names both in the clinic and in the classroom has arisen, which extends the range of children with labels and psychopharmacological medication inadequate, producing medicalization and pathologization of an age group as vulnerable as the children.

The problems of anxiety and depression are one of the main reasons for visits to a primary care physician. The current economic crisis has greatly increased in prevalence, and is expected that by 2020 will constitute the leading cause of disability worldwide [10].

Psychotropic Drugs

A psychotropic drug is defined as any natural or synthetic substance, capable of influencing the psychic functions by their action on the central nervous system, and psychotropic drugs as any pharmaceutical product containing psychotropic substances, used as an object of psychological or neurological treatment of the disorders of the nervous system [11].

Psychoactive drugs are drugs that improve, reduce, and/or mitigate the symptoms of mental illness. They are primarily used to treat three types of alterations: schizophrenia and other psychoses, depression and mania (i.e., mood disorders), and the pictures produced by excessive anxiety or distress.

Psycholeptics have depressant properties of mental activity and include antipsychotics, hypnotics, anxiolytics and sedatives, which are commonly, used for the treatment of psychosis and anxiety disorders. The psychoanaleptics are stimulant drugs that include antidepressants, psychostimulants used to treat attention deficit disorder or attention deficit hyperactivity disorder, and nootropics and drugs against dementia [12].

Consumption of Psychotropic Drugs

The enormous popularity of psychoactive drugs in recent years is due to the significant technological advances, the relative effectiveness of other intervention methods, dissemination of clinical trials, social changes, changes in psychiatric

nosology, and influence of the pharmaceutical industry. To all these factors, the extension of the idea that symptoms of mental illness depend on brain functions regulated by chemicals must be added, and that they can be modified in a controlled and socially acceptable manner [8].

The anxiolytic consumption has increased significantly worldwide, in Argentina it grew 5 % in 2013 in the legal market, excluding the parallel market, that operates without prescription in hospitals, or by contraband. In addition, the sale of drugs aimed at the central nervous system has overtaken the sale of drugs to diseases of gastrointestinal and cardiovascular systems, suggesting that there is an epidemic increase of anxiety and stress, as indicated by the billings. According to the latest survey by the National Drug Observatory over three million people use anxiolytics, indicating an increase of 40 % over the last 10 years [13].

Drug Rational Use or Prudent Prescription

According to the World Health Organization [14], the rational use of drugs (RUD) is that the patient receives the appropriate medication to their clinical needs, in doses corresponding to their individual requirements, for an adequate period of time, and at the lowest cost to himself and his community.

Furthermore, according to Mordujovich [15], the RUD is the application of the science knowledge backed by the evidence, quality, effectiveness, efficiency, and safety for the selection, prescribing, dispensing, and use of a particular medication when necessary and appropriate to a health problem, with the active participation of the patient enabling adherence and monitoring of treatment.

However, presently the RUD concept, promulgated at the conference in Nairobi, is being left unused to discuss prescription prudent, safe, or conservative, that means first prescribed under the principle of "*primum non nocere*". RUD is still used to define good prescribing practices; however, according to Albert Figueras [16], the prescription is far from a rational act, but is not in itself good or bad: just being rational is not the goal [17].

According Turabian and Pérez- Franco [18], the requirement is to "be useful for practice and patients". Beyond scientific rationality or resource management, the social event that involves the act of prescribing within a clinical context and genuine is considered, which is an encounter between persons and has a convenient, unique, and subjective sense.

Therefore, the requirement involves a commitment, where the former is to not do damage, to be honest, to know the limits and risks to which we are willing to go, and to share the uncertainties, mixing equal parts of art, values, and science with humility and humanity [17].

When considering psychotropic drugs, their rational use is based on helping the patient in the best possible way, taking into account the resources of the patient and the healthcare institution. It involves getting the best effect with the least number of drugs, during the shortest possible time, at a reasonable cost, which is essential to assess the willingness of the patient to take the medication.

The Argentine Union of Pharmacists and Biochemists estimated that the misuse or abuse of drugs causes 100,000 hospitalizations and about 22,000 deaths per year, especially in people over 65 years of age. The report estimates that 40 % of the population uses sedatives and stimulants without a prescription.

From the classical psychiatry there is a tendency to avoid anxiolytics prescription. It is recommended to use them with care, not exceeding 30 days because of their well-known addictive properties. The phenomena of tolerance have been reported, and it implies more doses for the same effect [13].

Medicalization

The use of drugs has been trivializing, particularly in the case of some psychoactive drugs such as anxiolytics. They have come to be used as a defense against the threats of everyday life.

People admit taking these medications to sleep, get some quiet, to overcome the stress of a

divorce, the pressures generated by their children, or job uncertainty. They are also used to reduce anxiety or depression, or to not be sad. Everyone is responding to the emotional hazards of the day with their own drug. Currently, society is not allowed to experience sadness, much less tolerate the situation of unease.

In general, self-administered psychotropic drugs work as a magical remedy and is not recognized as part of a treatment. Many people take it as they see fit. Typically, when a medication is prescribed for a short time, the patient tried it to restore emotional balance.

Given that society accumulates daily stress, and that anxiolytics are relatively inexpensive and apparently safe and effective, people trust, and seek quick solutions. Unfortunately, when it is not used as a treatment, this anxiolytic is not needed.

Anxiety is an emotional reaction to a perceived threat or danger. People feel that it lacks the resources to address them. There is a big difference between psychic resources and the individual responsiveness to the demands of society, and that difference is covered with quick fixes such as anxiolytics [13].

Medicalization is defined as the process by which nonmedical problems are treated as medical problems, usually in terms of disease or disorder [19]. Medicalizing the human condition involves applying a diagnostic label to unpleasant feelings or undesirable behaviors that are not clearly abnormal, but are placed in a difficult area to distinguish that occupy a range of experiences that often are inescapably attached to being a person [20].

On the other hand, a "non-disease" can be considered as a "defined process or human problem because any instance as a condition for the best results would be obtained if it were not considered and treated well". This requires accepting that not all suffering is a disease [21, 22].

Barros [23] believes that medicalization can be understood as the high and increasing dependence of individuals and society to the supply of services and goods by medical care and increasingly intensive use. That excessive interference of medical technology are considered as disease diverse problems such as physiological situations, or whose determination problems are fundamentally economic-social in its nature [24].

The medicalization of life is inseparable part of our society and matches the reductionist and co-modified concept of health, which tends to leave certain aspects of our lives exclusively in the hands of medical and health professionals, helping to cause significant adverse events, dissatisfaction, and also, from a global perspective, to increase not only the differences between "real" patients and "healthy" patients, but also the injustice between the strongest and weakest of society. This is a complex and multifaceted problem in which the relationship of health professionals and their scientific societies with the pharmaceutical industry is an important consideration from an ethical perspective [25].

Perception of Health, Disease, and Care

The medicalization of the disease has led to the medicalization of health, life, death, and consequently of the whole society. Today, many believe that if someone is declared a healthy person, it is simply because they do not know they are sick, because they have not been exhaustively examined, or because they have not had a genetic test to find out their heritage, or because it was not done with sufficient thoroughness [25].

A simplistic concept of mental illness and human behavior has also helped to reduce the stigma associated with treatment with psychotropic drugs, particularly antidepressants that have passed, in a few years, of being rejected or consumed in secret, to be used by patients directly [8].

The so-called "lifestyle drugs" are used to treat problems at the boundary, that are between the need to maintain the health, and the satisfaction of desires associated with lifestyle; that is, that are not strictly related to the notion of health [26, 27].

According to Pérez Leirós [27] and Brasesco [28], the boundary between health and welfare, between needs and wants, between patients and consumers, seems to be the key to understanding the difference between a drug and to "lifestyle medicine".

Originally, psychotropic drugs were not considered in the group called "lifestyle drugs". However, Solal [29] believes that they constitute

a group of great importance within the concept of medicine to lifestyle. Often the consumption of different types of drugs corresponds to recreational use, so a significant percentage of psychotropic drugs relate to this type of case [27].

In a study conducted in Buenos Aires, it was found that clinicians prescribed the first psychopharmacological therapy depending on whom they were prescribing for, and after it referred to a psychiatrist only when treatment failed. Gastroenterologists, gynecologists, and cardiologists are among the main prescribers of psychotropic drugs from other specialties. Furthermore, it is stated that more people flock to the clinician for complaints associated with stress, anxiety, and mood states such as depression [30, 31].

In recent years there has been a progressive increase in the abuse of psychotropic medications and takes precedence in subjects suffering from ailments of an indefinite cause [31].

Marketing of these drugs is controlled by those who promise to entertain pain and enhance pleasure. They advertise to those who search for chemical panaceas for social and psychological distress [32].

Despite the media pressure and the aggressive marketing of the pharmaceutical industry, medications are not always the best treatment for the majority of emotional issues that affect patients, even in severe problems. Many patients are being treated solely with medications that are of little use in the treatment of emotional disorders. Often the problem persists, there is no appreciatrive positive effect, and there are times when the problem is aggravated by drugs, particularly when taken for prolonged periods.

Clinical experience and scientific data indicate that, in most emotional and behavioral disorders, the medications, even if they are useful as a temporary solution, are not sufficient for the patient to obtain a lasting result and develop effective strategies to address their issues.

Anxiety may become a mental disorder, can have pathological symptoms when it is excessive or unnecessary and affects the life of a person. Anxiety is also enhanced by conditions that affect the daily life of society; mood changes with unexpected situations, which eventually deplete mental responses [13].

Business Interests

Multinational pharmaceutical corporations direct their advertising campaigns to new drugs, or new uses of existing drugs, designed to solve contemporary problems such us stress, anxiety, panic attacks, social phobia, among others. These drugs allow the individual to meet the requirements of large globalized cities, where a higher percentage of the population actually consumes psychoactive drugs [33, 34].

It should be noted that marketing of new drugs is based on controlled clinical trials. However, the provided information is often difficult to extrapolate to routine clinical practice, therefore research is needed to reflect more faithfully what occurs in real life, and help us make decisions. Recent studies have had a major impact on the psychiatric community, and have caused deep-rooted beliefs [8].

In his book "Unhinged: The Trouble with Psychiatry–A Doctor's Revelations about a Profession in Crisis," Daniel Carlat [4] explains what the interests are that drove the change in the conceptualization of mental disorders, generating a purely biochemical model, and the pernicious influence that the pharmaceutical industry has exercised on the practice of psychiatry. As the author detailed, we are in an era of "frenzy of psychiatric diagnoses" where a constant addition of new mental disorders in each edition of the DSM is observed, not only in adults, but more worrying still, in children and adolescents, despite the serious risks involved in these age groups.

Carlat recognized that psychiatry underwent a major change after the introduction of psychotropic drugs in the 1950s and its subsequent expansion in the 1980s [4]. Until then, psychiatry subscribed to the Freudian view which attributes the origin of mental illness to unconscious conflicts developed in the infant stage.

When psychoactive drugs initially arrived to the market, a biochemical theory of mental illnesses was present. The theory was that the mental disorder was mainly related to the idea of chemical imbalances within the brain. The next idea was that the mental disorders could be pharmacologically corrected and was widely accepted by the media, the general public, and the medical profession.

The American Psychiatric Association, pharmaceutical companies, and other stakeholders promoted this paradigm shift from psychiatry to a biochemical model.

The medicalization of psychiatry defended the biochemical model, identifying this specialty of medicine as a scientific discipline. On the other hand, psychiatrists came to occupy the top spot in the intervention of mental illness, to represent the legal authority for the prescription of psychotropic drugs, and show less interest in exploring the life stories of their patients while focusing their actions in the elimination or reduction of symptoms using medications that alter brain function.

This change coincided with the process of preparing for the third edition of the DSM by the American Psychiatric Association. The new model was introduced to establish the diagnosis of mental illness in order to ensure that a variety of psychiatrists seeing the same patient would agree with the diagnosis. With this purpose, each mental disorder was defined on the basis of a list of symptoms. The goals of the DSM-3 were to clarify that psychiatry is a medical specialty; to facilitate diagnostic agreement among physicians, scientists, regulators, and patients; and to adjust to emerging drug treatments. As a result of these changes, the DSM-3 became the "bible of psychiatry" with widespread use in all areas: community psychiatrists, insurance companies, hospitals, courts, prisons, schools, research teams, government agencies, and other medical groups.

This review has proposed that shyness and defiance were converted in new mental disorders. It has led to the opposition of thousands of mental health professionals, who have launched a campaign to collect signatures for the annulment of the proposals.

Carlat believes that psychiatry is "a profession in crisis," and recognizes that providing drug treatment allows psychiatrists to see more patients in less time, increasing economic performance [4]. The pharmaceutical industry, encouraged by psychiatrists, and the expectation of patients has contributed to the belief that psychopharmacology is complicated and accurate, and that psychiatrists are scientific experts. The responsibility of the psychiatrist is to ask patients about their symptoms and to see if they match any of the mental disorders referred to in the DSM, and thus "assigning labels". Patients usually meet the criteria for more than one diagnosis because there is an overlap in symptoms. Drug treatment is prescribed for major symptoms, and additional drugs are prescribed to treat their side effects. According to Carlat [4], a typical patient that is taking an antidepressant for depression will also take another drug for anxiety, another for insomnia, another for fatigue (manifesting as a side effect of the antidepressant), and yet another for impotence (also a side effect of the antidepressant).

Carlat [4] believes that psychotropic drugs may be effective in some cases, but is strongly opposed to excessive use and abuse. An alarming increase in childhood psychiatric diagnoses that respond to fads and data without scientific evidence, has been detected. This makes it easy to diagnose a child of 2 years because "it is sometimes irritable" or a fifth grader "who presents with an attention problem." Yet the most serious consequence is the prescribing of a drug whose efficacy and safety have not yet been established.

It is also important to note that Thomas Steitz, Nobel Prize winner in Chemistry, 2009, recently reported that pharmaceutical companies focus their interest on medicines to be taken for a lifetime, seeking chronic disease, and for patients to not heal [1, 35].

Alternative Therapies

The problems of anxiety and depression are the main reasons for visits to a primary care physician. The current economic crisis has greatly increased its prevalence and it is expected that by 2020 will constitute the leading cause of disability worldwide. It has been recognized that current pharmacological treatments are only effective in half of the patients, and in some, a residual disease remains.

It has been recently shown that psychological therapy and the cognitive behavioral therapy in particular, is more effective and economical, and poses no risk to health. It reduces symptoms of

anxiety and depression, establishes long-term changes, provides greater adherence to treatment, a significant decrease in the risk of relapse and a high recovery rate while avoiding chronicity, which decreases the number of doctor visits and inpatient days.

Major clinical practice guidelines based on scientific evidence such as those of the National Institute for Health and Clinical Excellence recommend cognitive behavioral therapy as the treatment of choice for mild and moderate depressive disorder, panic disorder, obsessive-compulsive disorder, generalized anxiety disorder, and specific phobias.

When the mental health problem of the patient is complicated by other medical conditions such as alcohol or drug abuse or chronic physical health problems, or in the case of children, adolescents and pregnant women, psychological treatment is advised [10].

The efficacy and safety of many psychotropic drugs has been questioned in recent years. There have also been important advances in understanding the causes of mental illness leading to unrealistic claims.

By contrast, according to Uriarte [8], although there are alternative therapies to psychoactive drugs (e.g., psychological and psychotherapeutic treatments), these have the same or greater deficiencies than psychoactive drugs.

Discussion and Conclusions

There have recently been multiple appeals to use caution in the practice of medicine, particularly in the use of medicines. After years of moving towards the most effective, fastest, and newest, now it is time to resume the principle of "primum non nocere" (above all else, do no harm), delve into its meaning and rethink practices that are ineffective, useless, and even harmful to patients [36–38].

Prescribing cautiously means thinking beyond drugs, practicing strategic prescribing, monitoring potential adverse effects, being cautious and skeptical of new drugs and new indications, working with the patient to establish common objectives, and considering the goal of long-term pharmacological treatment in a broad sense [37–39].

Some possible alternatives to gradually modifying this problem and obtaining a rational use of psychotropic drugs would be for health professionals to orient their patients in a wider manner. It implies:

- Understanding that not every problem requires medication, whether medical or not
- Attempting changes in lifestyle, such as eating healthier and exercising regularly
- Identifying search tools to cope with situations that lead to stress, anxiety, etc.
- Searching within the family, work or social groups for people with whom to speak with confidence about the problem or situation being experienced
- Assuming the disappointments of life, giving them the dimension they actually have
- Finding solutions to problems without the use of drugs for as long as possible
- Considering that the use of psychoactive drugs is sometimes necessary. However, it may be that the collateral outcome could be worse than the desired or expected effect. It must be taken into account that adverse events may occur and may be more serious than the problem that needs to be solved

Moreover, an ongoing review of the applied psychopharmacological treatments could be made. This would allow deciding whether the *deprescription* process is necessary and how to accomplish it. *Deprescription* is the process of removing prescription drugs through review and concludes with necessary dose modification, or elimination of some drugs while adding others [39].

Given all the warnings by various authors mentioned in this review, we consider necessary to modify the care provided in mental health, because the validity of the model of care based on the administration of drugs is now being questioned.

To ensure this important goal, health professionals should encourage autonomy and patient self-care with the goal of facilitating the incorporation of prevention and rehabilitation behaviors,

and to teach how to differentiate when a problem requires medical attention and when it does not, to suppress consumption of psychotropic drugs without a clear indication, avoiding unnecessary risks and expenses.

There is an urgent need to reflect and to revise the existing relations among health professionals and the scientific partnerships with the pharmaceutical industry.

It is extremely important to note that the existence of one or more symptoms such as euphoria, sadness, or anxiety do not always indicate the presence of mental illness.

We need to move toward a better understanding of the factors associated with greater efficacy and safety of our interventions, which is often achieved through a comprehensive approach that wisely combines the best of each type of approach, psychopharmacological and psychotherapeutic.

Finally, we must recognize that there are still nosological entities in which pharmacological therapies are not indicated. The competencies of psychotherapy and a comprehensive approach should not be forgotten in these cases.

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Part III

Neurosciences, Learning, Teaching and the Role of Social Environment

Learning Styles: From Basic Investigation to Learning in the Classroom

16

Gabriela Díaz-Véliz

Learning and Memory in Rats

Memory is a slippery concept because we still have not produced a complete, consensual notion about the physical nature of its trace. The only sure way to grab at such phenomenon is by measuring behaviors and their modifications, i.e., quantifying it in an indirect manner.

After training an experimental animal such as a rat or a mouse, the only way to be sure that a memory was formed is by evoking it back (i.e., by recalling it in a test session). Behavior that differs from the one emitted in the training session is what we call memory. Thus, the responses can be: amnesia (i.e., memory reduction or blocking/deficit), facilitation (i.e., memory improvement), and no measurable effect.

Behavioral tasks that promote associations between stimuli and responses, or between two stimuli, are known as associative. Through them animals learn how to predict future events to express a proper, anticipatory behavior. In the instrumental or operant conditioning, the environment is arranged to permit certain response of the animal, such as avoiding a painful stimulus (typically a footshock), so that the escape or evasion is an option available to demonstrate that the animal learned the task. These paradigms have the principle advantage of simplicity. Rats and mice can acquire the appropriate responses within a limited number of trials.

Avoidance conditioning, for instance, is a powerful model for studying the neural correlates of associative learning in a wide range of invertebrate and vertebrate preparations [1]. In two-way active avoidance the animal learns that a neutral stimulus (a tone, the conditioned stimulus [CS]) is a reliable predictor for a forthcoming aversive experience (a footshock, the unconditioned stimulus [US]), and can prompt an evasive action in order to avoid it, i.e., it moves to the other side of the shuttle box (the conditioned avoidance response [CR]) when the stimuli predict aversive events. Because there is the possibility of learning how to escape, this task can be classified as an operant (or instrumental) conditioning, i.e., the animal must learn the relation between CS (sound) and US (footshock) in order to anticipate US with a CR (escape) and avoid it.

Although traditionally a sound (tone) is used as CS, we investigated how some mesencephalic regions associated with both auditory system (inferior colliculus) and visual system (superior colliculus) could be related to a different aversive learning in rats [2]. Rats were subjected to the active avoidance conditioning test to compare avoidance learning when CS was an auditory (2,800 Hz tone) or a visual (28 V light) stimulus.

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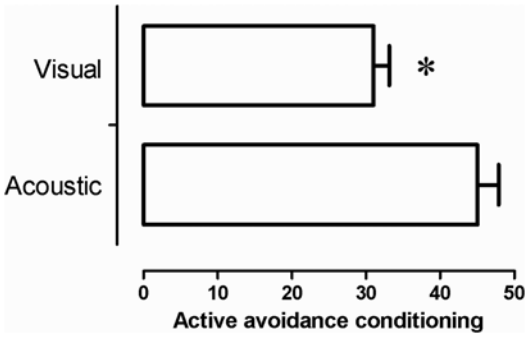

Fig. 16.1 Visual and acoustic avoidance conditioning. *Bars* represent the percentages of conditioned avoidance responses for 50 trials. The values are the mean ± SEM. of 8–10 animals on each group. For statistical comparisons was used the Student *t*-test [2]

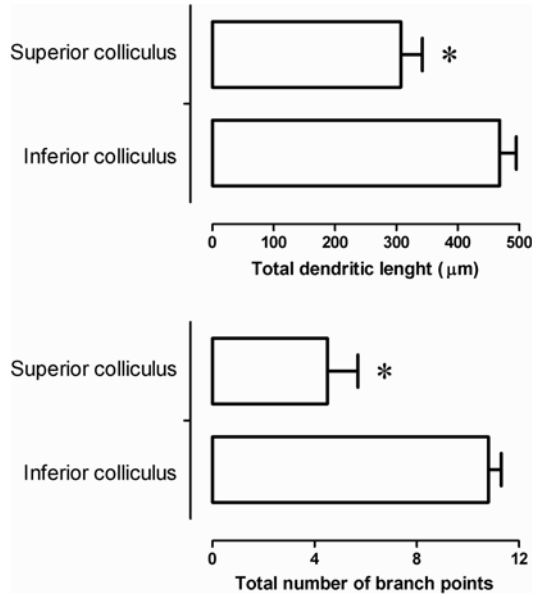

Fig. 16.2 Morphometric analysis of flat Inferior colliculus (IC) and wide-field superior colliculus (SC) neurons. Total dendrite length or the total number of dendrite branches of IC neurons, and total dendrite length or branch number of wide-field type neurons of the SC (Data obtained in $n = 80$ cells from $n = 8$ animals [2])

Each trial consisted of the presentation of CS that after 5 s was overlapped with a 0.20-mA foot shock until the animal escaped into the opposite chamber, with maximum shock duration of 10 s. A CR (learning) was defined as a crossing to the opposite chamber within the first 5 s after the tone or light. CRs were significantly lesser with visual conditioning compared with acoustic conditioning (Fig. 16.1), with no significant differences in the footshock thresholds between the two groups. Analyzing, in Golgi preparations, the neuronal morphology of the inferior and superior colliculi in order to explain the differences in avoidance learning, a lower total number of branches and less dendrites length was observed in superior colliculus (Fig. 16.2).

In conclusion, the different performances in conditioned behavior were associated with morphological changes in specific brain regions in rats. Given that these changes are correlated with learning, such plasticity seems to be an important predictor of learning-induced behavior [3]. Additionally, morphological and behavioral evidence indicates that the preferred channel for data input in rat brain seems to be the acoustic channel.

Neuroplasticity: Learning and Memory

We know that memory is a complex process necessary for cognition and that the ability to form memories requires changes in the synapses between neurons. Neurobiology development has shown that synapses structures are used not only to transmit information, but they are also extremely plastic and this plasticity is the basis for learning and memory. Synaptic plasticity, or the ability of synapses to modify their functional strength in an activity-dependent manner, also includes the ability of neuronal circuits to change as a result of certain patterns of neuronal activity. Neuroplasticity involves modulation of synaptic ion channels and receptors, dendritic branching, and spine density through genetic and epigenetic mechanisms. [4] Thus, learning could be considered as a change in behavior in response to environmental stimuli, and it depends critically on plasticity within the nervous system. As learning events occurs in the brain, physical changes are produced within brain circuitry and in its structure-function relations. Then, the most important factor in learning is the existing networks of neurons in the brain of the learner. Thus, knowledge induces physical changes in the brain [5].

Neural mechanisms that affect sensory function during states of attention, motivation, and vigilance (sleep and wakefulness) also affect how incoming sensory information is received (i.e., how neurons respond to sensory input), how neuronal responses are altered over time by changing sensory input (i.e., sensory plasticity), and how information about the environment is encoded, processed, stored for future use, and integrated with past experiences (i.e., memory formation [6]).

Memory is quite fluid, therefore, the brain continues to revisit and organize stored information with each subsequent experience in a cyclical manner, reprogramming its contents through a repetitive updating procedure known as brain plasticity. This is advantageous because knowledge is revised based on new input, resulting in a more accurate representation of the world.

Neuroplasticity could also be responsible for priming effects observed in various memory paradigms. For example, a single training trial may not be sufficient to elicit a memory of the trial, however, a subsequent trial may allow for memory formation in a time-dependent manner.

Sensory memory takes the information provided by the senses and retains it accurately, but briefly. Sensory memory lasts such a short time (from a few hundred milliseconds to one or two seconds) that it is often considered part of the process of perception. Nevertheless, it represents an essential step for storing information in short-term memory.

Short-term memory temporarily records the succession of events in our lives. However, this information will quickly disappear forever unless we make a conscious effort to retain it. Just as sensory memory is a necessary step for short-term memory, short-term memory is a necessary step toward the next stage of retention, long-term memory.

Long-term memory not only stores all the significant events that mark our lives, it lets us retain the meanings of words and the physical skills that we have learned. Its capacity seems unlimited, and it can last days, months, years, or even an entire lifetime.

Learning and Memory in Humans

In humans, learning can be considered as the process by which we acquire, develop, and process new information. However, it is clear that not all individuals learn in the same way, and the variability can be through age, motivation, prior cultural background, social context, and learning styles.

According Alonso et al. [7], learning represents the acquisition of a relatively enduring disposition to change the perception or behavior as a result of experience.

On the other hand, memory allows us to remember facts and experiences. It consists of encoding, storing information, and retrieval, making that information available for recall. When we see or experience something, it leaves a trace in our brain. Thus, learning is about acquiring information and memory is about storing it. In this way, we could say that learning is a process, and memory is the record of that process.

Zull [5] showed the connection between brain structures and learning and the relation between the functions of the cerebral cortex and the Kolb's learning cycle. At the beginning, the nervous system senses the environment through the sense organs, then these signals are recognized and integrated and, finally, a movement is generated as an appropriate response.

On the other hand, the learning cycle by Kolb arises from the structure of the brain, thus beginning with concrete experience that comes through the sensory cortex, continues with reflective observation what involves the temporal integrative cortex, then the abstract hypothesis occurs in the frontal integrative cortex, and active testing involves the motor brain [8].

Knowing the learning brain cycle induces to think in the importance that has the sensory input for adequate learning. The sensory cortex that receives input from the outside world correlated with concrete experience, depending on direct physical information from the world. The back integrative cortex that is related with integration of sensory information to create images and meaning, matches with reflection. The frontal integrative cortex that is responsible for organizing

actions for the entire body is related to the generation of abstractions and development of plans for future actions. Finally, the motor cortex that triggers all voluntary muscle to produce movement correlates with the necessity for action in completion of the learning cycle. Thus, learning requires conversion of ideas into muscular actions, including written and spoken language.

Student Learning Styles

There are different ways of understanding the learning process,one of them is provided by cognitive theories focused on how to learn and based on a constructivist postulate in which the subject constructs his knowledge of the world from the perception and the action.

Learning is considered not as a passive and receptive process, but as an interactive and dynamic process through which external information is interpreted and reinterpreted by the mind, gradually building increasingly complex explanatory models [7].

Even the best teachers have difficulty communicating knowledge to students when trying to apply the theoretical and pedagogical foundations in practice. The methodology used by the teacher and the evaluation method used can either promote or inhibit student learning strategies. In turn, in addition to using their cognitive abilities to structure the form of study, they must organize and prioritize their learning materials by providing adequate time for it.

How students perform these tasks depends, to a large extent, on their way of being and thinking and, above all, on their preference to use different learning strategies. Thus, defining the construct *learning style* is essential to delineate the areas covered, and particularly its possible applications.

The term "learning style" refers to the fact that each person uses their own method or strategies when learning. While strategies vary depending on what you want to learn, each tends to develop certain preferences or global trends, trends that define a particular style. Therefore, learning styles are like conclusions we reached about the way people act. However, when addressing the study of learning styles, it is difficult to provide a single definition that truly explains what this construct is. This difficulty occurs because it is a concept that has been addressed from many different perspectives, and in the literature we observe a large plurality of definitions according to various authors, some of which we note below.

> Some learn skills that stand out above others as a result of hereditary apparatus of their own life experiences and the demands of the current environment [8].
> Learning style is a particular set of behaviors and attitudes related to the learning context [9].
> The manner in which people gather, process, internalize, and remember new information [10].

One of the most inclusive definitions is from Keefe [11], who stated: "*Learning styles are cognitive*, *affective, and physiological traits that serve as relatively stable indicators of how learners perceive, interact and respond to their learning environments*". That is, the cognitive traits pertain to how students structure the content, form and use concepts, interpret information, solve problems, and select means of representation (visual, auditory, kinaesthetic). They are highly individualized preferences and trends that influence learning and they are dependent on the way that new acquired information is selected, represented, and processed. The previous cognitive structure provides meaning and organization to experiences and allows the student to go beyond the information given.

As noted, each person learns differently from another, using different strategies, at different rates, more or less effectively, even with the same motivation, the same level of education, the same age, or, they are studying the same subject.

Beyond this, it is important not to use learning styles as a tool for classifying students in closed categories because the way to learn is constantly evolving and changing. For this reason, some authors suggest discussing *learning styles preferences* rather than *learning styles*.

For Woolfolk [12], preferences are a more accurate classification, and are defined as the preferred way of studying and learning, such as using images instead of text, work alone or with

others, learning in structured situations, in different environmental conditions (room with or without music, the type of furniture, lighting type, etc.). It is important to note that the preference of a particular style does not warrant that the use of this style will always be effective. For this reason students may benefit if they develop new ways of learning.

However, despite the existence of a versatile range of classifications, *it* has achieved some consensus by noting that learning styles can be classified based on some basic criteria on how to select, organize, and process information.

In regard to the first criterion, it can be noted that neurolinguistic programming as the model refers to how the individual selects information [13]. The model called VAK (visual-auditory-kinesthetic) takes into account the neurolinguistic approach, which considers the route of entry of the information (eye, ear, body), which conditions the system of representation (visual, auditory, kinesthetic). The sensory modality preferred by each subject is undoubtedly a factor to be considered. Individuals rely on their senses to capture and organize information. However, most of us use representation systems unevenly, which will develop more when they are being used. The person who usually selects the type of information will easily absorb it and learn it, and the person who frequently ignores the information received by a determinate channel will not learn the information received from this channel, not because they are not interested, but because they do not pay attention to that source of the information.

Representation systems are not good or bad, but more or less effective for certain mental processes [14]. Visualizing helps us to establish relationships between different ideas and concepts. When a student has trouble relating these concepts it is often because he or she is processing information in a kinesthetic or auditory way. The abstraction and planning capacities are directly related to the ability to visualize. The auditory system cannot link concepts or develop abstract concepts with the same ease as the visual system does, also, it is not as fast. However, it is essential for learning languages and music. Kinesthetic learning is much slower than either of the other two systems, but it is deep. Once we know something that we have learned with the muscle memory, it is very difficult to forget it. Therefore, students who prefer to use the kinesthetic system need more time than others. We say they are slow, but that slowness has nothing to do with lack of intelligence, but with their different way of learning.

VAK Learning Style in the Classroom

Although everyone learns through a mixture of methods, one type of method is usually dominant in each person. Also, many methods are available for assessing the learning styles, with each method offering a distinctly different view of the learning style preferences. The VAK is a questionnaire developed with respect to the perceptual preferences in learning. This method defines the preference in the learning style in terms of the sensory modality in which a student prefers to take in new information [14].

The visual learners prefer the use of symbolic devices such as diagrams, graphs, flow charts, and models that represent the printed information. The auditory learners prefer hearing information and, thus, they learn better through discussions, lectures, tutorials and talking, through material, with themselves or others. Kinesthetic learning uses a combination of the sensory functions; such learners have to feel or live the experience to learn. They prefer simulations of real practices and experiences, lessons that emphasize performing an activity, case studies, real-life examples, role playing, and applications to help them to understand the principles and advanced concepts.

The knowledge about learning styles may help the educators in identifying and solving the learning problems among students, thus helping them to become more effective learners. This inspired us to conduct a study aiming to identify the VAK-preferred learning styles of all third-year students in the School of Nursing from the Faculty of Medicine, University of Chile [15]. We correlated the learning style with the academic

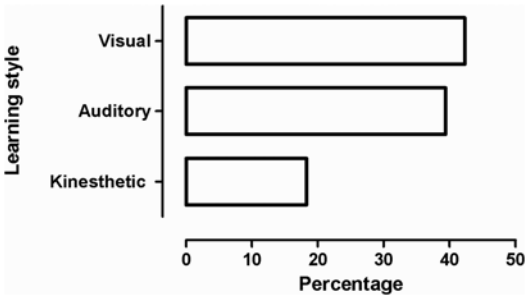

Fig. 16.3 Distribution of students according to their learning style. *Bars* represent percentage of preferred visual-auditory-kinesthetic learning style in 71 third-year students in the School of Nursing from the Faculty of Medicine, University of Chile [15]

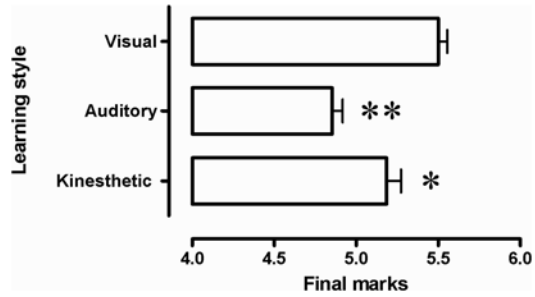

Fig. 16.4 Relationship between academic performance and learning style. *Bars* represent the mean ± SEM of marks according the preferred visual-auditory-kinesthetic learning style in 71 third-year students in the School of Nursing from the Faculty of Medicine, University of Chile [15]

performance of each student, trying to determine whether the different learning style among the students had any influence on their performance. Seventy one students (64 female and 7 male) with an age range of 19–22, years answered the completed questionnaire.

Data for performance was obtained at the end of the Pharmacology course. On a scale from 1 to 7, and pass mark 4.0, the average score was 5.2 ± 0.1, with the lowest score = 4.1 and maximum score = 6.2. The distribution of students' final marks follows a normal distribution.

By applying the corresponding questionnaire, it was found that the preference by the visual and auditory styles was similar (43 and 39 %) but significantly higher than the kinesthetic style (18 %) (Fig. 16.3).

The correlation between preferred VAK learning style and academic performance shows that students with visual learning style scored 5.5 ± 0.05, which was similar to students with kinesthetic learning style (5.2 ± 0.09), but significantly higher than marks obtained by the auditory learning style students: 4.9 ± 0.06 (Fig. 16.4).

This evidence is confirmed in Fig. 16.5, which shows that students with the visual style obtained the highest marks, with an average fluctuation between 5.1 and 6.2. In contrast, students with the auditory style had an average fluctuation marks between 4.1 and 5.3.

The results show a clear difference in the percentage of students who respond to the kinesthetic type of learning compared with the other

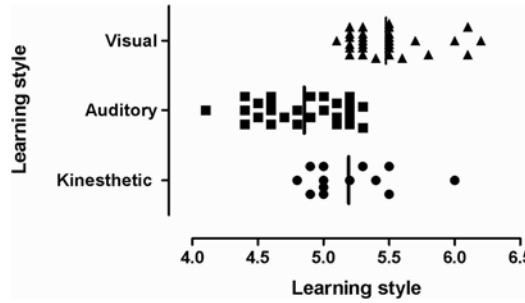

Fig. 16.5 Relationship between individual marks obtained at the end of the Pharmacology course and preferred visual-auditory-kinesthetic learning style. Seventy one third-year students in the School of Nursing from the Faculty of Medicine, University of Chile [15]

two types, as well as a good correlation between the academic performance of students and the preference for a particular learning style. The trend indicates that students with the visual style would be favored by the teaching style. In this study, the teaching methodology was carried out through lectures supported by visual materials (slides and data show).

It should be taken into consideration that, for better effectiveness of learning it should also analyze the style of presentation of information used by the teachers (teaching style preference). Many difficulties in learning can be associated with mismatches between the student styles of learning and the teacher styles of teaching. To promote meaningful learning, teachers should organize

classroom work with consideration to the learning styles of all students. Therefore, it could be considered to be desirable that the learning style of the students determine the methodology used by the teacher. Every student should experience several methodologies and have access to different learning contexts in order to accomplish skills.

Even being relatively stable, learning styles can be modified. It is the responsibility of the teachers to help students discover their own learning style with the goal of adapting themselves to different situations. Thus, in an ideal scenario, students should be able to use different strategies, choosing the most appropriate depending on the situation and context, which promotes learning to learn.

This study clearly showed that academic achievement is related to the learning process. However, the academic performance should be considered within a framework of complex variables: social and environmental conditions, intellectual factors and emotional aspects, technical and didactic aspects, organizational factors, etc.

Several authors [7, 8, 11, 15–17], both within educational psychology and in teaching in general, have emphasized the importance of considering learning styles as a point starting in the design, implementation, and monitoring of the process of teaching and learning, suggesting that accommodating teaching methods to the preferred styles of students can bring greater satisfaction and improve academic performance.

Knowing the students' preferred learning styles also helps in overcoming the predisposition of many educators to treat all the students in a similar way, as well as motivating the teachers to move from their preferred mode to using others. In doing so, they can reach out to more students because of the better match between the teachers and the learner styles [18, 19].

There is definitely a trend in teaching, to instruct all the students in the same way (lecture format), because of the relative ease of passing the information, the need to cover the content, a long history of traditional lecturing, and perhaps due to their own preferences in learning, which may not always be correct. The results of this study should convince the teachers to use multiple modes of information presentation.

Finally, it should be noted that if you desire a true diagnosis of preference for a particular learning style, it is advisable to use more than one instrument or questionnaire. Admittedly, none of the analyzed instruments is able, by itself, to provide a complete diagnosis of all factors involved in learning styles. The best strategy for accuracy would be the use of duplicate instruments and the choice of tools to meet a greater number of characteristics appropriate to the population that is diagnosed.

After years of theorizing about learning styles, it is clear that there are still issues to be investigated to progress and improve the teaching-learning process.

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Learning Styles and Strategies: The Importance of the Tool Selection

17

Jesús Fernando Escanero, Marisol Soria, Manuel Guerra, Jackson Silva, and Pascual Ángel Gargiulo

Introduction

Learning to Learn

The European Union (EU) [1] defines the competencies as a combination of knowledge, skills, and attitudes appropriate to the context, and the key competencies as those that all individuals need for personal fulfillment and development, active citizenship, social inclusion, and employment. The key competencies are all considered equally important, because each of them can contribute to a successful life in a knowledge society, consequently, these concern all citizens. 'Learn to learn' is one of the eight key competencies identified by the EU in the document "Competencies to be acquired by the 'longlife learner'". This competency, in a certain sense underlies all other ones.

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The European Commission defines the 'learning to learn' as the ability to pursue and persist in learning, to organize one's own learning including effective management of time and information, both individually and in groups. It also includes awareness of the needs and processes of their own learning, the identification of the available opportunities, and the ability to overcome obstacles in order to learn successfully. It presumes to obtain, to process, and to assimilate new knowledge and skills in addition to the finding and use of a guide. Learning to learn means that students commit to building their knowledge from their learning and life experiences in order to reuse and apply knowledge and skills in a variety of contexts: at home, at work, in education, and instruction (see Bologna reform). Motivation and confidence are crucial to the competence of the person, and European countries have incorporated it into their legislation. In Spain, the legislation includes the EU proposals. The LOMCE ("Ley Orgánica de Mejora de la Calidad Educativa", Spanish acronym for Organic Law on Improving Educational Quality, Organic Law 8/2013, of December 9), in particular, includes the EU proposals.

Acquisition of the Competency

The learning styles and strategies are the two bases or columns on which the acquisition of the aforementioned competency is structured. However, its application is not as simple as could seem a priori.

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DOI 10.1007/978-3-319-17103-6_17

The two great lines that dominate the learning styles and strategies, though apparently very distant is not so, neither in its principles nor in the measuring instruments that are designed to measure them [2]. The phenomenological perspective, advocated by Entwistle and Marton, as well as others, and located mainly in Europe, part of the perception that individuals have of their own context or situation following an ecological approach in the building of the theoretical framework of learning strategies, or as they prefer to call it, the *approach* that students use for learning, is none other than the style plus the context. The experimental perspective defended in America by Schmeck as well as others, is based on cognitive psychology and research on memory, learning, and particularly the "levels of processing". It emphasizes the role of learning styles and also analyzes learning strategies from an intercontextual point of view. From this perspective, learning styles are defined as long as students provide answers to how they learn in different contexts.

There are two important meeting points for both lines. The first is the interest by measuring learning strategies or styles quantitatively—for which everyone has developed their own instruments of evaluation. The second is the interest in confirming the relationship between academic performance and the scores obtained by students in the above-mentioned questionnaires.

The most widely used tools or questionnaires are analyzed in this chapter, and the most relevant are recommended, including a new one that it is being presented here for first time.

Learning Styles and Strategies

Terminological Delimitation

Styles and strategies of learning are two approaches to the same problem. There is a broad recognition that learning strategies are the base unit of analysis in the study of these procedural aspects [3].

It should be noted that there are numerous definitions with regard to learning styles, with the Keefe concept [4] being one of the most quoted. Keefe postulates that "learning styles are cognitive, affective, and physiological traits that serve as relatively stable indicators of how learners perceive, interact, and respond to their learning environments". Consequently, learning styles (cognitive traits) pertain to how students structure the content, form and use concepts, interpret information, solve problems, select means of representation (i.e., visual, auditory, kinesthetic), etc. The affective traits are related to the motivations and expectations. They influence learning. The physiological traits are linked to gender and biological rhythms, biotype, and biorhythm of each subject (i.e., sleep-wake rhythm of the students) [5, 6]. Overall, learning styles refer to typical and relatively stable rules that come into play in the act of learning [7].

Some authors suggest discussing "learning style preferences" rather than "learning styles". Thus, Woolfork (5) defines the preferences as the preferred ways of studying and learning, such as using images instead of text, working alone or together, to learn in structured situations or not, with or without music, etc. For us it is an approximation to the first-order strategies. Actually, the "preferential use of a particular set of strategies" [8] has been called learning style.

On the other hand, other authors have tried to establish the limits between *cognitive and learning styles* because in many publications, they are considered connected concepts and in others, overlapping concepts [9]. According to Castaño [9], in a generic way, there are the following differences:

1. Cognitive styles analyze the differences in the cognitive structure of the individuals, while learning styles analyze the individual differences when approaching the learning process.
2. Cognitive styles are in a deeper level of the mental structure of the individual if we compare them with learning styles.
3. The measuring instruments used to measure the style are different. While tasks are traditionally used to assess the cognitive styles, the self-report format is used to assess the learning styles.

Cognitive styles are typically described as characteristic modes of thinking, remembering,

and problem-solving [10]. They can be defined as an individual's preferred way of gathering, processing, and evaluating data. The duality of consciousness has been viewed in different ways. Many authors view intuitive and analytic cognition as representing the poles of a single dimension. This suggests that the cognitive style of a particular individual may fall at any point on the scale. It has enabled us to divide the diagram of the learning styles into two regions: the analytical region and the intuitive region. We believe that it is interestingly useful for the cognitive-pedagogic integration in the design of the new tool.

In attempting to establish the concept of learning strategies it can be noted as a synthesis of the different definitions that they are actions (organized and conscious) and procedures that the learner uses to perform specific learning tasks [2]. As characteristics of the strategies, following Esteban et al. [2], may be indicated:

- The strategies are selected in order to achieve a goal or objective.
- Consequently, they require a certain degree of control over their own cognitive activity, which involves deliberation and flexibility in the selection of their own resources and capabilities and the planning and the evaluation of actions or procedures. This feature refers to the metacognitive activity through which the subject interprets and controls their thinking in relation to the goal to be achieved and what he or she is doing to achieve it [11].
- Finally, all learning strategy requires the articulation of the selected processes (and their management) to achieve the goal.

Learning Styles

Learning Styles Inventories

As Gallego [12] indicates, it should be noted that one of the causes that have prevented further development and application of the learning styles lies in the plurality of definitions, approaches, and tools used. These opinions coincide substantially with the manifestations of Curry [13], who estimated that there were over 70 tools or instruments to determine the learning style of students.

As an example, we should mention the fact that different authors have made multiple taxonomies, with each having different styles and meanings. The following authors incorporated relevant proposals to learning styles knowledge. Dunn et al. [14] proposed visual, auditory, and tactile or kinesthetic styles. Schmeck [15] established deep, elaborative, and superficial categories. Kolb [16] classified them as convergent, divergent, assimilating, and accommodating. Honey and Mumford [17] divided them into activist, reflector, theorist, and pragmatist. Felder and Silverman [18] and Felder and Soloman [19] reported five dimensions, each one with two preferences: inductive/deductive, active/reflective, sensory/intuitive, verbal/visual, and sequential/global.

Applying Styles

The style application provides a student profile that allows acting in two directions [20]. On one hand, trying to improve the styles of the lowest scores in order to lead all students to have high scores in all constructs (styles), enabling them to become effective learners in all contexts. On the other hand, strategies would be trying to facilitate the knowledge, aiming to apply the best and most effective methodology for the learning/teaching interaction. It is designed as a matching/mismatching hypothesis. However, in this regard, Coffield et al. [21] indicated that there are no clear pedagogical implications in the field of styles. One of the problems rests with the lack of a single voice on the subject among researchers. There is rather widespread disagreement about the advice that should be offered to teachers and tutors, concluding that at present, there is no definitive answer to the question of whether the teaching style should be paired with the learning answer. The reason is a lack of rigorously controlled experiments and longitudinal studies to confirm the leading proponent claims.

In this chapter we focus on the first direction: improving the student learning styles.

Detecting the Problem

In our opinion, the application of different tools (i.e., index of learning styles (ILS), Felder and Soloman [19]; CHAEA—Spanish Acronym, Alonso et al. [22]) provides conflicting results as recently reported in various publications [21, 23]. These publications put us on alert for a possible discrepancy between the results obtained for the same groups explored. If we use the CHAEA, actives are those who in this case get lower scores and lower rates. By contrast, if we use the ILS, we find that it provides a predominance of this group (actives) over the reflexive one. It is difficult to find an explanation for this without a thorough analysis of the defining items in both tests, and makes it necessary to find the basis for this discrepancy. In a first approximation, major design differences are observed between both questionnaires. The CHAEA independently explores the active and reflexive styles with 20 items in each, while the ILS uses (reflexive/active) 11 items to determine the dominance of one of the two styles compared. Consequently, the ILS confronts styles (modality) and one variable subtract from another within the same dimension (i.e., active versus reflexive). In contrast, the CHAEA is designed in another way. In it, each construct is defined using 20 items, obtaining scores for each one independently, which is important to keep in mind when choosing the tool.

If the items defining the active style between both questionnaires are analyzed, the following can be observed:

- Only four items on the CHAEA (7, 35, 43 and 67) explore concepts equivalent to the ILS. On the last questionnaire they are formulated only in two items (9 and 25).
- Fourteen items on the CHAEA (3, 5, 9, 20, 26, 27, 41, 43, 48, 51, 64, 67, 75 and 77) do not have a complete transposition of the concepts defined on the ILS, and, of these, some items (numbers 5, 9, 13, 17, 25, 37 and 41) explore only partially equivalent concepts.
- The other items (4 of 11 on the ILS and 4 of 20 on the CHAEA) do not match between the two questionnaires. Some items on the ILS (1 and 29), which in our opinion clearly define profile of actives, do not present an equivalent one on the CHAEA.

In our view, some of the items on the CHAEA seem to better define rashness and impulsiveness traits than activity.

The New Questionnaire

To solve this problem a new tool has been created, based on the experiential learning established by Kolb [16, 24, 25], derived tools [17, 18, 22], and on the Allison and Hayes CSI—cognitive style index [26, 27].

Kolb's model [16, 24, 25] takes as its starting point the Lewin's learning cycle [28], which suggests that there are four states, one after the other. The concrete experience (CE) is followed by the reflection in that experience. After reflection several conclusions can be obtained about the experience or about the application of theories about it (abstract conceptualization [AC]). This is followed by finding ways to change the next idea of the experience (active experimentation [AE]), and results in a new particular experience. According to Kolb [16], learning is a process which enables deducing concepts and principles from the experience guiding the behavior in new situations. He defines learning as the process by which knowledge is created through the transformation of the experience. He identifies *two main dimensions (or axis)* of the learning: perception and processing. He points out that learning is the result of the way in which people perceive, and then processes what they have perceived. The poles, dialectically opposed, of perception axis are the CE and the AC. In the processing axis, the opposite poles are the AE (the implementation of the implications of the concepts in new situations) and the reflective observation (RO).

The juxtaposition of perception and processing is what led Kolb to describe a model of four quadrants to explain the learning styles [16, 24, 25]. Each quadrant is a different style: converging, diverging, assimilating, and accommodating.

Our model assumes the perception and processing axis. Perception, first stage of learning, is a cognitive process with two dimensions. At one extreme, it shows the senses of sight and hearing (listening, watching, and reading). At the other extreme, it is the kinesthetic dimension (trying, checking, and doing). Theorist and dynamic poles have been used to describe them.

The processing axis, which uses the same opposite ends as Kolb [16], establishes the AC as a stage between both opposite extremes. It goes from the critical and argumentative reflection (thinking), to the practical application and decision making (solving, resolving). The names given are reflexive and operative poles.

Interaction between the vertical and horizontal axes determines and defines four quadrants which correspond to the four learning styles, known as:

Style 1: **theorist-reflexive style**
Style 2: **dynamic-reflexive style**
Style 3: **theorist-operative style**
Style 4: **dynamic-operative style**

In turn, the two upper quadrants determine the cognitive **analytical** dimension, which includes the theorist-reflexive and theorist-operative learning styles, and the lower quadrants determine the cognitive **intuitive** dimension, formed by the dynamic-reflexive and dynamic-operative learning styles. In our opinion, cognitive dimension underlies and conditions the teaching styles (Fig. 17.1).

It is assumed, as Kolb did, that the learning cycle can start in any of the four poles, although generally it starts with the perception. The sequence (EC-OR-CA-EA) described by Kolb [16] does not always appear in the established order. In our model, there are two learning cycles which focus on every cognitive dimension:

- In the analytical cycle, the following elements are included: sight-hearing, reflection, abstract conceptualization, and resolution
- In the intuitive cycle the following elements are included: kinesthesia, reflection, abstract conceptualization and resolution

These cycles show action preferences, not implying an obligation, which enable the interaction of both regions (cycles). Furthermore, the end of one of them can be the start of either of the two regions.

Furthermore, each cognitive dimension is constituted by two quadrants, each one representing one pedagogical or learning style (see Fig. 17.1).

Results

The test was carried out with a total of 199 students from Physiology III (Heart, Circulatory, Respiratory and Digestive System), from the second academic year of the Degree of Medicine

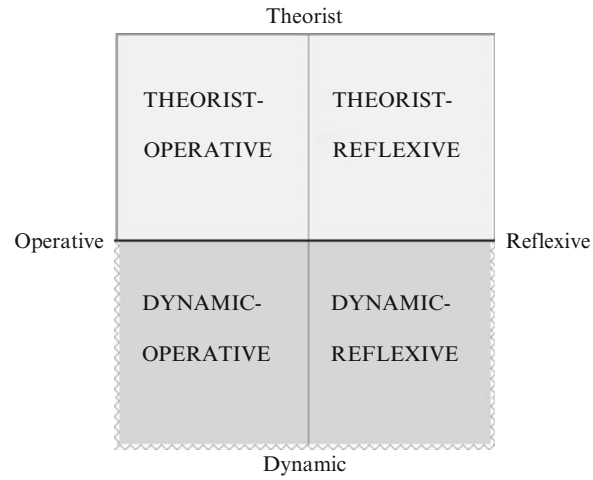

Fig. 17.1 Learning styles according to the questionnaire by Escanero and Soria (CESEA, Spanish Acronym of Cuestionario de Escanero y Soria de Estilos de Aprendizaje). *Source*: Escanero and Soria 2014. Intellectual Property Registration Z-21-14

Table 17.1 Characteristics of the learning poles

Theorist	Dynamic	Reflexive	Operative
Methodological	Creator	Conscientious	Solver
Logical	Explorer	Mediator	Decisive
Concrete	Experimenter	Compiler	Practical
Coherent	Inductor	Researcher	Realistic
			Receptive

(third semester) of the University of Zaragoza (Spain). In this group, 135 students (68 %) were women and 64 students (32 %) were men. The descriptive analysis showed that the theorist learning pole is the one with the higher average score (37, 25 points-maximum 48-) and the dynamic is the lowest score (34, 43). In general, the scores of the students are much more homogeneous than those obtained with other questionnaires that we think that best defines our students.

The consistency of the internal reliability was measured with the *Cronbach's alpha* and the analysis has shown that the scale used to measure the 48 variables has a good index (0.822). The scales used to measure each of the four learning poles were analyzed individually and the values have been satisfactory (between 0.6 and 0.8).

Use for Improvement

In the validation of the new questionnaire (Appendix) the characteristics which stand out in each of the poles were evaluated. The factorial exploratory analysis was used for each pole with 12 items. In this stage, the factors of each learning pole were identified following the *Kreiser-Gutman Test* (*eigenvalue* > 1). Each identified factor has been analyzed and has received a name related to a characteristic of the pole to which it belongs. As stated in Table 17.1, this has allowed the identification of the most important pole characteristics.

Moreover, each factor is defined by an item series. If they have low scores (0, 1, or even 2) the teacher must work with them to improve the style (pole) to which they belong.

Tables 17.2, 17.3, 17.4, and 17.5 list the questions that define each feature of the poles, as well as the factorial loadings of each one.

Table 17.2 Characteristics/factors of the theorist pole. Items that define each characteristic and factor loading of each item

	Factors			
Items	Methodological	Logical	Concrete	Coherent
10A	**0.743**	0.189	0.199	−0.079
13B	**0.743**	0.010	−0.032	0.257
6B	**0.641**	0.138	0.206	0.310
2B	−0.222	**0.704**	0.088	0.303
1A	0.222	**0.654**	0.294	−0.082
4A	0.193	**0.609**	−0.010	−0.003
24A	0.429	**0.483**	−0.183	0.106
15B	−0.035	0.141	**0.789**	0.129
16A	0.209	−0.062	**0.738**	−0.095
21A	0.074	0.323	**0.393**	0.352
19B	0.076	0.176	0.015	**0.734**
17B	0.236	−0.083	0.023	**0.728**
Eigen value	3.017	1.366	1.246	1.101
Variance	25.14	11.38	10.36	9.18

Table 17.3 Characteristics/factors of the dynamic pole. Items that define each characteristic and factor loading of each item

	Factors			
Items	Creator	Explorer	Experimenter	Inductor
17A	**0.755**	0.41	0.054	0.269
24B	**0.713**	0.103	0.169	0.035
13A	**0.710**	−0.217	0.055	0.206
19A	**0.651**	0.153	−0.081	−0.083
16B	−0.001	**0.794**	0.041	0.120
21B	−0.037	**0.603**	0.090	0.426
4B	0.290	**0.562**	0.285	−0.317
2A	0.066	0.183	**0.789**	−0.131
1B	−0.080	−0.081	**0.756**	0.309
15A	0.225	0.162	**0.332**	0.120
10B	0.088	0.212	0.046	**0.699**
6A	0.333	−0.047	0.156	**0.544**
Eigenvalue	2.767	1.612	1.121	1.071
Variance	23.059	13.436	9.340	8.929

Table 17.4 Characteristics/factors of the reflexive pole. Items that define each characteristic and factor loading of each item

Items	Factors				
	Conscientious	Meditator	Compiler	Researcher	Receptive
11[a]	**0.765**	0.159	–0.001	0.105	–0.078
18[a]	**0.662**	–0.141	0.378	–0.127	–0.016
8B	**0.593**	0.180	0.032	0.481	–0.012
20B	**0.472**	0.370	–0.154	–0.197	0.432
22B	0.037	**0.840**	0.051	0.043	–0.026
14B	0.048	**0.550**	0.274	0.311	0.190
23A	0.401	**0.517**	0.090	–0.344	–0.173
5B	0.044	0.133	**0.787**	0.187	0.090
12A	0.046	0.311	**0.550**	–0.401	–0.282
3A	0.401	–0.017	**0.464**	–0.006	0.263
9B	0.034	0.051	0.070	**0.812**	–0.088
7A	–0.080	–0.016	0.108	–0.020	**0.816**
Eigenvalue	2.711	1.381	1.160	1.113	1.044
Variance	22.593	11.506	9.668	9.277	8.701

Table 17.5 Characteristics/factors of the operative pole. Items that define each characteristic and factor loading of each item

Items	Factors			
	Solver	Decisive	Practical	Realistic
8A	**0.859**	0.034	0.002	0.035
9A	**0.857**	0.052	0.081	–0.019
7A	**0.213**	–0.051	0.175	–0.609
23B	0.131	**0.778**	0.013	0.154
22A	0.063	**0.691**	0.278	–0.098
12B	–0.471	**0.628**	0.000	0.023
3B	0.125	0.080	**0.688**	0.091
5A	0.093	0.129	**0.615**	–0.360
20A	–0.052	0.028	**0.586**	0.119
14A	–0.039	0.405	**0.507**	0.055
11B	0.196	–0.086	0.383	**0.678**
18B	0.236	0.418	0.193	**0.565**
Eigenvalue	2.586	1.878	1.279	1.085
Variance	21.549	15.651	10.658	9.044

Way to Act

Once a student has completed our questionnaire, the scores of the main factors of each pole must be analyzed. Estimation is necessary with the goal to orient work for their improvement. The mechanics are the same as those used to enforce certain behaviors: specific activities, registration of compliance/defaults, etc. The last goal, as it has been indicated previously, is that all students have a high score in all styles (or poles). It is equivalent to indicating that he/she is a good learner in any context.

From the above, it is evident that our recommendation is directed to the new tool presented.

Learning Strategies

Learning Strategies Inventories

When one approaches the subject of strategies, two things are needed. Conceptual precise location is required, but also needed is an understanding of the different denominations.

Thus, from the first classification by Flavell and Wellman [29], until the most commonly used inventories or questionnaires as the learning and study strategies inventory (LASSI) or Spanish acronym of adquisición, codificación, recuperación y apoyo (ACRA), there is an extensive number of names, which can discourage those who analyze this subject ignoring this problem. Flavell and Wellman [29] based their classification on the depth with which learning takes place. They distinguish between associative and restructuring strategies.

To analyze the different inventories commonly used in higher education, we will proceed by first analyzing those used to explore metacognitive

strategies. Then we will analyze the wider ones in which cognitive strategies are explored although other factors such as metacognitive and socio-affective variables may be added, as occurs in many cases. The reason for proceeding in this way is simple: our recommendation regarding its use is based on our practical experience.

Inventories for Measuring Metacognitive Strategies

The measurement of these strategies may present some difficulties because they form part of the mental processes of the individual. There have been several attempts to develop metacognitive inventories to date.

In 1978, Myers and Paris [30] created the first metacognitive inventory, corroborating strategies on the person, tasks, and categories developed by Flavell and Wellman [29]. They designed a structured interview format to allow older readers to respond freely to the open questions. Later, Paris and Jacobs [31] modified the instrument of Myers and Paris [30], using 15 open-ended questions containing three categories, namely planning, evaluating, and regulating and they developed an inventory for any reading situation. Miholic [32] used the Paris and Jacobs [31] questionnaire as a starting point to develop an inventory to measure metacognitive activities of young students. This inventory included 10 questions regarding the difficulties that the learners might face while reading. As opposed to the two previous ones, this instrument focused more on difficulties encountered in terms of whether readers show metacognition strategies in the process of reading. Subsequently, other authors have worked on inventories on metacognitive strategies of reading. Thus, Mokhtari and Reichard [33] have designed and validated an inventory on Metacognitive Awareness of Reading Strategies Inventory (MARSI) for teens and adult readers at the University of Texas. Pereira and Ramirez [35] evaluated the use of metacognitive reader strategies in university students in Venezuela. They translated to the Spanish the Survey of Reading Strategies (SORS, [35]), designed to determine the use of metacognitive strategies while reading school and academic English texts. At the Complutense University of Madrid, Jiménez et al. [36] measured metacognitive strategies of awareness reading, using an instrument called ESCOLA ("Escala de Conciencia Lectora", Spanish acronym for Reading Consciousness Scale). Dañobeitia and Ramirez [37] have designed and validated a set of meta-linguistic skills at the University of Talca. They did it following the postulates of Gombert, Guan-Qun and Meng (China), and Roehring and Mason (USA) [38]. They examined the psychometric properties of the MARSI instrument for measuring metacognitive awareness in reading [33].

Similarly, Jaramillo and Osses [39] validated an instrument on metacognition in terms of knowledge, metacognitive experiences, and cognitive self-regulation in students of the second cycle of primary municipal schools on reading comprehension.

A questionnaire by Schraw and Dennison ([40], Nebraska University) frequently used in research is the Metacognitive Awareness Inventory (MAI), developed to measure metacognitive awareness in adults. This 52-item inventory is a long, comprehensive scale assessing various facets of metacognition including metacognitive knowledge and regulation [40]. Items are classified into eight subcomponents under two broader categories of knowledge of cognition and regulation of cognition. Each component has different subcomponents. To clarify, knowledge of cognition includes at least three different types of knowledge: declarative, procedural, and conditional knowledge [41, 42]. Regulation of cognition, on the other hand, refers to a set of activities that help students control their learning. This component also has subcomponents: planning, information management strategies, comprehension monitoring, debugging strategies, and evaluation. Although a number of regulatory skills have been described in the literature, three skills stand out on all accounts: planning, monitoring, and evaluation [43]. Its use is simple because each question must be answered by remarking true or false.

The State Metacognition Inventory (SMI) was developed by O'Neil and Abedi ([44], University of South California). It has four subscales of metacognition, namely planning, self-checking, cognitive strategy, and awareness. The entire inventory was validated in a group of 219

community college students along with a 20-item math test. They are answered with a Likert-type scale (from 1 to 5). The Spanish translation and validation was performed with students from the Barcelona School of Psychology by Reinaldo Martinez Fernandez and published in his doctoral thesis [45]. Yildiz et al. [46] developed a new instrument called the Metacognition Scale (MS) by reviewing previous studies [40, 44, 47]. The questionnaire includes eight scales, namely declarative knowledge, procedural knowledge, conditional knowledge, planning, self-control, cognitive strategies, self-assessment, and self-monitoring. The results indicate that the MS is appropriate for researchers or teachers whose goal is to measure metacognitive awareness and metacognitive abilities of his or her students.

Finally, Labatut [48], in his doctoral thesis, described a "Metacognition Questionnaire" which was created based on the model of Mayor et al. [49]. The first four items are related to the personal data of each student—sex, age, speciality, and academic year. The instrument includes items relating to the three macrocomponents of metacognitive activity—awareness, control, and autopoiesis—combined with nine dimensions of cognitive activity. They are representations, processes, functions, duality, regulation, adaptation, systemic organization, flexibility, and reflexivity—resulting 26 items.

Nine items were added which corresponded to each of the variables in metacognition—subject (knowledge, skills-dispositions. and motivations), context (materials, situations, and sociocultural context), and activity (tasks, strategies, and attention effort). The last question of the questionnaire was incorporated after the author observed great difficulty answering questions in his students that they have regarding content with their teachers. The result is a questionnaire with 46 items that take into account all the basic aspects of metacognition. Students should answer the questionnaire, indicating one of the five alternatives in each item (Likert scale), as proposed at the beginning of the inventory.

Although our experience is centered on the questionnaire of O'Neil and Abedi [44], we believe that any of the latter three can be used to approach metacognitive knowledge of students.

Inventories Designed to Measure Cognitive Strategies

Consistent with this assessment framework, appropriate instruments should be used. However, we have not found any that would adequately cover the various strategies involved in learning. Additionally, some of those analyzed are the most used in our context research, also exhibit other problems.

1. The Spanish questionnaire of Learning Strategies ACRA of Roman and Gallego [50], validated in a non-university population (12–16 year old students) and also used at the university (Spain), cannot be transposed to the same because the work of university validation proves its unsuitability as the questionnaire is constructed [51, 52]. The authors report good internal consistency for the scales of the questionnaire, which ranges between 0.73 and 0.87 (Cronbach's alpha). However, it did not provide for strategies in which scales are composed. Moreover, the adaptation to the student population meant a reduction of the total number of items and this fact may result into a different validity of the questionnaire [52]. In fact, De la Fuente and Justicia [51] found three, not four, dimension/scales which were identified as cognitive and control strategies, support strategies and study habits with a reliability between 0.54 and 0.85 (Cronbach's alpha). In the latter paper, the authors argued for continued research on the validity of the scale. In the doctoral thesis by Ramirez Martinez [53], the strategies in college students were analyzed at the Universidad San Francisco Xavier de Chuquisaca in Sucre (Bolivia) with middle and high performance. An acceptable Cronbach's alpha for the subscales of ACRA was obtained. They were acquisition of information (0.836), coding of information (0.932), information retrieval (0.851), and support and processing (0.910). This instrument seems appropriate for use in the university population as was suggested

   The model underlying in this instrument hypothesized the existence of three groups of cognitive learning strategies in information

processing (acquisition, encoding, and retrieval), as well as a fourth group (processing support) which includes metacognitive and socioaffective variables. The four groups are translated into four scales (acquisition, coding, recovery, and support) which are subdivided into different types of strategies. The questionnaire consists of 84 items. The three scales of cognitive strategies cover the fundamental processes of information processing: attentional, processing, organization, replication, and storage.

From our point of view, the structure of the first two scales is debatable because storage strategies are located both in the first (acquisition of the information) and in the second (codification or storage). Moreover, the fourth level of support in the group includes socioaffective strategies with the same strategy (intrinsic and extrinsic motivation), both scored in the same direction, which is striking. This can give the impression that intrinsic and extrinsic motivation are equivalent or of similar value. While taking into account a set of key strategies such as search, selection and collection of information, others are excluded. This is the case of personalization strategies, creativity, and transfer. It also excludes the value of the task, self-efficacy, and control of the context among the support strategies.

2. The LASSI questionnaire by Weinstein [54] was designed for the academic population in the USA. It is popular with the Spanish research at the university, however, it does have some limitations. From our viewpoint, a first problem is that the author [54–56] to elaborate the questionnaire seems to not take into account the classification proposed by herself and that has been used widespread in research since its publication. This classification was developed, respect to the cognitive strategies, depending on the level of processing and cognitive control required, including in the most elementary level, repetition strategies followed by elaboration and organization strategies. It could be observed that in the three types of strategies a subdivision is stablished upon their development for elementary or complex learning tasks. To these strategies are added others of regulation of learning -metacognitive- and the affective-motivational.

   The classification has limitations dating back to the time of creation. When designing the questionnaire, Weinstein's goal was to correct the deficiencies of previous instruments and wanted to focus on the strategies related to the successful learning on which it could intervene educationally. It was created with an initial bank of 645 items. The process of the questionnaire elaboration concluded with the questionnaire which is internationally known and is composed of 77 items and 10 scales. They included attitudes, motivation, time management, anxiety, concentration, information processing, selecting main ideas, study aids, self tests, and exam strategies.

   Metacognitive strategies outlined in the instrument were not clearly detailed. The same was observed with fundamental cognitive strategies such as search and selection information as well as other important information related to processing strategies such as the case of storage, transference, customization, and creativity (in fact, on the processing scale only elaboration and organization strategies were included). The author also assumed that motivational and support aspects, now considered fundamentals, such as: the value of the task, the powers, the self-efficacy, the context control and the appropriate social interactions, were not included. In addition, in the questionnaire there are items formulated in an excessively general form as well as items that do not correspond to a genuine strategic activity. There are also items defined in terms of negative behaviors that express only what subjects do not do. The reliability of the scale is good, and scores between 0.68 and 0.86 (Cronbach's alpha).
3. The questionnaire CEAM II ("Cuestionario de Estrategias de Aprendizaje y Motivación II", Spanish acronym for Learning and Motivation Strategies Questionnaire II), translation and adaptation of the MSLQ (Motivational Strategies Learning Questionnaire) by Pintrich

et al. [57] to the Spanish university population [58], from our point of view also presents problems. The MSLQ is based on the model of self-regulated learning by McKeachie et al. [59]. This model integrates various factors that influence learning and emphasizes the cognitive and motivational factors and their relationships as well as the influence that they have on student involvement in learning and academic performance

The questionnaire consists of 81 items organized into two sections: a motivational section and a strategies section. The first is composed of six subscales (control beliefs, self-efficacy, intrinsic goals, extrinsic goals, task value, and anxiety on the tests) that are grouped into three dimensions (expectations components, value components, and affective components). The second consists of nine subscales (repetition, elaboration, organization, critical thinking, metacognition, time and place of study, regulation of effort, learning with others, and search for help) that are grouped into two dimensions (cognitive and metacognitive strategies and resource management strategies).

The questionnaire is set to include a specifically motivational dimension, with the same weight as the dimension of learning strategies (processing), but excludes search, collection, and selection of information strategies and does not give enough attention to metacognitive strategies. On the other hand, major strategies such as powers, interest, and physical and mental state are not evaluated in motivational subscales. Not included among the cognitive strategies are those related to memorization or transference.

Cronbach's alpha of internal consistency for the various subscales of the MSLQ ranges between 0.52 and 0.93 and Roces et al. [60] reported coefficients ranging from 0.57 to 0.84.

4. CEVEAPEU (Spanish acronym of Questionnaire for the Assessment of Learning Strategies of University Students, "Cuestionario para la Evaluación de Estrategias de Aprendizaje de los Estudiantes Universitarios") is a solid and well-structured questionnaire that could collect more complete information than the others already alluded to. Included in its design are two scales, one of affective support and control strategies and the other related to the processing strategies [61]

   In the first questionnaire the authors integrated subscales of motivational strategies that incorporated components not included in other questionnaires. They were affective strategies and control of the context, social interaction and resource management, as well as metacognitive strategies (explicitly assemble the components of planning, self-assessment and control/self-regulation, not shown with sufficient clarity on the other questionnaires). In the second included were a search subscale, collection, and selection of information, which are not included in any of the instruments analyzed. Also, the second subscale of processing and information use incorporated the most relevant processing steps (acquisition, elaboration/development, organization, and storage, not to mention personalization strategies and creativity, nor of the transfer and use of information).

5. At the Meeting of the AMEE (Association for Medical Education in Europe), held in Zaragoza [62] in 1995, the ESEAC presented a questionnaire developed by Bernad [63] and implemented at the University of Zaragoza by different partners [64] and subsequently applied to the students of Physiology of the Faculty of Medicine of the University [65]. Unlike the instruments described above, the ESEAC determines the behaviors rather than the "opinions" of the student. In this sense, this scale tries to obviate the aforementioned drawbacks of other scales. First, the items of the above instruments are made based on the opinions of the students -"what they say they do", "what do they think"-, and they forget about the aspects of the behavior shown in the "execution" of concrete tasks, which by their nature are directly observable and therefore quantifiable ones. The second drawback, not present in the ESEAC, is that the items of the mentioned questionnaires are formulated in a

general or decontextualized way ("Do you take notes in class?, Do you seek to have prestige among my colleagues, friends and family?, When you read, do you differentiate the important to the accessory aspects?, etc."). The inclusion of numerous items such as those in the above-mentioned questionnaires suggests that the behavior of the learner would have a single undifferentiated reference frame in which behavioral differences arising from the variety of factors within the specific context in that learning actually occurs (different disciplinary structures, different types of relationships within the group, distinct personality and teacher training, etc.) are not covered, that is, everything is now included under the label of "contextualized" learning

According to the authors the ESEAC questionnaire is defined by four characteristics [63]:

(a) It implies a holistic or global conception of learning (what is intended with this questionnaire is not to study the influence of each variable, one by one, in the learning process of students, but rather to note the set of a representative sample of the same with the different steps and nuances that can differentiate into this global process when learning is analyzed in its strategic version)
(b) It is an ecological approach to learning (ESEAC as an instrument aimed to facilitate and enrich the activity of teachers in their task of assessing students as part of their normal activity)
(c) It considers attention focused on student activity
(d) It warrants the generalizability of the processes analyzed

Accordingly, the ESEAC questionnaire is developed and is intended to be applied within the framework of the learning of the matter, and therefore poses school evaluation considering two main contextualizing aspects. The first is the specificity of the corresponding content to the different courses taken by students and, within them, the second, the corresponding academic level (primary, secondary, and tertiary). The creators of the ESEAC have taken the context as a fundamental component in the process of elaboration and development of it.

The ESEAC questionnaire breaks down the overall process of student learning into seven dimensions, corresponding to as many subfields which can be grouped into cognitive processes from the model of information processing where each dimension involves common and/or similar characteristics. These are understanding the task, planning and executing it (I dimension), translating it into meaningful mental content through the various languages available and complementary (II dimension), organizing the data processed by inference or reasoning of various kinds (III dimension), avoiding committing mistakes (IV dimension), placing at different levels of abstraction or distance relative to the data directly offered to the learner and processed by him (V dimension), acting with varying degrees of cognitive awareness (VI dimension), and finally, acting with more or less ability when regulating or controlling their own learning process (VII dimension).

These dimensions group the learner behavior into two blocks, one which refers to their behavior directly related to the content that learns or processes (processing strategies), and the other explains his or her personal conduct in the performance of the task, control or management of resources and personnel feelings, such as those derived of self-concept, anxiety, interest, motivation, etc. (support strategies). Each dimension, in turn, is broken down into strategies. There are a total of eight strategies and with their techniques, each of them represents the grouping of concurrent processes that are responsible for partial and objectified achievements within the overall process of the learning process. They include interpreting, planning and executing the task, adequately representing the information using thinking codes, organizing information logically, understanding the failures or mistakes, measuring the level of abstraction that moves in their interpretation of the information and the level of metacognition or self-regulation, and control of affective-emotional processes in their actions as a learner.

Finally, strategies are analyzed in smaller units: the variables (17 in total) are equivalent to more specific behaviors involved in each strategy, understanding that in the opinion of the experts on the subject of strategies, the distinction between learning "strategy" and "technique" is often difficult to establish and is irrelevant. Finally, each variable is estimated by the ESEAC in three performance levels: high, medium, and low.

Way to Act

As we have previously explained, we recommend working separately with metacognitive and cognitive strategies. First, it is important to know what they are and how they represent metacognitive strategies. Based on our experience with medical students overall, the scores in the scale of control of regulation are lower than in the scale of knowledge of the strategies directly applied to learning. This fact is probably a result of the fact that medical students reached high performances in their studies. Consequently, they trust in their own strategies. Subsequently, one tool of those contained in the section of cognitive strategies was applied. Although the ESEAC is the best tool for us because it explores behaviors against the others that consider opinions, we think that its application requires some training at the initial level. The ESEAC could be used when supported by other tools as described above. Until ESEAC handling is known, we recommended the use of the other tools described to obtain high performance and in this way, all students are provided with the best learning strategies in the way of self-training.

Appendix

	Questions	A	B
1	Where do you feel better when you learn?	Classroom	Laboratory
2	When you learn, what activity do you prefer?	Practising	Knowing
3	When you learn, how do you classify these activities?	Analysing	Applying
4	When I am learning I try to	Analyse	Try/Check
5	And also	Implement	Collect information
6	I think I am	Intuitive	Methodological
7	And also	Fast	Slow
8	And also	Planner	Analytical
9	And also	Organizer	Researcher
10	I proceed	Structurally	Looking for a global vision
11	I act	Conscientiously	Realistically
12	I prefer to	Have time	Improvise
13	I think I am	Spontaneous	Perfectionist
14	When I learn, I feel	Practical	Observer
15	I learn better	Solving problems	Schematizing
16	When I learn, I like to	Summarize	Contrast
17	And also to	Improvise	Analyse
18	I face the problems	Comprehensively	Specifically/Directly
19	When I have a problem I seek	An immediate result	To master the subject/To draw conclusions
20	Learning I try to answer to	What for?	Why?
21	And also to	What?	How?
22	Facing a problem I	Act	Think about it
23	I am	Cautious	Decisive
24	How do you define yourself?	Reasoner	Restorer

Items (questions) which correspond to each pole

Pole	Items (questions)
Theorist	1A, 2B, 4A, 6B, 10A, 13B, 15B, 16A, 17B, 19B, 21A, 24A
Dynamic	1B, 2A, 4B, 6A, 10B, 13A, 15A, 16B, 17A, 19A, 21B, 24B
Reflexive	3A, 5B, 7B, 8B, 9B, 11A, 12A, 14B, 18A, 20B, 22B, 23A
Operative	3B, 5A, 7A, 8A, 9A, 11B, 12B, 14A, 18B, 20A, 22A, 23B

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Attentional Capacity in Children: Intervention Programs for Its Development

18

Mirta Susana Ison

The goal of this chapter is to offer a brief conceptual overview on the complex attentional mechanism and to present one of the psychoeducational intervention programs developed by the team conducting research on children's developmental psychology at the INCIHUSA-CONICET and the School of Psychology at Aconcagua University. All the intervention programs implemented by this team were designed with the purpose of optimizing and strengthening attentional resources and cognitive control processes in school children of the province of Mendoza.

The Attentional Mechanism: A Brief Conceptual Overview

Although at present there is no unified theory of children's attention, several authors agree to define it as a control mechanism which is responsible for the hierarchical organization of the processes involved in the development of information [1–3].

Posner [4] identifies three functional components of attention, namely alerting, orienting and, executive attentions. They are supported by separate neuroanatomical networks: the posterior attention network, the anterior attention network and the vigilance network [5, 6].

The vigilance or alerting network is the most basic element of attention, and it implies the arousal level of an organism. It allows the activation level needed to carry out any action, for stimulus receptivity and for response preparation, that is, for the attentional tone. In addition, it is the necessary prerequisite for cognitive functioning. The anatomical structures involved are the locus coeruleus, the right area of the frontal and parietal lobe and the cortex, and the neurotransmitter involved is noradrenaline [2, 4, 7].

The posterior attention network is responsible for the orientation and localization of visuo-spatial stimuli. It is involved in the visuo-perceptual and visuo-spatial recognition of objects (what and where they are) and in the execution of visuo-motor tasks. The anatomical structures associated with this network are the superior parietal lobe, the temporal-parietal junction, the superior colliculus, and frontal eye fields. Acetylcholine is the main neurotransmitter [2, 4, 8, 9].

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Finally, the anterior attentional network is associated with executive attention, the attentional control responsible for resolving conflicts between thoughts and feelings before producing a response. Volitional control, referred to by Norman and Shallice [10] as the supervisory attentional system (SAS), intervenes in this network to appropriately deal with various situations. The anatomical structures operating in this network are the lateral ventral prefrontal cortex, the basal ganglia and the anterior cingulate cortex (connected with structures involved in emotional processing) and the neurotransmitter involved is dopamine [2, 4]. The SAS becomes activated to face new tasks for which no solution is known, and therefore, it requires planning, making decisions, and inhibiting automatic responses [11].

Posner [4] suggested that SAS is involved in error detection. A behavioral indicator of error detection and correction is the reaction time after committing an error.

It could be said that the function of executive attention is deciding to which stimuli to direct perceptual resources. It intervenes, also establishing the activation or inhibition order of the processes which develop and organize information in accordance with the situational requirement. This function is closely related to the motivational and autoregulatory mechanisms [1, 3, 11, 12].

The development of attention is considered to be essential for the functioning of other cognitive processes. It is also an indicator which enables prediction of cognitive levels in childhood. It represents the gateway to the possibility of triggering a series of cognitive functions. It may be mentioned in this way memorization and retrieval of contents, the ability to plan, organize and monitor an action to check its adjustment to the task by inhibiting inappropriate and dominant responses. It intervenes also in the cognitive flexibility required to correct mistakes or generate new behaviors in response to contextual demands, and the completion of the action once the objectives have been accomplished, and in the evaluation of results [2, 9, 11, 13–16]. These cognitive functions are also referred to as executive functions or cognitive control functions, and they allow individuals to self-direct their behavior to achieve specific goals. Therefore, attention plays a key role in children's school performance because it participates in the selection of relevant information. Its role is also relevant in the maintenance of that information, allowing for the manipulation of mental representations by modulating the responses to the various stimuli [17]. A higher attentional capacity has been found to be associated to a better execution of tasks which involve cognitive control, both in children and adults [18–20].

Children's attention development is a gradual, developmental process which has been proved to become more organized, flexible, and independent from context over time [2, 21, 22].

The main contexts for child development, namely family and school, need to promote the continuous development of cognitive, emotional, and social competencies. They may enable the child to gradually strengthen and use the following functions: (1) attention control; (2) maintenance and manipulation of information to behave in accordance with that information; (3) regulate one's own behavior to act in a reflexive manner; (4) establish courses of action involving a certain degree of planning, organization, and monitoring of such actions; (5) identify a problematic situation and find possible solutions anticipating likely outcomes of the action; and (f6 decision-making.

School children need to use a higher or lower level of attentional control, which constitutes a fundamental requirement for cognitive functioning. The level of attention control depends on the interrelation between neuropsychological patterns, socio-psychological factors, and external physical conditions. They interact together and may enhance or hinder cognitive functioning.

In summary, attention plays a key role in children's school performance because it participates in the selection, integration, and understanding of a broad amount of information [23]. It is understood as an active, constructive mechanism, whose capacity may be modified through continued practice. Indeed, each subject may generate his or her own attentional potential, which depends on the interaction of cognitive, conative, and affective factors [24].

This demonstrates the relevance of additional research showing the effectiveness of different intervention strategies aiming to boost the functioning of different socio-cognitive-affective processes that have not yet achieved, for various reasons, an adequate level of development for a certain maturation stage.

Can Attentional Capacity Be Enhanced?

Various studies have shown that different types of intervention were effective when it came to improving performance levels in tasks requiring cognitive control in preschool children [25, 26] and school children [13, 27–30] living in socially vulnerable conditions.

Research conducted in cognitive neuroscience reveals that differences existing in the neural structures and circuits are related to differences in the development of cognitive and socio-emotional skills, which would lend support to initiatives aiming to provide targeted educational interventions [31].

All the programs that have proved to be effective involve stimulating attentional control, creativity, cognitive flexibility, self control, and discipline by means of repeated practice activities that present gradually, increasing levels of challenge for their resolution. Therefore, it may be advisable to use both programs stimulating cognitive control functions and programs promoting social and emotional development [27]. In that way, children with a poorer cognitive control performance could draw greater benefit from these activities, and the gap existing between them and those children showing a better cognitive performance can be narrowed. This is the basis for psycho-educational intervention programs.

Our research team has conducted several studies on the efficacy of various intervention programs targeted to school children raised in socially vulnerable families in the province of Mendoza, Argentina, by evaluating their social skills and cognitive control processes [13, 29, 30, 32, 33].

The first study included the participation of school children 7–8 years of age who were affected by attentional impairment in a computer-based intervention program, and revealed significant improvement in their sustained attention as compared with the control group [34]. A later study showed that sustained attention, working memory, and alternative thinking attained improved retrieval at earlier ages when the computer-based intervention program was used in combination with a program aimed at strengthening children's cognitive skills to solve interpersonal problems [13, 30].

This section will describe one of the programs used to stimulate and optimize school children's attentional control capacity.

Computer-Based Program to Optimize Children's Attention

For research conducted between 2004 and 2010, a computer program was used to improve focused and sustained attention in children previously identified as having lower attentional efficacy [35].

Attentional efficacy is defined as the child's ability to accurately discriminate among stimuli that are identical to a cue, among a group of similar stimuli, during a certain period of time. When it comes to performing a task involving the visual search for a key stimulus, apart from the ability to select the stimulus correctly, it is necessary to sustain the focus on that stimulus for the appropriate execution of the task. Then it should follow that attentional selectivity and sustenance operate simultaneously [9]. Reduced attentional efficacy can thus be defined as a reduced ability to effectively focus and sustain attention during the period required by the assigned task, with respect to what is expected for the child's developmental age.

The results of a study conducted in 2010 are summarized below (see Ison [29] for further information).

A group of 138 school children (22.8 %) with low attentional efficacy (67 boys and 71 girls), between the ages of 7 and 12 years (9.25 ± 1.52) were identified. Two groups were then formed: (1) a study group composed of 72 school children (34 boys and 38 girls), and (2) a control group,

formed from 66 school children (33 boys and 33 girls). The children were randomly assigned to each group by means of the following procedure: once the children with low attentional efficiency were identified, they were assigned a number which was written on a piece of paper; the paper was folded in four and placed in a box. The pieces of paper were then drawn one by one to form the study group and the control group. The same procedure was used in each of the school grades.

According to the postintervention results obtained through the Analysis of Univariate Variance (ANOVA) procedure, in the younger group (7–9 years of age) the effect of the gender variable was not significant ($F(1, 75)=1.50, p<0.224$); however, significant differences in attentional efficacy were found between the study group and the control group. The attentional efficacy of the study group was significantly higher than that observed in the control group ($F(1, 75)=9.61, p<0.003$). Similarly, in the older group (10–12 years of age), the gender variable did not have a significant effect ($F(1, 63)=0.08, p<0.777$). The results of the test showed a significant improvement of attentional performance in the study group in comparison with the control group ($F(1, 63)=7.59, p<0.008$).

The intervention program used was the Computer-Based Attention Test for Children designed to stimulate focused and sustained attention in children [35]. This test was developed at INCIHUSA-CONICET and was used during the research studies carried out between 2002 and 2010 [13, 29].

More recently, between 2012 and 2013, an updated version of the 2003 test was developed.

Brief Description of the Program

The program consists of three tests aimed at stimulating focused and sustained attention through visual search tasks. In addition, each test includes training sessions geared for helping children to understand instructions correctly and to become familiar with the program. At this stage, the expert can guide the child and explain to him or her everything that is required to complete the second phase, which is the training itself. During the training sessions, the expert explains the instructions of the task to the child and shows him or her what the task consists of on the computer screen. Then, the child is given time to practice until the expert can verify that the instructions have been completely understood. Following that, the child completes the task on his or her own.

Each test presents three levels of difficulty (low, medium, and high) and different numbers of stimuli for the child to work with, it also offers the chance to save that information and the reaction and total times. In relation to the latter, the expert may allot a certain time to each task. All these variables can be selected by the expert, who sets the test configuration for each particular case.

Examples of Test 1

The cue is presented on the left and random identical, similar, or different stimuli appear on the right. If the stimulus appearing on the right coincides with the cue, the child will click "sí" (yes) and immediately hear a sound every time the choice is correct followed by the "muy bien" (very good) phrase said by a bird. Then a new figure will appear (See Fig. 18.1a).

If that new figure is different from the cue, the child will click "no", and the sequence described above will ensue.

If the child makes the wrong choice, a different sound will signal the mistake and the bird will say "presta más atención" (pay more attention) (See Fig. 18.1b).

Examples of Test 2

This task consists of searching and selecting the stimulus that is identical to the cue which, in this case, appears as "buscado" (wanted). It presents two categories of stimuli: (1) a farm with animals, and (2) a city with cars.

The task consists of looking for similarities and identifying differences within a broad and varied stimulus field. The screen shows a farm with different animals among which one is the key stimulus. The child needs to click on the animal that is identical to the "buscado" (wanted) cue. A green tick will appear on the animal when the choice is correct, followed by the corresponding sound, and the bird will say "muy bien" (very good) (See Fig. 18.2a).

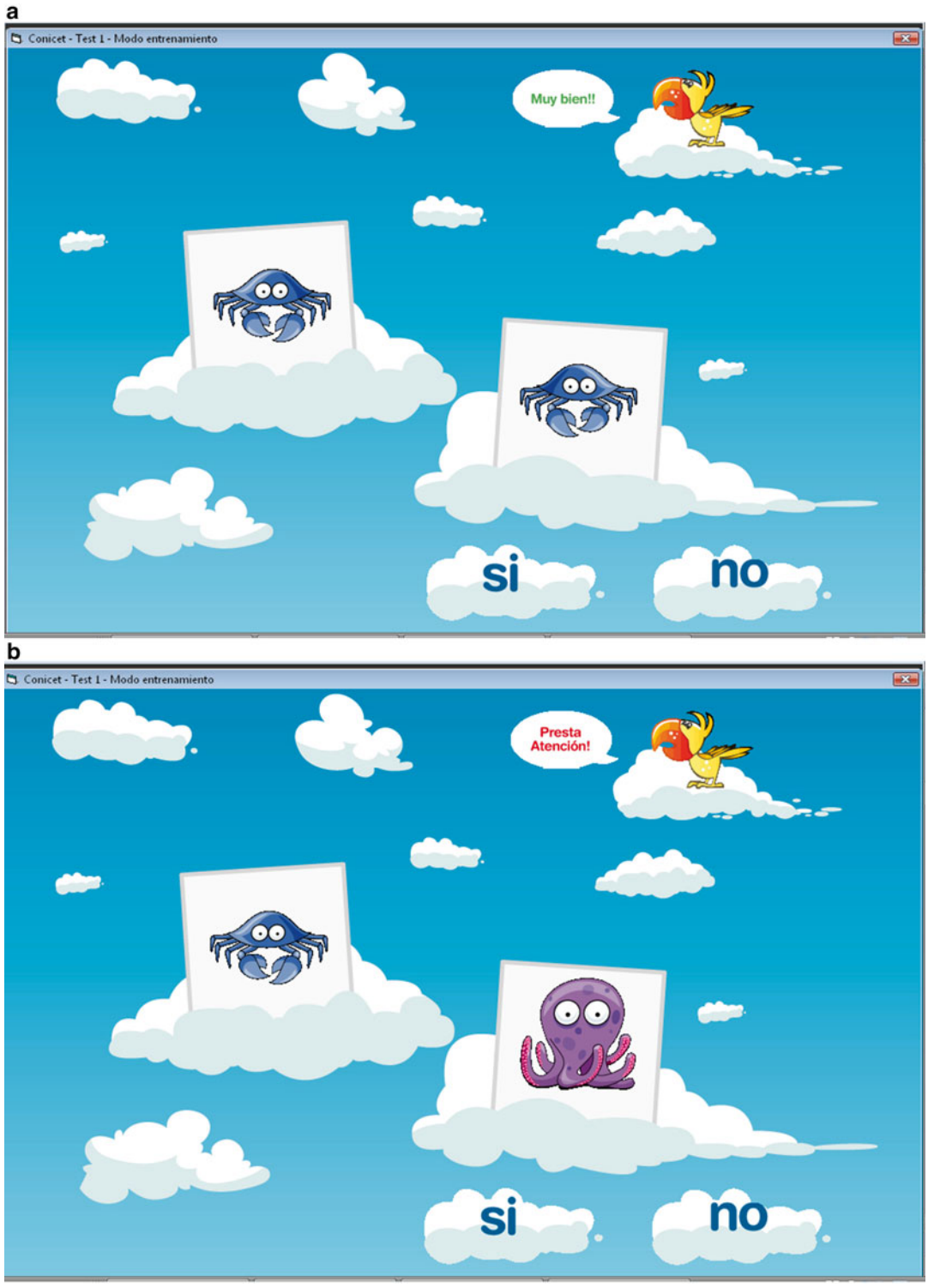

Fig. 18.1 (**a**) Attentional focus test when the correct stimulus has been chosen. (**b**) Attentional focus test when the incorrect stimulus has been chosen

Fig. 18.2 (**a**) Focused and sustained attention test, right choice. (**b**) Focused and sustained attention test, wrong choice

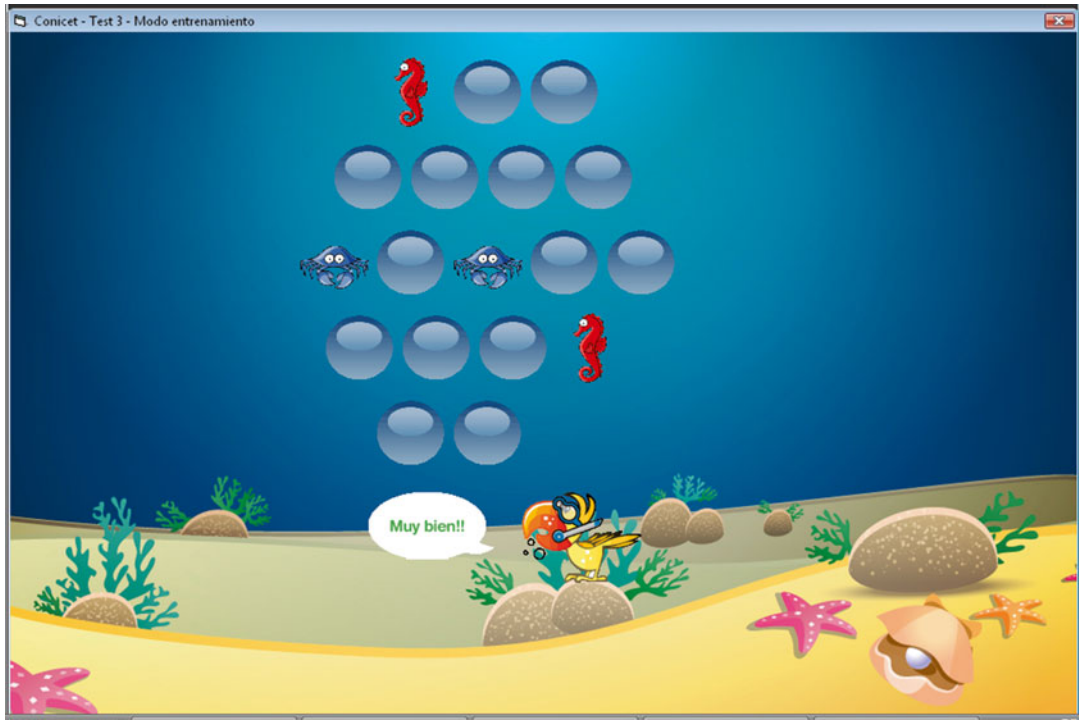

Fig. 18.3 Sustained attention and working memory test

If the child happens to select the wrong animal, a red cross will appear on the animal, followed by a sound that will indicate that the choice was incorrect, and the bird will say "presta atención" (pay attention) (See Fig. 18.2b).

Examples of Test 3

This test is based on the "memo test" game logic. A series of bubbles appear, and when children click on them, a stimulus pops up, sea animals in this particular case. And the task consists of finding the pair for each animal. If the correct pairs are found, the bird will say "muy bien" (very good), or else "presta atención" (pay attention). This is the most complex test because the child needs to remember where a certain stimulus is in order to find the corresponding pair, a task that requires not only concentration but also working memory. When both stimuli are matched, they will remain on display on the screen as the child looks for the other animal pairs (See Fig. 18.3).

For all tests, the program keeps record of the right and wrong choices and the omissions, as well as of the total time used to complete the task. It also keeps a record of the stimuli to which the child was exposed, the number of correct and wrong choices and omissions—stated in raw scores and percentages—and the total time needed for the completion of the task. Finally, a graph showing the performance of each child appears on the right. It shows the results of the tests (See Fig. 18.4).

Conclusions

Starting school poses new challenges for children, which involve developing a series of cognitive, emotional and social skills.

In order to adapt to the school context and achieve learning goals, school children need to deal with conflicts and organize their behavior

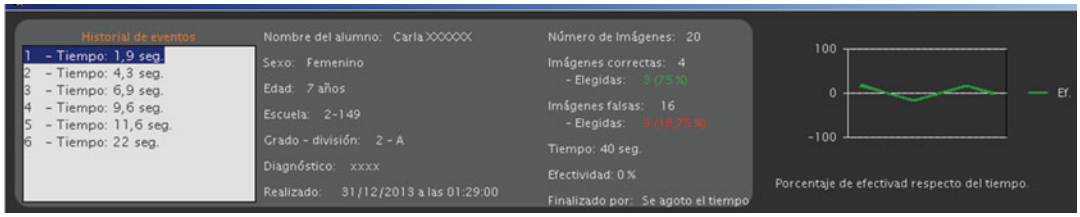

Fig. 18.4 Individual information record. The program records the child's personal data, in addition to information about the school, diagnosis, and date

according to working goals, plans, and rules. During the first years of school, children will only be able to achieve these goals under the teachers' guidance. However, they will gradually internalize cognitive strategies, routines, and habits that will enable them to gain autonomy when managing behavior and learning. In other words, they will need to begin to control their own behavior to better respond to contextual demands.

In this regard, attentional control has been found to be an important component of self-regulation [2].

The results obtained by our team show that intervention programs have greater effectiveness if applied to small groups of school children, and greater recovery of attention is achieved if stimulation starts at earlier ages. Additionally, greater effectiveness is attained if such programs are implemented systematically and sustained over time, with greater probabilities for them to help all children to develop cognitive and socioaffective skills that may enhance their educational opportunities.

If we consider executive attention to be a system involved in the voluntary control of actions, the benefits of attentional training could also extend to the cognitive and emotional regulation manifested in children's behavior [2, 27, 36].

Finally, the intervention program presented in this chapter finds its rationale in the interplay of the school child, the classroom context, and the child's family. Therefore, in addition to providing specific tools to effectively interact with children, the joint effort of parents and teachers promotes greater commitment to, and cooperation with, the teaching–learning process.

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19 Social Representation and Imagery of Labor: Evaluation Process of the Psychosocial and Labor Vulnerability and Its Relation with Mental Health

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Introduction

Vulnerability is a construct that our research team formulated from the research conducted since 1990 on the effects of unemployment and work on the mental health of workers.

We define psychosocial and labor vulnerability (PSLV) as a construct determining an interdisciplinary research field which includes the study and addressing the problems and consequences that have work processes, both from "work" and "no work" perspectives about life in general and mental health in particular. The importance of establishing this concept as a specific chapter of the psychosocial vulnerability relies in the necessity of another look at the design and implementation of comprehensive plans for addressing social and health problems affecting the working population.

Literature about psychosocial vulnerability has, in our understanding, a biased perspective about the problems that unemployment and working conditions can cause to the mental health of individuals.

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From this point of view, we have been able to observe that a large number of the studies that have been done, both in our country as well as all over Latin America, mainly focus their results on the effects that socioeconomic conditions have on the mental health of workers.

We also consider it necessary to make a qualitative leap in relation to the field's current policies, which are limited to identify occupational diseases, accident mechanisms of production and financial compensation for active workers or, in the case of unemployed people, monetary assistance in the form of subsidies.

In reviewing plans and programs to assist the unemployed, we have observed that there is a deficit of state policies regarding care of the sectors at risk of PSLV. On one hand, this deficit is the result of focusing most of the studies on almost exclusively some aspects of worker's problematic, and taking as the target population only to the lower income population sectors, being only a few studies on workers with a higher socioeconomic status and higher qualifications; and on the other hand, the lack of broader, longer term plans proposed according to the territorial conditions where those plans will be developed. Also, those in the social management processes require more data to efficiently implement the scarce resources devoted to social action, making it necessary to develop effective measurement tools to determine the areas that may be included within this state and to define its

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scope and consequences on health and psychological distress of citizens.

It is necessary to design boarding actions directed at reducing the serious consequences that PSLV can have on workers.

Over the several years over which we developed a research project endorsed by the National University of Rosario, we made an approach to the isolation of the most significant aspects of the PSLV construct we are proposing. This was made in two main population sectors in regard to PSLV; unemployed workers and those who are exposed to harmful aspects of the work process organization.

In the case of employed workers we can mention as PSLV indicators, the deteriorating social relations that the worker sets with their peers in work organizations, the harmful effect of what Neffa [1], among others, has appointed as Conditions and Working Environment (CYMAT), exposure to new working conditions such as stress, burn out, mobbing, the time urgency syndrome (described as "hurry sickness" by Ulmer and Schwatzburd [2]), the effects of job insecurity (especially in what Piore [3] called internal labor markets), the influence of family and social conflicts on the labor activity, etc.

For unemployed workers, we believe that the following indicators should be taken into account: drastic changes in daily lives, work skill desynchronization in relation to labor market requirements, decline of family ties, isolation from the immediate environment, health factors related to age, certain aspects of job demands appointed by cultures and business trends, disabling effects of social policies, rupture of the imaginary progress that possession of paid jobs had on other socio historical periods, lack of prevention programs and primary care appropriate to their health problems, lack of training programs, lack of social programs involving the development of self-managing business projects.

Notes About the Concept of Work

The main discussion around the concept of work lies both on what we understand as work and its importance in the social history of humanity and the current capitalist society.

Therefore, the discussion on what is meant by work is a major debate in contemporary society, as it relates to mental health and mental suffering of workers; and it is in this crossing of both concepts (work and mental health) where we expect to find some leads to advance in reformulating the attention of PSLV workers.

From a traditional perspective, "work" is every human's productive activity aimed at changing the nature and the process to transform itself. However, it would be a mistake to think that people work just for the sake of having an occupation, or for any ethical or moral mandate. In simple terms, we could say that humans do not work for fun but out of necessity.

People in today's society must work to buy the items necessary for survival of themselves and their families. These needs are not the same for all workers in a given society, the higher the renumeration to a worker by his/her activity, the greater the needs that maybe covered. Therefore, in the process of work, social groups construct representations that are determined by the strong significance job has as a tool for workforce reproduction and in some cases, as a mean of social advancement. For much of the second half of the twentieth century, this thought process was reinforced by high levels of social inclusion existing in the capitalist world. It seemed that having a job guaranteed accreditation of social citizenship.

With the depletion of the accumulation model based on the welfare state (around the 1970s) and the advent of the hegemony of neoliberalism which involved the conservative restoration of the 1980s, the possibilities of the work world changed. The processes of deindustrialization, outsourcing, and re-industrialization that developed new computing and robotic technologies involved a new model of organization of production and increasing labor market exclusion of large masses of workers [4].

In developed countries, this process had certain rationality and methods of different types were tested to mitigate the impact of this process (reduction of working hours, creation social safety nets, increase of contributions to the development of small and micro enterprises, etc.). In contrast, in countries belonging to what is generally called The Third World in which a simple

pneumatic or hydraulic change in the machines meant the loss of lots of jobs, a wild deregulation of the labor market was chosen with the consequent rise of unemployment and poverty.

Employment is currently a right increasingly scarce in the world, the organization of production is oriented toward forms of self-employment and development of enterprises with social labor-assisted insertion. The prototypical cases are in northern Italy, the English industrial districts, Spanish business incubators, German micro business tutorials, etc.

In some societies, and responding to the increasing demands of the sectors affected by current policies, those with management responsibilities chose the simple but useless way of subsidizing those who were most affected by the so-called "temporary employment plans" [4, 5] developed an extensive criticism of these type of coping strategies of labor problems, in which they emphasized that the plans became hidden subsidies in addition to not solving the employment problems of those who had them, further segmented the labor market producing a paralyzing effect on the social players.

Now, if we reformulate the concept of work that was discussed above and in addition, define it as "any human's productive activity aimed at changing the nature and in the process to transform oneself" and we add to the definition "in order to satisfy their basic needs and continuously improve their quality of life" we believe the concept is more accurate. In this train of thought, temporary employment would no longer belong in the category of work becoming a single subsidy, and neither are those tasks that involve only survival strategies (wash windshields, open car doors, etc.) but do not cover the needs of the subject, would fall into the category of precarious subsidies: every informal or low-paying job that does not allow the worker to meet their basic needs with the salary received.

This new concept of work involves redefining labor (and obviously economic) policies and beginning to develop serious strategies for human resource training, ongoing surveys by region of training, work and employment; development of interactive and computerized job searching systems, programs to support micro and small businesses, etc.

However, reformulating the concept of work would be an incomplete task unless reference is made to the necessity of promoting physical and mental health in work processes. Assisting those with work and those who lack it temporarily, requires taking into account the health variable. We want to highlight the necessity for a comprehensive concept of work that contains within itself the reference to health care.

It means recognizing that the effects of unemployment not only involve the lack of work, the problem leads to a variation of social relations and affects the individual and collective health of the inhabitants of the region affected by it.

Therefore, in a new setting for the concept of work we could say it is every human being's productive activity aimed at changing the nature and in the process to transform itself in order to meet their basic needs and continually improve its quality of life, attending fundamentally to the consequences and requirements that it produces in health and particularly mental health.

A second topic of discussion is found in the debate about what work means for social development.

First of all, we should mention that centrality of work in the social system is a concept that emerges in modernity, serving the needs of capitalism which requires a free work force to expand itself.

In addition to a free work force, capitalism needed to develop a strong work discipline that would allow structuring this work force in the manufacturing organization and to be able to control and supervise it in order to ensure the increasingly complex industrial manufacturing activities.

All the new theories about production organization such as Taylorism and Fordism were not enough to discipline the work force, it was necessary to achieve the development of imaginary social meanings to facilitate the acceptance of disciplining the working class, which goes beyond the discipline in the manufacturing context, involving the social control of subordinate classes (i.e., that these classes accept the order of

things as natural and progressive), developing a positive feeling for the social organization through the hope of social mobility that bourgeois ideology was building since its structure as a social class and particularly since it took control of the political superstructure after the French Revolution.

The worker should feel that they do more than make a living while working, they must feel like they are a part of a process of a social system and should consider that their work force is a fundamental condition for social development.

It is there that the imaginary social signification that allows considering the work as a central element of society appears more than ever.

History shows that work was not always central to human societies. In an interesting analysis, Meda [6] refers to the existence of a type of pre-economic societies were the closest word to refer to work was the indigenous lexeme "tarak", which denotes the arduous physical activity required to perform a coherent set of technical operations to produce all the necessary means of subsistence the necessary means of subsistence. In these societies, we find the obligation to cooperate as a community, not the distribution of economic goods. In them, we can find the law of least effort and the set of daily activities can be reduced to three: (1) playful competition, (2) aesthetic care and willingness, and (3) activities that respond to the sacred and social logic.

According to Mauss and Malinowski [7], ties that maintain social cohesion in these communities are not economic but social in nature, involving kinship links, symbols, a relationship with nature, and traditions.

With the pass from maternal to paternal right and the triumph of patriarchy, also begins the separation of domestic work from production and increases the importance of the second to the detriment of the first one former.

Primitive accumulation will be (in addition to other factors such as slavery, differentiation of social functions–religious, military, farmers) developing social classes. Capitalism is the most developed form of a class society, but also the most unequal of all, according to Kligsberg [8] using data from OXFAM, 85 people have as much wealth as 3,570 billion poor in the world and the richest 1 % of the world population owns 46 % of global assets.

Therefore, with the only requirement being that you need to work, or the obligation to sell the work force to survive is not enough to sustain a system of so much inequality, this is why capitalism was building a cultural apparatus within which the work is a source of personal fulfillment, opportunity for social mobility, organizer of social life, etc., ultimately central to the cohesion of society and a guarantee to reduce poverty.

These imaginary social settings would collapse if all workers would meditate on the hard facts of the international economy; economy in which one of the proponents of the work centrality theory, the International Labor Organization (OIT), had to coin a new term to define "good job", the notion of "decent work".

In the words of Ghai [9], ex-director of the Research Institute of the United Nations Development, the term "employment" refers to "… Four elements of this concept: social protection, workers' rights and social dialogue. 'Employment' covers all types of work and has quantitative and qualitative aspects. Thus, the idea of 'decent work' is valid for both workers in the formal sector employees and workers in the informal economy (independent), self-employed and working at home. The idea includes the existence of enough jobs (work opportunities), compensation (in cash and in kind), and safety at work, and healthy working conditions. Social security and income security are also essential elements, although dependent on the capacity and level of development of each society. The other two components aim to strengthen the social relations of workers: the fundamental labor rights (freedom of association and eradication of employment discrimination, forced labor and child labor) and social dialogue in which workers exercise the right to express their views, defend their interests, and negotiate with employers and with the authorities on matters related to work activity.

Notes on Mental Health

To carry out a health analysis in which a theoretical position is taken, the starting point value.

We will start working the conceptualization of the health-disease process from the paradigm from which the process is socially determined [10]. In this regard, we recognize that our development is biased by the above decision made consciously because we ignore the biologist "derived from a single cause" analysis, considering current ecological "derived from multiple causes" adopt a position of greater breadth and understanding of the process; we believe that they do not express the social conditioning to the full extent because they do not assign differential value to the various factors involved in the health-disease process.

Considering that the health/illness processes as socially conditioned involves criticizing the concept of health as merely the absence of disease. Moreover, from this perspective, we disagree with the World Health Organization's health concept that originally defined "health" this way, thus adding the idea that it involved a positive of well being.

Continuing to achieve a health concept that is entirely satisfactory, we extrapolated ideas from Noriega [11], who believes that health is a relative term, it will vary according to the different actors' social interests in which social centrality is occupied by subjects, and health involves the ability and capacity for people to control and direct their life processes (e.g., work, consumption, recreation, cultural processes, etc.), such as our way of living and reproducing in society.

Following the dynamism ideas [12], we can consider the health-disease process as relentless, socio-historical, and continuous.

Assuming these features are the most relevant in the health-disease process (i.e., as a process of relative character, dynamic, multicausal) which occurs within a socio-historical continuum, socially conditioned and that to its continuation the self-managed community participation is essential, we now begin to analyze the health specificity of mental health.

We are interested in conceptualizing mental health because work has a strong effect on it. A concept that can serve as a consideration starting point is found in the Argentinian National Mental Health Plan [13] where it was developed from the National Mental Health Direction, a mental health concept that is transcribed below:

> State of relative balance and integration of conflicting elements constituting the subject of culture and groups–progredients balance and integration, with predictable and unpredictable crises, with possible objective and subjective registers–in which individuals or groups participate actively in their own and their environmental changes [13].

Through this definition the notion of mental health linked to the balance issue can be emphasized. This is not a notion of equilibrium in an absolute sense but it is considered as relative, in the same terms as Piaget [14] considered development of personality to be a search and permanent disruption of the balance in search of other balance that overcomes the previous one.

In addition to the conflicts, the individual is subjected to the action of social ambivalence. The society sends mixed messages that affect their balance. What one hand gives you (through advertising), the other pulls back (for lack conditions to submit it).

It has been observed that the balance referred to is progressive, because when you break a round, it resets above surpassing the previous stage. The subject would be a whole that "rewholes" at every moment, allowing us to reflect on a subject that is not a sum of parts, but an ongoing process of integration.

When referring to the integration process, a reference to culture is necessary. We talk about a subject who is culturally given, integrated as a person, but also in the process of integration with the social and cultural whole of which is actively involved through groups, organizations, institutions and other cultural forms (accept it or reject it by the subject).

This definition considers people in their life cycle who are exposed to vulnerable situations or crises, in which health and illness are brought into play.

According to Jaime Breilh [15], social conditioning of every space and time, i.e., processes in which society and group modes of life unfolds, acquire protective or beneficial (healthy) and

deleterious or destructive properties (unhealthy) that are triggered simultaneously. When a process becomes beneficial, it becomes flattering of defenses, and supports and encourages a favorable directionality to the human individual and collective life, whereas when the process becomes destructive, it causes deprivation or deterioration thereof.

These concepts must be understood in the context of social development because they are the product of a particular social economic situation in which they are embedded. In the case of the third millennium capitalist society we note, as stated by Asa Cristina Laurell [16], the most important features of economic adjustment programs inspired by neoliberal theories are constituted, among others aspects, by strict and drastic reduction of public expenditure, increase in goods and services, a new definition of the exchange rate, opening (trade and investment) regional to global markets, privatization of public enterprises economies, deregulation financial activity and the casualization of the labor market.

Neoliberals reject the welfare state because it espouses values such as individualism, competition, and the market as a starting point of any economic program. Strategies to reduce the action of the state are cutting social spending, with the consequent elimination of programs and the reduction of benefits for the weaker sections of society, spending targeting, privatization of the production of services, and decentralization of public services at the local level. However, we caution that neoliberal approaches have prompted debates about the real problems of many public institutions such as centralism, bureaucracy, authoritarianism, and other undemocratic practices.

Beyond any debate, the important thing to note is what Bonantini, Simonetti, and Quiroga stated [4, 17], that these policies only made economic actions (providing subsidies to unemployment as work plans, occupational, etc.), but in no way attended alterations of mental health and psychological suffering resulting of unemployment and working conditions developed as a result of labor flexibility programs, that subjected workers to disastrous economic conditions, but mainly conditions of uncertainty regarding their future careers.

The Importance of Measuring Psychosocial Labor Vulnerability and Its Relationship to Mental Health

As stated earlier in this chapter, by understanding PSLV as social vulnerability applied to the field of work, we intend to advance the understanding of the relationship between vulnerability in the working process and the effects that it has on mental health and psychological suffering.

The point of our work is to recognize that vulnerability is not just about economic conditions and the worker's quality of life, but what we intend is to develop knowledge that can be applied to primary care processes and prevention in the mental health of working populations, so as to improve the conditions of life and work of society.

It is about achieving a qualitative leap in the policies that currently exist in the field, which are only limited to determining occupational diseases, mechanisms of production, and economic accident repair for active workers, or in the case of unemployed workers, to monetarily assist through different types of subsidies. In our studies, we have observed that there is a deficit of state policies regarding care of the sectors that are at risk of PSLV, making it necessary to develop effective instruments of measurement to identify the sectors that may be vulnerable and define the scope and consequences thereof.

In doing so, it is necessary to recognize that tools that people have for dealing with situations of vulnerability risk are not only the variable income level or the conditions of their habitat.

Modern pathologies that have arisen as a consequence of life vicissitudes in modern technological societies (stress, burn out, workaholism, mobbing, burnout syndrome related to empathy, etc.) show that changes in mental health and mental suffering are not a problem that exclusively concerns the lower sectors of the social pyramid, but different levels and workers are affected by these socio-professional pathologies. Workaholism is a condition that affects mostly freelancers, entrepreneurs, and senior business leaders. Burn out, as evidenced by the extensive literature on the subject, has a strong impact on

teachers and health workers. Burnout syndrome related to empathy also affects other sectors. Professionals working in palliative care units are exposed to potential sources of stress because they need to address the suffering of the patient and family, and prepare for the death of the patient. Mobbing particularly affects employees of bureaucratic sector companies. As we see, the PSLV is a social situation for workers to which different social sectors and different types of workers are exposed.

It is for this reason that we consider it necessary to create boarding actions designed to reduce the serious consequences that psychosocial labor vulnerability can have on production workers. In our work, we have made an approach to the isolation of the most salient aspects of this construct we are proposing.

We can mention some indicators of PSLV in the case of employed workers. One indicator is the deterioration of social relations that the worker sets with their peers in work organizations, the harmful effect of what Julio Neffa [1], among others, has appointed as "Working Conditions and Environment" (CyMAT, Spanish acronym). Another important factor is the exposure to new working pathologies such as stress, burn out, mobbing, or the time urgency syndrome (described as "hurry sickness" by Ulmer and Schwatzburd, [2]). The effects of job precariousness, especially the "internal labor markets", as designed by Piore and Doeringer [18] can also be considered as important factors . The influence of family and social conflicts on the labor activity of the production workers may also be considered here. The capacity to establish territorial and trade networks is also relevant, as well as other additional factors.

For unemployed workers the following indicators should be taken into account: drastic changes in their daily lives and their work skills and desynchronization in relation jobs market requirements. Additionally, the declination of family ties and the isolation with respect to the immediate environment should also be mentioned. The health factors related to age (physical damage such as crushing of the fourth lumbar vertebra), aspects of job demands by certain cultures and business trends (leading to dismiss workers for issues of gender, family responsibilities, age, etc.) cannot be forgotten. Several factors related to policies must also be considered. It is the case of the disabling effects of social policies (welfare dependency, political patronage, subjection to political leaders, etc.), and the rupture of progress that carried the possession of paid jobs in other socio-historical periods. Adequate programs are also needed for personal and social development. The lack of prevention programs and primary care appropriate to their health problems, lack of training programs, and the lack of social programs involving the development of self-managing business projects must be avoided.

We developed an instrument for use in screening tasks in large populations based on the conceptualization of PSLV and considering the exposed indicators.

This measuring instrument was built to be applied to a non-random convenience sample, in which the size was determined based on the characteristics and heterogeneity of the study population.

Three calibration groups were formed. Employed, unemployed, and precarious workers were matched by demographic characteristics. These calibration groups were formed in order to establish the validity of the instrument's construct.

Once we collected the data and they were loaded into a database, we proceeded to perform an exploratory analysis using the Principal Components Analysis in order to find the underlying structure of the items that relate to the Psychosocial and Labour Vulnerability (PSLV). Those components with more weight in the extraction were selected. A Varimax rotation factor analysis was used to better reveal the structure of components. A logical regression model was used to study the association between dimensions or specific factors of the above procedures and groups calibration previously established. The internal consistency of the instrument and the rating scale were assessed by calculating the Cronbach's alpha coefficient yielding a 97 % confidence. Finally, a receiver

operating characteristic curve was constructed with the goal of obtaining sensitivity, specificity, and predictive values. Stability or repeatability of responses was controlled. The criterion of significance was $p < 0.05$ for all analyses.

We began the last step of the application once the instrument was validated. It consisted of applying the instrument to populations that worked in direct contact with the user of a public services company from the Santa Fe province. This target population was chosen in order to take into account the experience with the bank employees in 2001 during a great financial crisis. We believe this is a sector with highly exposed workers and therefore PSLV states. As a result of this research, the team hopes to register this instrument.

Mental Health and Work Process

We will now address the effects of work, and not work on mental health matter, from our own data collected in various investigations conducted between 1999 and 2012, the results of which have been presented in different publications.

In this work we performed a journey through different theoretical points of view involved in the existing process of the relationship between health and work. We have reviewed the concept of work and the literature regarding it, establishing a position in the centrality of work controversy, noticing that this concept is a starting point for the capitalist machinery which needs to develop models of subjectivity to achieve consensus about general bases of the support system.

Work is a source of mental health disturbance and a suffering factor for workers. These work characteristics and their consequences are pointed out, under the capitalist mode of accumulation, on the lives and health of workers.

There is a wide range of studies on the problems that the work process produces on health in general and mental health in particular.

The capitalist organization of production and the need to continuously increase corporate profits are systems that have been set up based on rotating shift work where the worker has high psychological distress as a result of living with different schedules, both of their family and society in general. We can also refer to the effects of different pollutants experienced by employees in the work process, or the effects on their psychological state, because of the extreme demands of the organization of production ranging from conditions in rarefied environments (e.g., mining) to the extraction of dust, chemical, physical contaminants (other engineering industries, iron and steel, petrochemical, etc.). This has led to different Ministries of Health and Labor making lists of occupational diseases involving smaller work schedules, risk payment, heat exposure payments, and differential rates in retirement ages, etc.

From labor psychology, inputs are not less, especially because through all the work previously done, it is possible to detect disorders and psychological suffering that are not considered occupational diseases, but which deeply affect the mental health of workers. In a series of works, the effects of stress on workers were analyzed. Mandolesi [19], Quiroga [20], Suaya [21], and Filippi [22], among others, have conducted research in Argentina on different topics related to the effects that the manufacturing process has on workers mental health. In these studies, carried out with different participants in the production (teachers, nurses, administrative, banking), it has been revealed that multiple disorders are suffered by workers as a result of burnout, mobbing, bullying, workaholism, etc.

In our own research, we have been able to establish that workers with direct access to the public have higher psychological distress as a result of being the most visible face of organizations concerned with the performance for the service they provide.

There are paradigmatic examples, as was the effect on the mental health of the banking crisis of 2001 in Argentina, or the traumatic effects suffered by the workers of the maintenance of power lines energy sector during the energy crises of 2011 and 2012 when the electricity cuts became widespread throughout Argentina.

A separate chapter is needed for working accidents. Bonantini [23] communicates some of the information obtained in an accident investigation in Villa Constitución city (Santa Fe province).

In that study our goal was to go beyond the simple analysis of the frequency and incidence of accidents. We would like to analyze these data as Turner [24] does in anthropology, who thinks the accident is "social drama", i.e. as a field full of "dramatic significance". It has the sense of the emergence of tensions and conflicts in social participants' daily life, which are constrained in short time intervals to various and contradictory "messages". In the accident at work besides those who were directly involved (the worker or workers suffering from the accident), a wide range of participants are also affected. These participants are in contact with people injured or they are injured themselves, and this affects their daily routine, adding to the catastrophic effects that the accident has on their mental health (other collaborators of the organization, family, community members, etc.).

Therefore the statistic that we will show must be analyzed in this dramatic scene, involving working accidents.

In Table 19.1, we can see the evolution of accidents in recent years in Argentina.

Given the severity of the effects of industrial accidents on worker mental health and suffering, we have to consider this not as a cold chart with numbers, but as a starting point for meditation. We can observe two key issues. The first is the magnitude of the social and health impact of accidents. The second, and this is a critical point, is the importance of state policies designed to prevent accidents and to make primary care compulsory in mental and physical health once an accident happens and the drama starts.

Observing the table above, it is possible to visualize some of the consequences of the impact of labor policies from different economic perspectives applied in Argentina. They are the neoliberal paradigm and the Keynesian paradigm, both economic prospects applied since the return of democratic governance. The first one lasted from 1988 (year of the fall of the "Plan Primavera" [Spring Plain]) until 2001, when the "exchange rate crisis" took place (also known as the "1 to 1" [1 dollar = 1 peso]). The second paradigm is applied to exit the "convertibility" and extends up to the present.

Table 19.1 Work accidents in Argentina

Work accidents in Argentina					
Year	Incidence rate (in thousands)	Rate losses (in thousands)	Lost labor duration (in days)	Rate of deaths (per million)	Days not worked
1996	84.3	1,701.9	20	233.2	6,173,376
1997	77.4	1,063.2	13.7	220.9	4,639,087
1998	72.9	1,067.6	21.5	223.4	7,740,303
1999	76.7	1,556.3	20.3	204.7	7,763,370
2000	77.5	1,579.1	20.4	185.9	7,771,910
2001	69.0	1,414.2	20.5	159	6,956,680
2002	62.4	1,427.1	22.9	152.1	6,381,975
2003	72.7	1,642.8	22.6	152.2	7,748,171
2004	80.2	1,913.2	23.9	150.1	10,245,610
2005	81.4	2,003.6	24.6	142.8	12,022,892
2006	80.7	2,212.2	27.4	149.1	14,765,377
2007	82.5	2,458.2	29.8	149.8	17,818,104
2008	80.6	2,455.8	30.5	123	19,012,471
2009	78.9	2,212.6	30.6	130.6	17,366,014
2010	71.3	2,206.8	31.2	138.1	17,581,681
2011	73.3	2,453.9	33.6	140.9	20,395,891
2012	69.4	2,470	35.7	147.6	21,390,013

Source Superintendence of Workplace Hazards. Ministry of Labor, Employment and Social Security

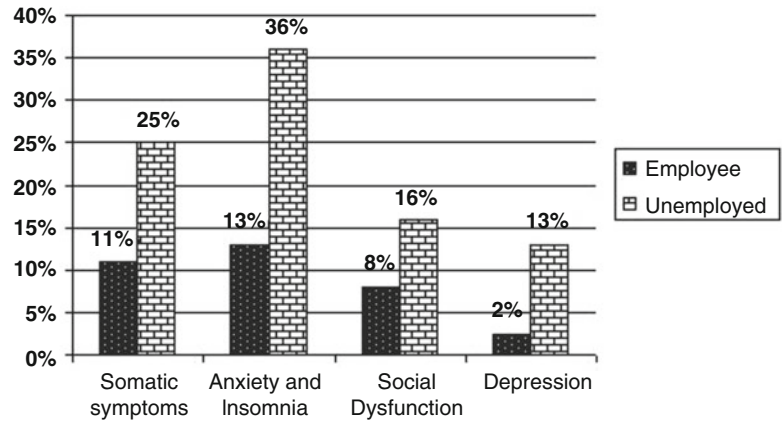

Fig. 19.1 Perception of psychic discomfort by occupational status and subscales of the GHQ-28. Rosario, Argentina (*Source* Bonantini C. Simonetti G. et al. (2003). "Vulnerability and mental health. An analysis of unemployment and its effects on mental health." Cuadernos Sociales 5. June 2003. UNR Editora. Rosario [17])

While in the 1990s the neoliberal paradigm gave priority to cash replenishment of any affectation suffered by the worker, through the privatization of occupational risks, creating ARTs (Spanish acronym for insurance of occupational risks, "Aseguradora de Riesgos de Trabajo"), in the first decade of the twentieth century, we find a decrease in the incidence rate of accidents and the number of deaths by millions increased economic losses, days off work, and days not worked. We consider this as a result of prevention policies supported by the Ministry of Labor and Social Security, which amended the legislation and increased controls and implementation of workplace safety standards. The increase around lost sick leave and working hours could also be due to the changes in the organization of the working world, which gave greater importance to health care and worker safety.

Unemployment and Psychological Distress

Much more striking are the consequences of the lack of work on the health of the unemployed. In the follow charts we show some data obtained by Bonantini et al. [4, 18] regarding the changes in worker mental health.

When we relate the perception of psychological distress among the employed and unemployed, we get Fig. 19.1.

As can be clearly see from the figure, the unemployed workers are in a state of much greater vulnerability than the occupied population. It indicates the effects of the employment situation. It may be observed that the unemployment condition is much more negative.

To specify the conditions that we could gather among unemployed workers, we took a chart showing the most common mental disorders found in the unemployed workers from the cited publication.

We note that the unemployed suffer a marked increase in somatic symptoms, anxiety, and insomnia while simultaneously showing higher rates of social dysfunction and depression.

This could be due to social and personal constraints. The unemployed worker may feel vulnerable for several reasons. In his or her imagination, they could feel the risk of being stigmatized for not having a productive work activity. Anxiety and insomnia are indicative of a state of mental alertness, which when converted to somatic level, would result in an increase in symptoms of this nature.

Depression can be considered a result of a subjectively perceived stigmatization by the unemployed worker who loses the ability to occupy the family group support role, which in some social environments, is perceived generally as a personal failure.

The data shown in Table 19.2 were collected using the CADEPA Questionnaire, in its reduced and approved version of the GHQ (Goldberg Health Questionnaire) in a sample of unemployed workers belonging to the Gran Rosario Metropolitan System (SMGR). This version of

Table 19.2 Mental health of the unemployed (Rosario, Argentina)

	No (%)	Doubtful (%)	Slight (%)	Moderated (%)	Intense (%)
Obsessive compulsive disorder	57.52	38.57	2.61	1.30	
Simple phobia	66.00	24.19	7.85	1.96	
Panic attack	52.29	24.84	11.11	11.76	
Depression	47.06	15.69	17.64	13.08	6.53
Generalized anxiety	40.53	39.22	3.07	4.57	2.61
Major depression	66.7	17.63	10.45	3.92	1.30
Average values	55.00	26.67	8.78	6.08	3.40

Source Bonantini C. Simonetti G. et al. (2003). "Vulnerability and mental health. An analysis of unemployment and its effects on mental health." Cuadernos Sociales 5. June 2003. UNR Editora. Rosario [17].

the questionnaire was developed by the Department of Clinical Psychiatry of the Clinical Hospital "José de San Martín" (which is part of the University of Buenos Aires) and was based on the descriptive criteria from the DSMIV.

The current results were obtained from the analysis of the information gathered from a database of 54,000 unemployed workers. A standardized questionnaire was applied. In almost 20 % of respondents it was possible to detect mental health disorders. A population with almost one million people at that time had about 25 % unemployed workers and almost 40 % of subjects with employment problems. We evaluate the possibility that about 120,000 people could be suffering health disorders.

The progression from panic attacks as acute claudication, to depression and major depression as chronic claudication, could be related to the effects of a persisting state of lack of employment, and to a social situation of high rates of unemployment. It also may be related to persistent somatic affectations, as damage of the fourth lumbar mainly found in people over 45 years is what invalidates them (in occupational pre-practice diagnoses) to access a new source of work.

A Provisional Synthesis

In this chapter we have tried to weave the concepts of work, vulnerability, and mental health.

We believe that the capitalist enterprise should be interested in mitigating the adverse effects that work organization has on the physical and mental health of workers.

Additionally, we want to point out the necessity of developing prevention programs, particularly for unemployed people, who also perceive the risks to their health.

As observed in the various tables presented and retrieving the information from the texts cited in this chapter, not only is the mental health of unemployed affected, but also that of the employed workers. It is a valid reason to consider it important to develop research leading to proposals to achieve significant reductions in the damage that labor in all its forms produces over workers.

The concept of PSLV has allowed us to understand that workers have different resources and tools to deal with the risk that working means.

Therefore, it is necessary to have instruments to generate information for using on physical and mental health policies, to enable preventing the risk, but also, if it has already occurred, to have strategies for primary health care in order to give early assistance to affected ones.

We consider it essential to have instruments to perform a screening on different populations that might be in a PSLV position so as to determine the risk's characteristics and the steps to follow to prevent it.

In our research, we have developed an instrument which, as indicated above, on the basis of a number of defined variables belonging to the PSLV concept, allows the screening and would provide valuable information to develop the state policies to which we are referring.

It is necessary that society, besides acting on the negative consequences that has the work and the lack of it on the workers' health, begin to promote the necessary changes in its organization in order to act preventively against the possible negative effects, such as many societies have done (especially in Scandinavian countries), adapting legislation aimed to moderate the adverse effects of different forms of the organization of production.

It is rational for the company that profit constitutes its fundamental objective because the existence of it as it is defined by his objective. However, we must begin to redefine it in the context of new forms of social organization, as a mediating element between the company and workers which at times of decisions must do so in favor of the most vulnerable sectors of society.

The state has an ethical, moral, and social obligation to protect those who are in a defenseless situation from large organizations of capitalist production. Protective legislation in these sectors should be developed to ensure equal social opportunities, the right to work and be fairly paid, and the right to enjoy optimum health, living, and working conditions.

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Part IV

Explaining Human Pathological Behaviors: From Brain Disorders to Psychopathology

The Ying and Yang of Pain: Protective Versus Damaging

20

Juan C. Cavicchia and Cristian G. Acosta

Introduction

From day-to-day experience (be it direct, for we feel it ourselves, or indirect, because of what we are told about someone else's experience) everyone knows what it feels like to be in pain. Localized, episodic injuries such as scraped elbows or knees or breaking a bone; toothaches, giving birth, heart attacks and headaches are all forms of acute pain, while migraines, cancer, and heart pain are examples of more permanent forms of pain. In all these cases, however, pain permeates our entire lives. It is easy to assume that this "perception" is the end of the story: 'pain-is-pain', and that is all there is to say about it. It clearly is not. In fact, the way in which people react to what they describe as something 'painful' has changed considerably over time. In the eighteenth and nineteenth centuries people believed that pain served a specific function [1]. It was seen as a message from God or Nature; its influence would perfect the spirit. 'Suffer in *this* life and you wouldn't suffer in the *next* one', was a common way of summarizing the prevalent beliefs at that time. Submitting to pain was required. This view could hardly be more removed from twentieth and twenty-first century understandings, where pain is regarded as an unremitting evil to be 'fought'.

One of the first researchers to offer a definition of what constitutes pain (and by extension, the stimuli likely to be responsible for this evoked pain) was Charles Sherrington. He stated that "harmfulness is the characteristic of the stimuli by which [the nerve endings] are provocable, for physiological reference therefore they are preferably termed nocicipient" [2]. A few years later Sherrington expanded his definition of a noxious stimulus as one with 'an intensity and quality sufficient to trigger a reflex withdrawal, autonomic responses, and pain' collectively representing what he called the 'nociceptive reaction'. In that work [3] he introduced the notion of a neural apparatus constituted by nociceptive nerves or nociceptors which were responsible for detecting noxious stimulus. That new term implied that pain was a specific sensation with its own sensory machinery and was directly contrary to the then widely accepted theories stating that pain resulted from either a central summation resulting from excessive sensory stimulation or that all nerve endings are similar and that particular (undefined) patterns of activity provoked by intense stimulation evoke pain. This divergence of opinions reflected the competition between the so called 'specificity' and 'pattern theories' of pain that

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somewhat define the state of pain sensory biology in much of the nineteenth and early twentieth centuries. By the 1960s and 1970s the debate reached a climax with two well-defined positions: on one hand, Ed Perl strongly argued that pain is mediated by specialized high-threshold nociceptor sensory neurons [4], while on the other hand, Pat Wall and Ron Melzack emphasized central processing as generating pain [5].

It is now clear that pain is not an either/or situation: ***nociceptors*** are the peripheral path to nociceptive pain, and altered central processing does contribute to pain hypersensitivity in patients. It is also certain that we can discard the notion that sensory specificity is somehow encoded by non-specialized primary sensory neurons.

Currently, the accepted view is that stimuli that are damaging or potentially damaging to the tissues are said to be noxious, and primary afferent neurons that respond only, or preferentially, to such stimuli are called ***nociceptors***. As quoted by Light and Perl, "Nociceptors are defined as primary afferent units that uniquely signal stimuli intense enough to cause damage to the tissue" [6]. Furthermore, we now know that nociceptors are pliant and modifiable, particularly in response to both injury of its axon and exposure to a large number of inflammatory influences. This intrinsic flexibility is central to their role as pain-generating units, as will be discussed later in the chapter.

Importantly, in their interaction with the environment, living organisms must recognize and react to harmful stimuli in order to avoid them. To achieve this, nociceptors have a high stimulation threshold and therefore normally respond only to stimuli of sufficient energy to potentially or actually damage tissue. This high threshold for nociceptor activation is often found to be significantly lowered in conditions leading to chronic and pathological pain.

As was previously mentioned, nociceptors are a type of primary afferent sensory neuron and a thorough description of these neurons is available [7]. In this chapter we merely skim through some of the basic aspects of nociceptors. To begin with, these neurons are pseudo-unipolar. That is, in their mature form they have only one process leaving the soma. This process is called the initial segment and it branches at its T junction into a peripherally and a centrally projecting process, where they synapse on nociceptive second order neurons. Their cell bodies are grouped to form dorsal root and trigeminal ganglia. Some nociceptors are thinly myelinated (Aδ-fibers) but most are unmyelinated [8], and these slowly conducting afferents represent the majority of sensory neurons in the peripheral nervous system. The nociceptors may respond to mechanical, thermal, and/or chemical stimuli; and they may project to skin, muscle, and blood vessels of the trunk and limbs or to visceral organs in the thorax and abdomen. If we link cellular morphology and function, we recognize that the nociceptor unit has four functional compartments: the peripheral terminal that transduces external stimuli and it is where action potentials initiate; the axon that conducts these action potentials; the cell body (or neuronal soma) that controls the identity and integrity of the neuron (and where most of the biosynthetic activity underlying neuronal plasticity takes place); and finally the central terminal which forms the pre-synaptic element of the first synapse in the sensory pathway in the central nervous system (CNS) (Fig. 20.1).

Remarkably, the nociceptor is also subjected to influences emerging from their innervations targets, nerves and also the spinal cord. These extrinsic signals contribute to the function and phenotype of the nociceptor and are added to the intrinsic properties of the nociceptor itself.

That nociceptors and the ability to sense pain are central to survival in normal individuals can be illustrated by the unfortunate patients carrying a mutation in TrkA, the receptor for nerve growth factor (NGF). These patients suffer from hereditary sensory and autonomic neuropathy type 4, in which because of the lack of a functional TrkA, nociceptors failed to survive [9]. This condition produces congenital pain hyposensitivity, causing the patients to burn and chew their tongues and lips, and as a result of undetected damage, lose the tips of their fingers and damage their joints. Clearly, ignorance of noxious stimuli is not bliss: this is the Yin side of pain, a necessary mechanism set to protect us from inflicting self-damage (either by action or omission) or a warning signal to alert us that something in our body is not right. Other examples in which there is an innate inability to sense pain not associated

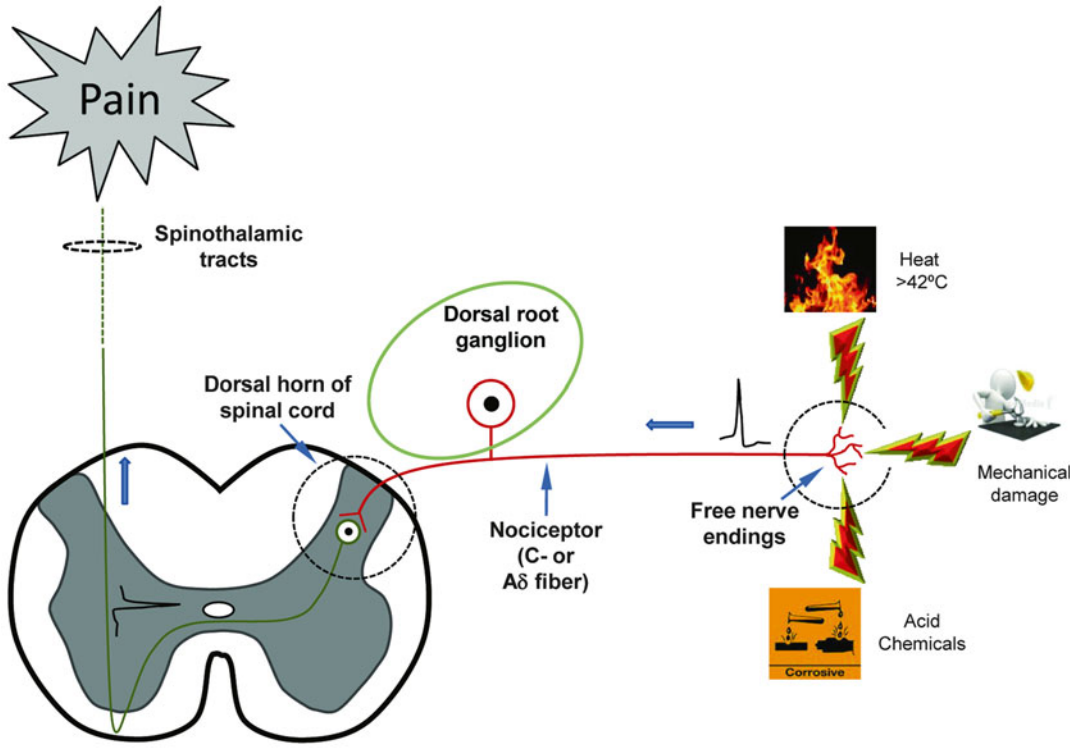

Fig. 20.1 Diagrammatic representation of a classic pain pathway. In this simplified representation only the commonest of noxious stimuli have been included (chemically-triggered irritation, burning and mechanical damage)

to nociceptor loss but rather to a lack of key molecular mediators of pain in these neurons, are those carrying mutations in voltage-dependent Na^+ channels such as SCN9A (the gene encoding the alpha subunit of Nav1.7 voltage-gated sodium channel) [10, 11] as well as SCN11A (which encodes for Nav1.9) [12–14].

Finally, we must bear in mind that, beyond the events occurring at the cellular and molecular level, pain is a phenomenon that has a physical and a psychological dimension. In the specific case of chronic or maladaptive or pathological pain, these two aspects are characterized by the occurrence of vicious circles (understood as self-sustaining and self-preserving mechanisms that reinforce undesirable/uncomfortable behaviors).

The psychological vicious cycle begins with feelings of anger, anxiety, fear, etc. arising from the presence of pain (particularly if it is chronic, moderate to severe in intensity or if it is spontaneous and unpredictable). These feelings drive the individual to a bad, poor mood, which if prolonged in time, could lead to depression. In turn, depressive syndromes can accentuate the subjective side of pain, leading to increased pain perception (even when the intensity of the pain remains unchanged over time). This takes us back to the beginning and the cycle is then perpetuated unless the pain is effectively suppressed.

The physical vicious cycle typically begins when the person avoids doing physical activity because of his/her pain. The longer this avoidance goes on, the more deconditioning occurs. The lack of activity has several implications: the patient becomes less active, hence with lesser social life and growing isolation—this feeds back to the poor mood and the depression and also leads to further activity avoidance. Again, the longer this state lasts, the more difficult it becomes to return to physical activity, which nurtures a heightened level of physical discomfort and eventually causes more pain. This cycle is then closed, and links to the psychological one (see Fig. 20.2). That type of pathological pain is

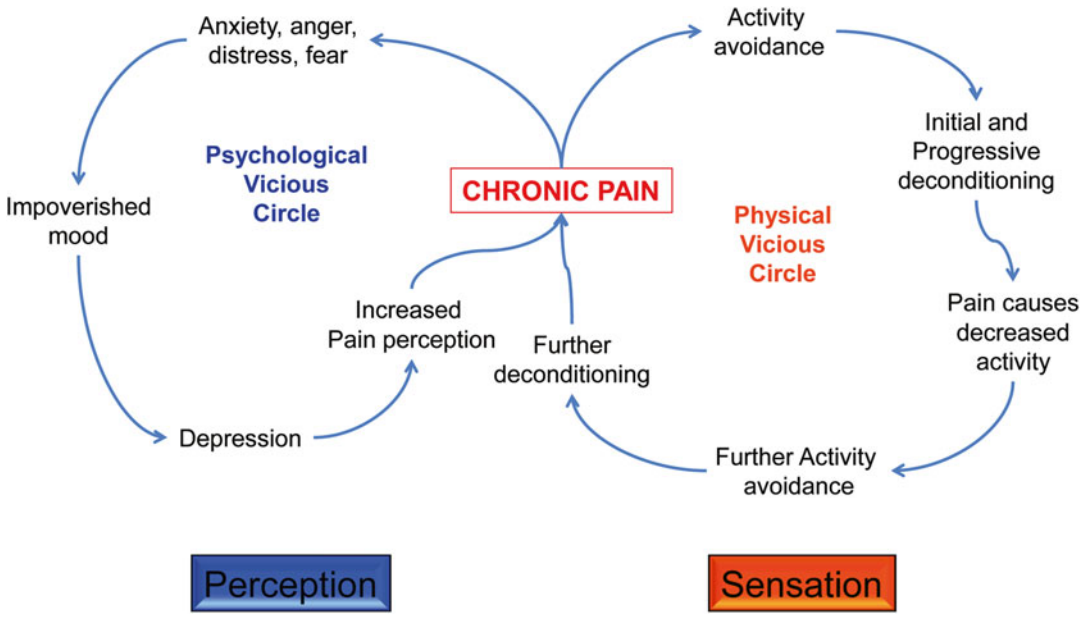

Fig. 20.2 The *vicious circles* of chronic pain. The flow diagram illustrates the likely sequence of events leading to depression and avoidance of physical activity, two of the most important side effects of chronic pain with a significant impact on patient's quality of life

seen as the Yang, the one that no longer serves the protective role of the warning system, and instead becomes a debilitating and hard to treat medical condition with significant clinical relevance [15, 16].

In summary, in this chapter we briefly present the main aspects of pain. First we present evidence on how nociceptors (as the mediators of pain) acquire their specialized molecular phenotype. Second, we comment on how they transduce noxious stimuli and transfer input to the CNS. Finally, we elaborate on how some of the adaptive and maladaptive functional and phenotypic changes that occur in them in conditions of inflammation and illness lead to spontaneous pain and pain hypersensitivity.

The Terminology of Pain

There are a number of key concepts in the field of pain and pain research that we need to define as best as we can so as to properly understand the extent of the problem and also to put into context many of the challenges that physicians (and other health professionals) face during diagnosis and treatment of pathological pain.

According to the *Online Etymology Dictionary*, the word pain probably originated from the Latin *poena*, meaning "punishment, penalty, retribution, indemnification" (in Late Latin also "torment, hardship, suffering") and from the Greek *poine*, that is "retribution, penalty, quit-money for spilled blood" and also possibly from PIE **kwei-* "to pay, atone, compensate". The earliest sense in English survives in the phrase *on pain of death*. The word pain also has a root in the Old French (eleventh century) *peine* "difficulty, woe, suffering, punishment, Hell's torments". *Pains* as in "great care taken (for some purpose)" is first recorded in the 1520s (in the singular in this sense, it is attested from c.1300). The first record of the term *pain-killer* dates from 1853. These concepts of pain as being essentially associated to the idea of hardship and punishment is in agreement with its endurance bringing spiritual elevation and purification, ideas that were predominant in Medieval and Modern times.

Contemporaneously, the International Association for the Study of Pain (IASP) set a

permanent committee in charge of periodically reviewing the definitions for a number of key terms. Here we pursue those definitions, which are presented as listed in the IASP website (http://www.iasp-pain.org/AM/Template.cfm?Section=Pain_Definitions) with some additional comments where it is pertinent to make clarifications or where debate is ongoing about the exact meaning of a term.

Pain: An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.

First must note that pain is always subjective. Therefore, individuals report their perception as being painful, and they do so verbally. However, the *inability* to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment.

We learn about the meaning of the word "pain" through experiences in early life. The episodes linked to the use of the term are normally related to injury and possible tissue damage. Accordingly, *pain is that experience we associate with actual or potential tissue damage.* It must be noted that although pain is unquestionably a sensation in a part or parts of the body, it is also always unpleasant and therefore also an emotional experience. In line with this argument, experiences which resemble pain but are not unpleasant, e.g., pricking, *should not be called pain*.

Unpleasant abnormal experiences (called dysesthesias and defined below) may also be called pain but are not necessarily so because, subjectively, they may not have the usual sensory qualities of pain. Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this is attributable to psychological rather than physiological reasons. Unfortunately, there is usually no way to distinguish their experience from that due to actual tissue damage if we limit our investigations to the subjective report. The position of the IASP Committee is that if the patients regard their experience as pain, and if they report it in the same ways as pain caused by tissue damage, it should be accepted as pain. This definition clearly avoids tying pain to the stimulus causing it. Activity induced in nociceptors and nociceptive pathways by a noxious stimulus is not considered pain, which is always a psychological state.

***Neuropathic pain*:** Pain caused by a lesion or disease of the somatosensory nervous system.

We must allude to the fact that neuropathic pain is a clinical description (and ***not*** a diagnosis) which requires the presence of a demonstrable lesion or a disease that satisfies the established neurological diagnostic criteria. *Lesion* is commonly used when diagnostic investigations (e.g., imaging, neurophysiology, biopsies, laboratory tests) reveal an abnormality or when there was obvious trauma. The term *disease* is typically used when the underlying cause of the lesion is known (e.g., stroke, vasculitis, diabetes mellitus, genetic abnormality). *Somatosensory* refers to information about the body per se including visceral organs, rather than information about the external world (e.g., vision, hearing, or olfaction). The presence of symptoms or signs (i.e., touch-evoked pain) alone does not justify the use of the term *neuropathic*. Some diseases, such as trigeminal neuralgia, are currently defined by their clinical presentation rather than by objective diagnostic testing. Other diagnoses such as postherpetic neuralgia are normally based on the clinical history of the patient. It is common when investigating possible neuropathic pain that diagnostic testing yield inconclusive or even inconsistent data. In such instances, clinical judgment is required to reduce the totality of findings in a patient into one putative diagnosis or concise group of diagnoses.

The main difference between ***central neuropathic pain*** and ***peripheral neuropathic pain*** is that the former is caused by a lesion or disease of the central somatosensory nervous system, while the latter is caused by a lesion or disease of the peripheral somatosensory nervous system.

In sharp contrast to **neuropathic pain,** ***nociceptive pain*** is pain that arises from actual or threatened damage to non-neural tissue and is a result of the activation of nociceptors. In fact, this term is used to describe pain occurring with a normally functioning somatosensory nervous system to contrast with the abnormal function

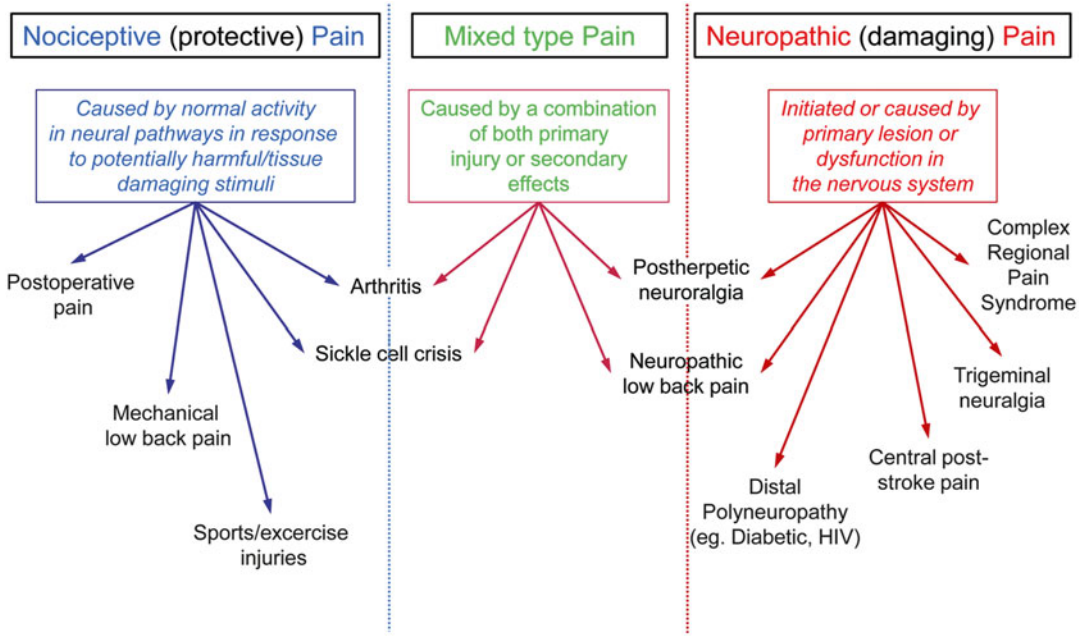

Fig. 20.3 Comparative definitions of nociceptive, neuropathic and mixed pain with examples of clinically relevant conditions typically classed into each type

seen in neuropathic pain. In other words, the protective, normally arising acute pain caused by a sudden injury for example, is called nociceptive while pain arising from an underlying malfunction or damage in the somatosensory system is termed as neuropathic (provided the damage has been diagnosed and established as the cause for the reported pain, usually chronic in duration). A patient may occasionally exhibit a combination of symptoms which in turn can be described as being partly neuropathic and partly nociceptive. Figure 20.3 summarizes what we have described and provides a few examples of each type of pain.

When the pain is located in the distribution of a single nerve or nerves, it is referred to as ***neuralgia***, while a demonstrable inflammation of a nerve or nerves is called ***neuritis***. Note that pain associated with inflammation of tissues other than nerves could be termed ***inflammatory pain***. However, this may lead to confusion because chronic inflammation resulting from conditions such as arthritis often causes pain syndromes that fit well within the definition of neuropathic pain. Furthermore, many clinical conditions that present with chronic pain are associated with ongoing chronic inflammation, which is believed to play a role in both, causing and maintaining this type of pathological pain.

Neuropathy is a disturbance of function or pathological change in a nerve: in one nerve, mononeuropathy; in several nerves, mononeuropathy multiplex; if diffuse and bilateral, polyneuropathy. Note that neuritis is a special case of neuropathy.

Patients occasionally present with a combination of symptoms which constitute a syndrome. Such is the case of ***causalgia***, a syndrome of sustained burning pain, allodynia, and hyperpathia after a traumatic nerve lesion, often combined with vasomotor and sudomotor dysfunction and later trophic changes. ***Hyperpathia***, on the other hand, is a painful syndrome characterized by an abnormally painful reaction to a stimulus, particularly a repetitive stimulus, presenting with an increased threshold and the pain is often explosive in character.

Additionally, there are signs or symptoms that are associated with the different types of clini-

cally relevant pain and need to be defined. They include allodynia, hyperalgesia, dysesthesia, hyperesthesia, hypoalgesia and paresthesia. Next, we briefly introduce each term, followed by its accepted IASP definition.

Allodynia is pain resulting from a stimulus that does not normally provoke pain. In this case, a stimulus that normally does not cause pain, leads to an unexpectedly painful response. This is a clinical term that does not imply a mechanism. Allodynia may be observed following application of different types of somatosensory stimuli to various other tissues. *Allo* means "other" in Greek and is a common prefix for medical conditions that diverge from the expected. *Odynia* is derived from the Greek word "odune" or "odyne" meaning "*pain*" which is used in "pleurodynia" and "coccydynia" and is similar in meaning to the root from which we derive words with *-algia* or *-algesia* in them.

The term *allodynia* was originally introduced to separate hyperalgesia from hyperesthesia, the conditions seen in patients with lesions of the nervous system where touch, light pressure, or moderate cold or warmth evoke pain when applied to apparently normal skin.

There are a number of potential problems with the definition of allodynia. First, how can we be certain that a strong pinch applied to the skin of a normal individual does not cause significant tissue damage, even in the short term? Can we consider that sensitized skin (e.g., sunburn) constitutes a sort of peripheral pain amplifier that can result in light tactile stimulation leading to pain? And yet, sunburned skin is technically normal skin that has transiently been affected by excessive solar irradiation that is unlikely to result in any permanent damage. These complicating factors are By "implicated" we mean that the definition of allodynia implies an abnormal pain processing being associated to abnormal tissue (or damaged tissue) whereas in the example of sunburn skin, the skin is physiologically normal.

It is also important to recognize that allodynia involves a change in the *quality* of a sensation, whether tactile, thermal, or any other sort. The original modality is normally non-painful, but the response it triggers is painful. Thus there is a loss of specificity of a sensory modality. By contrast, ***hyperalgesia*** (see later) represents an augmented response in a specific mode, that is, pain. In allodynia, the stimulus mode and the response mode differ, unlike the position with hyperalgesia.

Hyperalgesia is increased pain from a stimulus that normally provokes pain; in other words, hyperalgesia reflects increased pain on suprathreshold stimulation. As with allodynia, this is a clinical term that does not have any mechanistic implications (i.e., it does not convey information about the actual ontogeny of the phenomenon being described by the term). For pain evoked by stimuli that usually is not painful, the term *allodynia* is preferred (see above), while *hyperalgesia* is more appropriately used for cases with an increased response at a normal threshold, or at an increased threshold (e.g., in patients with neuropathy). It should also be recognized that with hyperalgesia the stimuli and the response are both in the same sensory mode. Current evidence suggests that hyperalgesia is a consequence of perturbation of the nociceptive system with concurrent peripheral or central sensitization, or both. However, it is important to distinguish between the clinical phenomena, which this definition emphasizes, as well as the interpretation, which may well change as knowledge advances.

Hyperesthesia is an increased sensitivity to stimulation, excluding the special senses. *Hyperesthesia* can refer to various modes of cutaneous sensibility including touch and thermal sensation without pain, as well as to pain. The word is used to indicate both *diminished* threshold to any stimulus and an *increased* response to stimuli that are normally recognized. In this sense, *hyperesthesia* includes both allodynia and hyperalgesia, but the more specific terms should be used wherever they are applicable.

Dysesthesia is an unpleasant abnormal sensation (often painful) while ***paresthesia*** is just an abnormal sensation (e.g., tingling under the skin), in both cases regardless of whether these sensations are spontaneous or evoked. From these definitions it is clear that hyperalgesia and allodynia are both special cases of dysesthesia. It may also be true that dysesthesia is a particular form of paresthesia, however,the reverse is not true.

Hypoalgesia is a diminished pain in response to a normally painful stimulus. This term refers only to the occurrence of relatively less pain in response to stimulation that normally causes pain. On the other hand, ***hypoesthesia*** refers to the case of diminished sensitivity to stimulation that is normally painful.

Finally, ***analgesia*** is understood to be absence of pain in response to stimulation which would normally be painful. As with allodynia, the stimulus is defined by its usual subjective effects.

Some aspects of pain are relevant to the understanding of the mechanisms underlying its occurrence. They suggest what physiological parameters are likely to be affected at the cellular level, and are therefore, useful to guide research efforts to what is causing pain. In this category, we encounter concepts such as ***pain threshold***, defined as the minimum intensity of a stimulus that is perceived as painful. Although this definition is accurate, in practice the *threshold* itself is really the experience of the patient, whereas the *intensity measured* is an external event. It has been a common mistake for many pain researchers to define the threshold in terms of the stimulus, which should be avoided. Nonetheless, the threshold stimulus can be recognized as such and measured. In psychophysics for example, thresholds are defined as the level at which 50 % of stimuli are recognized. In that case, the pain threshold would be the level at which 50 % of stimuli would be recognized as painful. We should keep in mind that the stimulus is not pain and it cannot be a measure of pain. Other subjective experience of the individual is the ***pain tolerance level***, that is, the maximum intensity of a pain-producing stimulus that a subject is willing to accept in a given situation. As with the pain threshold, it is not a measure of pain. However, both measurements are important because lowered pain thresholds or tolerance levels suggest changes in the excitability of the nociceptors, which are likely to involve, for example, alterations in the electrical properties of the neuronal membrane. Hence their importance as tools to gain insights into the mechanisms underlying pain must be pointed out.

There has been extensive research into the ability of nociceptors to exhibit increased responsiveness to their normal input, and/or recruitment of a response to normally sub-threshold inputs. This complex phenomenon is called ***sensitization*** and can include a lowered threshold and an increase in supra-threshold responses. Spontaneous discharges and increases in receptive field size can also occur. This is a neurophysiological term that can only be applied when both input and output of the neural system under study are known (e.g., by controlling the stimulus and measuring the neural event). Clinically, sensitization can only be inferred indirectly from phenomena such as hyperalgesia or allodynia. It has been shown that these sensations involve a degree of increased nociceptive responsiveness to external stimuli. If the sensitization affects the function of central neurons only while peripheral neurons function normally, we refer to ***central sensitization***. When what we observe is an increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation of their receptive fields, we are observing ***peripheral sensitization***.

Up to this point we have used terms such as "nociceptor" or "nociceptive" without properly defining them. For that matter we have not as yet clearly stated what we understand by the term ***nociception.*** The simplest possible definition states that nociception is the neural process of encoding noxious stimuli. Note, however, that this definition does not necessarily imply pain sensation. As a matter of fact, consequences of encoding may be autonomic (e.g., elevated blood pressure) or behavioral (e.g., motor withdrawal reflex or more complex nocifensive behavior). The former is not usually linked to pain per se. Having said this, we need to establish the difference between a nociceptive neuron and a nociceptor. In general, a ***nociceptive neuron*** is a central or peripheral neuron in the somatosensory nervous system that is capable of encoding noxious stimuli. Notice that this definition does not explicitly state any specific physiological properties for these neurons, and therefore can be applied to a number of neuronal types not necessarily linked to pain sensation. This is not true for nociceptors,

whose function is to encode and transduce noxious stimuli, and to do so normally in response to high stimulation thresholds.

The Nociceptor

In all clinically relevant situations, and at the core of pain sensation, lies the specialized neuron we call nociceptor (reviewed in detail by [17]). As excitable cells with receptive fields projecting to the external as well as the internal environment of the organism, they possess a large number of receptors acting as sensors. In turn, the activation of these receptors leads to signalling events terminating in a number of possible targets: the nucleus, where they promote synthesis of new proteins; the proteins of the cell membrane involved in controlling neuronal excitability, e.g., ion channels or even other receptors, also membrane bound, that can be sensitized to react to the presence of small quantities of certain chemicals; and so on. We must add to this cellular complexity that a given subtype of nociceptor (e.g., mechanoceptive, thermoceptive, polymodal, etc.) carries its own, specific complement of receptors and effectors. These phenotypical markers are quite often used to functionally and histochemically define a nociceptor . Note that sensory ganglia contain more than one type of nociceptor. Finally, there is the added complication of the temporal dimension of the neuronal responses to noxious stimuli: some are quite prompt, such as those triggered by burning of the nerve endings of the skin, while other influences work over extended periods of time, such as chronic underlying inflammation or axonal degeneration resulting from serious ongoing medical conditions such as diabetes. It seems obvious that more than one mechanism must be at work at the cellular and physiological levels for nociception to be accurate and reliable. It is also not surprising that being so complex and involving such a large number of molecular players, it can go wrong fairly often.

In the next sections we will briefly discuss some of the main aspects of the cellular biology and physiology of nociceptors and their link to pain. However, this is not intended as a full review and neither is it an in-depth description of the topics: it is only a guide to point out the most salient and active areas of interest and research in the field with some mechanistic insights and future perspectives.

The Nociceptors: Its Development and Maturation

Nociceptors develop from those neural crest stem cells that migrate from the dorsal part of the neural tube and form late during neurogenesis, whereas neurons born earlier become proprioceptors or low-threshold mechanoceptors [18–20]. All newly formed embryonic nociceptors express TrkA, the NGF receptor [20]. However, the transcription factors that determine nociceptor cell fate remain poorly understood. Differentiation of most TrkA+ neurons depends on the pro-neural transcription factor Neurogenin1 (Ngn1) [21, 22]. However, Ngn1 activity is not specific for nociceptors—it is also required for formation/differentiation of TrkB+ and TrkC+ cells, which eventually mature to become low-threshold mechanoceptors [21, 22]. However, the Runx1 runt domain transcription factor is expressed exclusively in TrkA+ neurons at early embryonic stages [23–26] but because its expression is initiated some time after the onset of TrkA expression, it is unlikely to be involved in early nociceptor cell fate determination [23]. Here it is important to mention that 1) some large, myelinated, fast conducting TrkA+ neurons become Aβ-nociceptors in adulthood, but are likely to have been TrkA- early in embryonic life; and 2) it is now clear that there is a degree of phenotypical switch in the dependence on trophic factors and hence its receptors (TrkA, TrkB, TrkC, etc.) happening between late embryonic and early postnatal stages [27–30]. It is also important to bear in mind that following sensory neurogenesis, potential nociceptors undergo two distinct differentiation pathways leading to the formation of two main classes of nociceptors: peptidergic and non-peptidergic nociceptors. These two sets of nociceptors express distinct repertoires of ion channels and receptors and innervate distinct

peripheral and central targets [31–33]. This topic is briefly discussed in more detail in the next section, followed by a more in-depth description of the main differences existing between peptigergic and non-peptidergic nociceptors.

Segregation of Peptidergic Versus Non-peptidergic Nociceptors

During the perinatal and postnatal period, about one half of developing nociceptors switch off TrkA expression and start expressing Ret, the transmembrane signaling component of the receptor for glial cell-derived neurotrophic factor (GDNF) and other GDNF-related growth factors. The neurons undergoing this phenotypic switch in their trophic factor dependence become the so-called non-peptidergic nociceptors, most of which (>95 %) bind isolectin B4 (IB4+). The remaining nociceptors retain TrkA (a few also co-express Ret) and develop into the peptidergic class of nociceptors that do not bind IB4 and express the calcitonin gene-related peptides (CGRP) and substance P (SP) [34, 35]. The dynamic expression of Runx1 appears to be an important participant in this process [23, 24, 26]. Early embryonic nociceptors share a similar molecular identity, co-expressing both TrkA and Runx1 [23]. During the period when nociceptor segregation occurs, cells from Runx1 persist as nonpeptidergic neurons. Conditional knockout of Runx1 in the dorsal root ganglia (DRG) transforms these nonpeptidergic cells to a TrkA+ CGRP+ identity, and in this situation most nociceptors develop into peptidergic nociceptors [23, 26]. Conversely, constitutive expression of Runx1 in all nociceptors is sufficient to suppress embryonic peptidergic differentiation [24]. Runx1 also coordinates afferent targeting to the spinal cord; in mice that lack Runx1 prospective IB4+ non-peptidergic afferents adopt the projection pattern typical of peptidergic afferents [23]. These observations suggest that persistent Runx1 expression promotes the Ret+ nonpeptidergic cell fate, whereas loss of Runx1 is essential for peptidergic differentiation. Several studies have suggested that Runx1 and TrkA/Ret signaling pathways form a complex interaction loop for establishing nonpeptidergic nociceptor cell fate [36, 37]. TrkA-signaling is required to activate Ret, partly it appears by maintaining Runx1 expression at perinatal stages [36]. However, despite progress in teasing out the determinants of nociceptor specification, several issues remain to be resolved. Because both TrkA and Ret are required for afferents to innervate peripheral targets [36, 38], a loss of either TrkA or Ret signalling prevents nociceptors from receiving other target derived signals. Consequently, it is not known if TrkA signalling directly or indirectly controls expression of Runx1, and Ret, or if Ret signalling is directly involved in TrkA expression suppression. Additionally, while TrkA signalling is required to maintain Runx1 expression at embryonic stages, Runx1 expression is extinguished from TrkA+ peptidergic nociceptors during perinatal/postnatal development, therefore, we need to determine whether TrkA signaling switches from activating to suppressing Runx1 expression at different developmental stages or if a peripheral innervation defect in the absence of TrkA signalling indirectly extinguishes Runx1 expression. A further problem is that the intrinsic transcription factors that establish peptidergic nociceptor cell fate still remain elusive. Runx1 can, therefore, exert opposing activities depending on the cellular context. It will be extremely interesting to establish if changes in context-dependent transcriptional activities contribute to the phenotypic switches in nociceptors that occur in pathological conditions.

Subpopulations of Nociceptors

It is frequently stated that IB4 binding and TrkA expression define separate subpopulations of small, putative, nociceptive neurons. In the DRG, most small neurons (defined as those neurons showing slow action potential conduction velocity and a total cell area at the level of the nucleus of <400 μm^2 in the rat—this cell area is different in other species). These small neurons express

either TrkA or bind IB4 or both. It is therefore worth comparing these two populations. IB4 is a lectin from the plant *Griffonia simplicifolia* that binds to β-D-galactose residues in glycoconjugates on the cell surface and Golgi apparatus of small, neurofilament–poor DRG neurons in a variety of species [39]. That there is some degree of co-localization between TrkA expression and IB4 binding [40, 41] was confirmed with intracellular recording and dye injection studies in rat DRGs. These showed [1] that a third of C-fiber neurons were positive for both TrkA and IB4, with a tendency for reciprocal staining intensities for these two markers [2]. Most nociceptors strongly expressed TrkA or IB4 binding sites [3]. IB4 binding sites were present on C-fiber but not A-fiber nociceptive neurons, whereas TrkA expression was in both C- and A-fiber nociceptors [4]. Some weak positive labelling for TrkA and IB4 was seen in some D hair units. TrkA-, and not IB4-, positive neurons express SP and CGRP, as we stated in the previous section. Other differences between these neurons include projection of TrkA-positive neurons to laminae I and IIouter and IB4-positive neurons mainly to IIinner [42] of the dorsal horn [39] Compared with IB4-negative small neurons, IB4-positive neurons have longer duration action potentials and a smaller noxious heat-activated current [1], and the tetrodotoxin-resistant (TTXR) Na^+ channel subunit Nav1.9 is preferentially expressed in IB4-positive cells [43]. It has also been shown that IB4-positive neurons selectively express the K^+ leak channel TREK2, causing these neurons to be more hyperpolarized than IB4-negative nociceptors, and preventing these neurons from firing spontaneously [44], which is assumed to be the underlying cause for spontaneous pain [45, 46]. In summary, A-fiber nociceptors express TrkA but not IB4 binding sites, while most C-fiber nociceptors express one or the other, or both of these (Fig. 20.4). Apart from the NGF and GDNF dependence of TrkA expressing and IB4 binding neurons respectively, the functional differences between these groups of nociceptors are still relatively poorly understood.

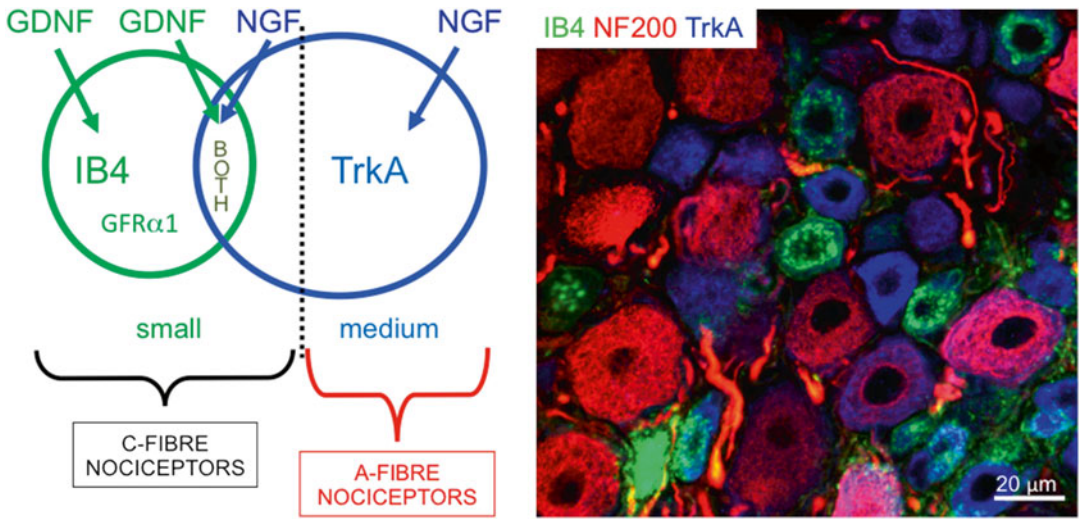

Fig. 20.4 Nociceptors are classified according to cell size (small, medium) and phenotype (binding of isolectin B4 or expression of the NGF receptor, TrkA) into C-fibre and A-fibre nociceptors. Note that IB4 binding small nociceptors express the GDNF-receptor GFR α 1. The right side panel shows triple immunofluorescence staining of a section of L5 normal rat DRG. Note the heterogeneity of subpopulations present in this small section of tissue. Neurons labelled with Neurofilament of 200 kDa (NF200, in *red*) are large and myelinated. Of these, a few express TrkA (in *blue*) which defines them as Aβ-nociceptors. Small neurons are either stained for TrkA or bind isolectin B4 (IB4, *green*) or rarely for both. All of these are nociceptors, unmyelinated and putative C-fiber neurons

Physiological Bases of Neuronal Excitability and Its Consequences for Pain

The mature nociceptor expresses dozens of ion channels and receptors, and the correct establishment of their expression is essential for nociceptors to detect specific noxious stimuli. There are two notable features about the developmental control of sensory channels/receptor expression. First, many sensory channels/receptors are expressed in only a partially overlapping or mutually exclusive manner, including TRP class thermal/chemical receptors and Mrg class G protein-coupled receptors [33, 47–49]. Second, the emergence of individual sensory channels/receptors is subject to complex temporal control. For example, expression of three TRP channels, TRPV1, TRPM8, and TRPA1, is initiated at E12.5, E16.5, and P0, respectively, while TRPA1 expression in peptidergic nociceptors is established at P0 and non-peptidergic nociceptors at P14, respectively [49]. Albeit of considerable interest in basic research, these complex developmental processes are beyond the scope of this chapter and have already been thoroughly reviewed in the literature [17].

As stated above, the mature nociceptor expresses a large number of ion channels and other associated receptors. These ion channels are carried through the cell membrane ion currents that are responsible for the excitability of the neuron. They play a pivotal role as direct or indirect controllers of or contributors to action potential generation and propagation along the nerve fibers. It is therefore important for us to look at the growing body of knowledge accumulated about these ion channels and to discuss the implications that their electrophysiological properties have for pain physiology normally and in models of chronic pain.

Na^+ Currents and Channels

Voltage-gated sodium channels (called Nav) are essential for generation and conduction of action potentials. The currents are subdivided into tetrodotoxin-sensitive (TTXS) and TTXR. The channels designated Nav1.1, 1.2, 1.3, 1.6, and 1.7 are TTXS, while Nav1.5, 1.8, and 1.9 are TTXR. Three of these proteins, Nav1.8 and Nav1.9 (both TTXR), and Nav1.7 (TTXS) are preferentially (but not exclusively) expressed in small DRG neurons. Described next were the main known properties of these channels and how their function and/or expression are altered in models of inflammation and nerve injury.

Nav1.8 (Also Called SNS/PN3)

Nav1.8 is expressed in rats in small- to medium-sized DRG neurons [50] most of which are nociceptive [51]. Nav1.8 gives rise to a TTXR Na^+ current that is believed to contribute the majority of the inward Na^+ current in action potentials of small DRG neurons and it is most likely responsible for the TTXR current at nociceptive receptor terminals [52, 53]. Its electrophysiological properties probably contribute to properties of nociceptive neurons, including its high activation threshold of about −35 mV. Consider that normal resting potential in C-fiber nociceptors is ~55 mV and therefore a threshold of −35 mV implies that a neuron must be made 20 mV more positive in order to fire an action potential. This is a significant change in membrane potential and it suggests that such a nociceptor will not fire easily unless the intensity of the stimuli is high enough to be registered as a threat or it is noxious. The slow kinetics of Nav1.8 give rise to long-duration action potentials, and contributes to the large action potential overshoot, and its rapid repriming. All this may enable firing even in depolarized fibers [50–52, 54]. In essence, the slow inactivation of Nav1.8 means that these channels may be able to sustain repetitive firing even at depolarized membrane potentials. That is why they are thought to underlie spontaneous pain which requires ongoing firing in C-fiber neurons at very slow rates [45].

Inflammation and Nerve Injury

Several inflammatory mediators can acutely (minutes to hours) decrease the activation threshold, and/or increase the kinetics or magnitude of the TTXR Na^+ current (presumed to be Nav1.8

related) [55] and may contribute to acute hypersensitivity at nerve terminals. In the longer term, Nav1.8 mRNA and TTXR Na^+ current in small DRG neurons and in cutaneous fibers are upregulated during most studies on inflammation [56, 57]. In some models of neuropathic pain, such as 7-day axotomy of the L5 nerve, TTXR current density and Nav1.8 mRNA and protein are decreased [58]. This reduction in Nav1.8 may explain why axotomized C-fiber neurons are incapable of action potential firing despite being relatively depolarized [44].

Nav1.9

Nav1.9 is also known as NaN/SNS2. Similarly to Nav1.8, Nav1.9 gives rise to a TTXR current. Nav1.9 immunoreactivity is mostly present Sin small DRG neurons [59, 60], preferentially in those with IB4 binding [43] (and therefore, non-peptidergic) and along C-fibers and at nodes of Ranvier of thinly myelinated fibers [61]. It gives rise to a persistent, depolarizing TTXR Na^+ current in small DRG neurons as a result of its low activation threshold and ultra-slow inactivation [62], thus it is likely to influence membrane excitability, although the magnitude and mode of this effects remain poorly understood. Interestingly, GDNF, but not NGF, upregulates Nav1.9 mRNA [43]. This is in agreement with GDNF being the main trophic factor required to maintain the phenotype of IB4-binding small C-nociceptors in vivo [41]. Interestingly, Nav1.9 expression is linearly and positively correlated with TREK2 expression (a K^+ leak channel, see below) in DRG neurons, which suggests that both channels are part of the same control mechanism of neuronal excitability in IB4-binding neurons [44].

Inflammation and Nerve Injury

Nav1.9 mRNA in DRG neurons is increased after *inflammation* [59], after exogenous GDNF administration [43], and it is also activated by the application of a cocktail of pro-inflammatory mediators [63]. *Axotomy* induces a decrease in Nav1.9 mRNA and protein in the DRG [43, 58, 59] that is reversed in IB4-positive neurons by exogenous GDNF [43]. A lack of function mutation of the gene encoding for Nav1.9 in humans (SCN11A) has recently been reported [12, 14]. Patients with this rare mutation experience lack of pain, and are prone to suffer extensive burns. This highlights the importance of the protective role of acute pain which prevents us from suffering life-threatening injuries.

Nav1.7 (or PN1)

Nav1.7 is expressed more highly in small rather than large DRG neurons [64] despite the fact that its mRNA is present in neurons of all sizes [65]. It is thought to carry much of the TTXS inward current in action potentials in small neurons [66]. Nav1.7 protein is present in fibers and terminals of cultured DRG neurons [67]. Its slow inactivation, combined with its low activation threshold (closer to −50 mV) [66], may be important in the generation of receptor potentials and contributing to the generation of action potentials. NGF causes a long-lasting (weeks) increase in Nav1.7 protein in DRG neurons in vivo [68].

Nav1.7 expression drops substantially after axotomy while essentially remaining unchanged after acute cutaneous inflammation [44]. The role of this channel in pain is normally emphasized by the finding that a lack-of-function mutation of its gene derives a lack of pain sensitivity and the consequent exposure to injury (e.g., breaking bones). This seems to be a genetic trait that is inherited [10, 69, 70]. It has also been shown that a scorpion toxin causes a gain of function of Nav1.7 leading to pain hypersensitivity [71].

It is important to note that certain types of pain (e.g., oxaliplatin-induced neuropathy), do not require either Nav1.7 or Nav1.8 [72]. Thus, proper patient stratification and accurate diagnosis is essential to correctly treat chronic pain using modulators of Na^+ channel function.

Other Na^+ Subunits

mRNAs of several other TTXS subunits are more abundant in medium and large neurons. These include Nav1.1, 1.2, and 1.6 and Nav2.2 (NaG) [73]. Following *axotomy*, the appearance of a more rapidly repriming TTXS current is thought to contribute to hyperexcitability in axotomized small DRG neurons in vitro [74, 75]. This has been ascribed to increased expression of Nav1.3 (also known as brain type III), but roles for other TTXS channels have not been ruled out.

Na^+ Channel β Subunits

Na^+ channel β subunits may interact with the cytoskeleton or extracellular matrix and play roles in Na^+ channel trafficking within cells and insertion into membrane, thought to be mediated by annexins and the auxiliary protein p11 [76–78]. When co-expressed with β subunits, subunits can alter the kinetics, peak current, and/or voltage dependencies of β subunits [79].

K^+ Currents and Channels

K^+ channels are central to the control of resting membrane potential, after-hyperpolarization, and firing frequency and they influence adaptation. They tend to increase membrane potential stability (i.e., less likely to oscillate), and at least some of the K^+ channels that contribute to long duration after-hyperpolarizations may prove to be more highly expressed in nociceptors. Despite much work in this field in recent years, there is still a lack of complete understanding of two main questions. 1) Which K^+ channels are expressed by different functional subpopulations of primary afferent neurons, and 2) How these channels work together to maintain membrane potential stability and to provide re-polarization/after-hyperpolarization in firing nociceptors [80, 81].

Voltage-Gated K^+ Currents and Kv Channels

The two main groups of calcium-insensitive voltage-gated K^+ currents are the depolarization-activated delayed rectifier (IKv) and fast transient (IA) currents. The protein subunits of the channels that underlie these currents are the Kv subunits.

Delayed Rectifier Currents

Delayed rectifier currents (also called IKv) serve to rapidly terminate the action potential in the soma and inhibit repetitive firing in myelinated axons [82]. They are particularly prominent in some large cutaneous afferent neurons [83], but are also present in small DRG neurons [84].

Fast Transient (A-Type) K^+ Currents

Fast transient K (IA) currents tend to clamp resting potential at hyperpolarized voltages until they inactivate, thus prolonging the after-hyperpolarization and slowing/preventing repetitive discharges [85], IA currents are present in both large cutaneous afferents and small DRG neurons [84] but are more prominent in slowly conducting afferents [86]. IA can be subdivided into rapidly (fast IA) and slowly [87] inactivating types [88]. Slow IA is particularly prominent in small DRG neurons with TTXR action potentials (see [88]), and may therefore contribute to the broad after-hyperpolarizations of nociceptive neurons.

In DRG neurons IKv and slow IA are both sensitive to dendrotoxin, but fast IA is not [88]. Kv1.1 and 1.2 are associated with the delayed rectifier, whereas Kv1.4 is associated with fast IA [89, 90], and it has been suggested that Kv1.1/1.2 associated with Kv1.4 (dendrotoxin insensitive) may give rise to the slow IA in DRG neurons [88, 89], Kv1.1, 1.2, 1.3, 1.4, 1.5, and 1.6 and Kv_2.1 are all expressed in DRG neurons; of these, Kv1.1 and 1.2 are more abundant and highly expressed in medium- to large-sized neurons, and Kv_2.1 is also more highly expressed in medium to large neurons, whereas Kv1.4 is more highly expressed in small neurons [88, 89, 91]. Kv1.1 has recently been shown to play a central role in mechanosensation [92].

M Currents

M currents (I_M) are voltage and time dependent, noninactivating, and activated at negative voltages (beginning at approximately −70 mV). Their inhibition by acetylcholine or other agents leads to increased neuronal excitability [93]. I_M are associated with KCNQ2/3 and −5 subunits [93–95], all members of the six transmembrane superfamily that are distantly related to Kv channels. I_M as well as KCNQ2, −3, and −5 have been detected in both small and large DRG neurons [94, 96]. Blocking of I_M with linopirdine causes increased firing in response to current injection in small DRG neurons, indicating that the I_M may normally act as a brake to firing in these neurons. After cutaneous inflammation, there is inhibition

of I_M leading to depolarization and exacerbated firing [97]. More recently, I_M present in nociceptors (probably including peptidergic and non-peptidergic C-neurons) have been attributed to the expression of Kv7.2 [97, 98] and Kv7.5 [8, 99], although evidence is contradictory and requires further study.

Inwardly Rectifying K^+ Channels

Inwardly rectifying K^+ (Kir) channel [100] current contributes to the resting membrane potential in a number of cell types, including DRG neurons, where Kir current is mostly in medium-size neurons [101]. Little is known about Kir-related channel subunits in DRGs. However, there is evidence that paclitaxel reduces the expression of Kir1.1 and Kir3.2 in sensory neurons, leading to neuropathic pain and nociceptor hiperexcitability [102]. Kir3.2 has also been involved in the response to opioids [103]. Other Kir channels (2.1, 2.2 and 2.3) have been proposed as key controllers of the pacemaker activity of lamina I spinal cord neurons part of the pain circuit [104].

Ca^{2+}-Activated K^+ Currents

Ca^{2+}-activated K^+ currents (IKCa) are of three types, related to BK [87], IK, and SK channels (big, intermediate, and small conductance channels, respectively), all activated by elevated intracellular Ca^{2+}; BK is also voltage dependent. Functionally BK is associated with after-hyperpolarizations that develop rapidly and decay in 10–100 ms, and SK with slower after-hyperpolarizations that may last for seconds and limit firing frequency [105, 106]. Either or both of these could therefore contribute to the long after-hyperpolarization in nociceptors, although this remains to be established. BK and SK currents are found in DRG neurons [107–109], with BK currents observed in two thirds of small DRG neurons. Immunoreactivity for SK1 and IK1 channels was found in many human and rat DRG neurons [110]. A related channel, SLACK (sequence like a Ca^{2+}-activated K^+), is expressed in rat DRG neurons [111]. SLACK has a conductance similar to that of BK, with which it can interact to generate an I-KCa channel (different from IK1). Interestingly, SLACK (and its partner, SLICK) is required for the depolarization of afterpotential in medium DRG neurons [112]. Protein kinase A induced internatilization of SLACK causes neuronal hyperexcitability [113]. It has been demonstrated that a reduced SLACK expression leads to increased thermal and mechanical sensitivity, a process regulated by the chloride channel TMEM16C [114].

Background K^+ Channels

The greater part of the time-independent resting conductance in a variety of neurons is contributed by background K^+ channels. They are two pore domain (called K2P), homo- or heterodimeric channels. They are mainly responsible for setting resting membrane potential (Em) and are constitutively open at rest, generally voltage independent, and respond to a number of different factors [115, 116]. Thus, through setting Em, K2P channels strongly influence neuronal excitability and firing [117, 118].

The K2P channels are encoded by the K2P (originally termed KCNK) family of genes; 15 distinct isoforms have been cloned, with 12 apparently functional [119]. K2Ps are grouped and named in families according to their functional properties: TWIK, weak inward rectifiers; THIK, halothane inhibited; TREK, lipid, stretch and temperature activated; TASK, acid inhibited; TALK, alkaline activated; and TRESK, Ca^{2+} activated [119–121]. Some (e.g., TRESK) are inhibited by arachidonic acid while others (e.g.,TRAAK, TREK2) are activated by it and also by G-protein coupled receptor agonists [122]. Thus, far from being passive, K2P channels are acutely modulated by ligands or environmental factors, resulting in altered leak K^+ current, and thus altered Em.

mRNA studies have found high levels of TRESK, and variable levels of TRAAK, TREK1, TREK2, TWIK1 and TWIK2 in rodents (rat or mouse) DRGs [123, 124].

There is growing evidence that K2P channels are implicated in nociception and pain. TREK1 is colocalized with TRPV1 in some nociceptive DRG neurons and its mRNA expression is reduced after inflammation in neurons innervating the colon [125]. TREK1 knockout also reduces inflammation-induced mechanical and

thermal hyperalgesia [126]. TRESK knockout enhances DRG neuron excitability [127], and a dominant negative TRESK mutation is implicated in migraine [128]. In 2006, Kang and Kim showed that at 37 °C, TREK2 contributed ~69 % of the K^+ standing current (responsible for the majority of Em) in a third of small sized cultured neonatal rat DRG neurons; TRESK, expressed in all sizes of DRG neurons [124] contributed 16 % and TREK1 12 % of the total K^+ leak current recorded in these neurons. The relatively high TREK2 mRNA in rat DRGs [129] supports TREK2 contributing substantially to Em in adult DRG neurons. We recently demonstrated that TREK2 hyperpolarizes IB4-binding C-nociceptors and limits pathological spontaneous pain. Similar TREK2 distributions in small DRG neurons of several species suggest that the role(s) of TREK2 may be widespread [44].

Axotomy and K^+ Channels

Changes in the in vivo electrophysiological properties of different subpopulations of DRG neurons after axotomy suggest altered (mostly decreased) K^+ channel expression or activation. Decreases in IK-immunoreactivity and fast IA occur after axotomy in large cutaneous afferent neurons [130] and in the former in small neurons [88]. Reductions in expression of Kv1.1, 1.2, and 1.4 and Kv2.1 have all been reported [88, 89, 91]. Overexpression of Kv1.2 impairs axotomy-induced neuropathic pain in rats [131]. Kv2 channels dysfunction after axotomy enhances sensory neuron responsiveness to stimuli exacerbating pain [132].

Reverse transcription–polymerase chain reaction showed increased KCNQ−2, −3, and −5 in both large with retigabine resulted in decreased electrophysiologic and behavioral changes in a model of neuropathic pain [94]. A reduction of K(ATP) currents after axotomy in both small and large DRG neurons has also been reported [133].

Interestingly, IK(Ca) is reduced after axotomy in DRG neurons a finding linked to increased neuronal excitability and possibly pain [134].

Inflammation and K^+ Channels

After acute (2- to 3-h) inflammation, activation of KCNQ/I_M with retigabine resulted in animals putting increased weight on the inflamed foot [94, 96]. K^+ leak channels (K2P) mRNA levels have also been shown to change as a result of CFA-induced cutaneous inflammation for 1 or 4 days. Some of these changes in mRNA (for TASK1 and TASK3) were correlated with spontaneous foot lifting, a measure of spontaneous pain [129]. However, most studies suggest that changes in expression resulting from inflammation may occur bilaterally, and affects DRG neurons projecting to sites not directly affected by cutaneous inflammation. This is believed to be caused by circulating cytokines and hormones whose release into the bloodstream is triggered by local inflammation, making it a systemic event [135, 136]. It is often referred to as global effects and may explain why underlying clinical conditions associated with chronic inflammation have widespread pain symptoms that can encompass multiple organs and systems, and not only those directly affected by the ongoing inflammatory process.

Hyperpolarization-Activated Currents and Channels

The H current (Ih, also called the funny current when first described in the heart, and therefore also termed If) is a hyperpolarization-activated, time and voltage-dependent, non-selective cation current. When activated this current causes depolarization of the membrane, reducing afterhyperpolarization duration, increasing firing frequency and decreasing adaptation [137, 138]. An Ih is prominent in most or all large DRG neurons but in fewer small neurons [86, 101, 139]. The channels that give rise to Ih are made up of HCN (Hyperpolarization-activated cyclic nucleotide-gated channel) protein subunits, four isoforms (HCN1 through HCN4) of which have been cloned [138, 140, 141]. In DRG neurons there is strong expression of HCN1 mRNA in all large- to

medium-diameter and most small-diameter DRG neurons, lower expression of HCN2 mRNA in approximately 80 % of large and approximately 60 % of small neurons, and low or undetectable levels of HCN3 and HCN4. HCN1 through HCN3 proteins are concentrated at the membrane especially of large neurons [142, 143]. In vivo, rat DRG neurons express HCN1 and HCN2, with the latter being more abundant in C and Aβ-nociceptors and remarkably high in muscle spindle afferents [144]. Detailed kinetic analysis of Ih in vivo shows that in most neuronal subtypes, Ih is made up of heteromeric HCN1 + HCN2 channels [145, 146] as described for DRG neurons [139]. There is growing evidence for involvement of HCN channels in chronic pain [147–149]. It has recently been shown that HCN2 expressed in small, putative C-neurons in mice is important to sustain chronic, inflammatory pain [150, 151]. However, expression of HCN2 is also altered in medium and large neurons after inflammation [144, 152] which suggests a more complex mechanism for the involvement of HCN2 in chronic pain. *Nerve injury* causes increased Ih in large-diameter neurons dissociated in vitro, and ZD 7288 (a specific Ih blocker) blocks ectopic discharge in axotomized A-afferent fibers [142]. Ivabradine (an approved Ih blocker with similar affinity for all 4 HCN isoforms used in the treatment of cardiac arrhythmias) has been studied as a potential treatment for inflammatory pain linked to changes in the expression of HCN2 [153–155]. However, the lack of selectivity of the drug and its tendency to reduce the heart rate in normal individuals may limit its clinical usefulness.

Ca^{2+} Currents and Channels

Ca^{2+} has crucial roles as a second messenger (therefore it is involved in signal transduction), in transmitter release (mediated by Ca^{2+} influx at presynaptic terminals), and in inhibiting firing by activation of IKCa. Also, the inflection seen on the falling phase of some of the broader (longer duration) action potentials in DRG neurons is partly due to an inward Ca^{2+} current. Based on electrophysiological and pharmacological criteria, several voltage-gated Ca^{2+} currents have been found and described in DRG neurons. These include L (nimodipine-sensitive, high voltage activated), T [156], and N (intermediate properties) [157]. Additional information on the properties of these currents can be seen in the review by Catterall [158]). Other currents are expressed in some DRG neurons; these are the P- type (sensitive to inhibition by low doses of ω-agatoxin IVA), the Q-type (blocked selectively by ω-conotoxin MVIIC) and a toxin-resistant fraction that has been termed R-type Ca^{2+} current [156, 159, 160]. Their amplitudes differ in neurons of different sizes, with relatively large L-type and N-type and smaller T-type currents in small cells, larger T- but little L- and N-type currents in medium-sized neurons, and little T-type current in large neurons [161]. T-type Ca^{2+} channels (Cav3.2) are thought to be necessary for the normal mechanosensitivity mediated by Aδ-fiber D hair LTMs [162]. Additionally, L- and N- but not T-type currents cause substance P release from isolated DRG neurons [163]. The secretory activity of some DRG neurons has been taken as an indication that these neurons behave physiologically like small neuroendocrine units. Importantly, Ca^{2+} currents can be modulated by a variety of agonists. For example, activation of δ-opioid receptor II on cultured early postnatal rat DRG neurons reduced N-, L-, P-, and Q- but not R-type currents [164], and 5-HT inhibits Ca^{2+} currents in small DRG neurons probably via 5-HT1A receptors [165]. Reports of expression include the following channel subunits demonstrated both immunocytochemically and by in situ hybridization (current type associated with the subunit in parentheses): Cav2.1 (P/Q), Cav2.2 (N), Cav1.2 and Cav1.3 (L), and Cav2.3 (R) [158, 166–168].

Nerve Injury and Future Treatments

There is currently substantial interest in calcium channels as new targets to treat neuropathic and inflammatory pain [167–169]. For instance, in relation to nerve damage it is now known that the T-type current in medium-sized neurons, as well as all Ca^{2+} currents decrease 10 days after CCI (chronic constriction injury) of the sciatic nerve [170]. Furthermore, the α2δ1subunit is upregulated [143] after various types of nerve injury [171, 172]. Additionally, regulation of α2δ1function and expression has been proposed

as a major contributor to mechanical and thermal hyperexcitability. This may be an important site of action of the analgesic gabapentin [173–175]. There are expectations that a synthetic peptide called ziconotide will be the first in a new class of neurological drugs: the N-type Calcium Channel Blockers, or NCCB. This drug (based on a snail toxin) had a novel mechanism of action and acts as a non-opioid analgesic. This feature gives it the potential to play a valuable role in treatment regimens for severe chronic pain [176]. However, N-type calcium channels are widespread throughout the body and preliminary clinical data suggests that ziconotide may be far too toxic to be used orally.

Conclusions

It is now well known that changes in the electrical properties of the neuronal membrane in DRG neurons underlie the changes in excitability associated with both acute and chronic pain, albeit the changes are different in nature depending of the type of pain involved. A good example of this is the demonstration that the rate of spontaneous firing in C-fiber nociceptors is directly and significantly related to the amount of spontaneous foot lifting in rats after cutaneous inflammation or partial nerve injury [45, 46]. This behavior is used as a marker for spontaneous pain. It decreases in animals in which C-fiber nociceptors express higher levels of the protective channel TREK2 (which exerts a hyperpolarizing influence on their membrane potentials) [44]. Altered expression of HCN channels is also associated with spontaneous firing in C- and Aδ-fibre neurons [152]. These are just but a few examples of the interplay between ion channels, excitability and pain.

To put the importance of the ion channels and their role as regulators of neuronal excitability into perspective, there has been a recent report of changes in C- and A-nociceptors, and Aα/β--cutaneous LTMs that are consistent with the uninjured neuron hypothesis [46]. These changes could contribute to different aspects of peripheral neuropathic pain as follows: spontaneous firing in C- and A-nociceptors to spontaneous burning and sharp-shooting pain, respectively; spontaneous firing in Aα/β-cutaneous LTMs to paresthesias. Finally, if decreased A-nociceptor electrical thresholds contribute to sensory hypersensitivity, they would result in greater evoked pain (hyperalgesia and/or allodynia).

Future Perspectives

The field of pain, and our understanding of its causes, has advanced a great deal since the time of Sherrington. From a historical perspective, we have moved from perceiving it as a test of faith that ought to be endured to the present notion that in itself pain acts as a warning that prevents us from harm unless it becomes maladaptive, persistent and therefore, pathological. This in turn imposed the need for treatments that can either suppress or at least provide temporary relief for pain. In the process of developing therapeutically effective ways of treating pain, knowledge about the nociceptor cell and its projections and integration to the CNS has been gained. We now know a lot about the molecular and cellular bases of how the sensation of pain is detected, transduced, transmitted and eventually, perceived by the individuals. This knowledge has been key to developing pharmacological tools that target specific receptors, ion channels, or signaling pathways that are involved in the genesis and maintenance of pain (be it acute or chronic).

Future treatments should be aimed at taking into account the complex temporal and spatial dynamic of the nociceptor and its molecular players. It is the sum of their expression patterns in specialized neuronal subpopulations plus their regulation by multiple endogenous and exogenous factors (ranging from hormones and cytokines to cold and pressure) that ultimately determines what type of pain we feel, its threshold and duration, as well as its intensity and physical location.

The new generation of pain treatments will most certainly target the cell machinery that synthesizes, assembles, and sorts ion channels and pain receptors to the cell membrane and nerve terminals. It should also contemplate the key role played by trophic factors and genetic determinants in the phenotype of primary sensory neu-

rons, a field of active research which has still to produce a useable drug, despite promising starting points such as monoclonal humanized anti-NGF proteins and others.

Finally, innate protective mechanisms against pain should be preserved and even stimulated as a more natural way of achieving clinically relevant results with the bare minimum of secondary, adverse effects. This will most certainly be achieved by combining selective pharmacological tools with a more holistic therapeutic approach including concomitant physical and psychological therapies.

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Neuropsychological Disorders After Mild Traumatic Brain Injury or Concussion

21

Garazi Bermúdez Vilar and José Vicente Lafuente

Introduction

Mild traumatic brain injury (MTBI) is considered presenting with 13–15 points on the Glasgow Coma Scale (GCS), with or without brief loss of consciousness immediately after impact. *Concussion* is defined as the physical injury to the head resulting with a transient altered mental function for less than 24 h. It is associated with symptoms that are expected to recover within 2–3 weeks. In some cases, concussions can persist longer as one or more symptoms (somatic, cognitive, or behavioral/affective). This syndrome is what we call "*post-concussive syndrome*" (PCS) (1).

A concussion may appear after a head injury and it typically shows impairment of neurological function that resolves spontaneously. Its acute symptoms are believed to reflect functional disturbance of the cerebral tissue, rather than structural changes, as it presents with grossly normal neuroimaging studies (1).

Although it is a well-known pathology, there is little evidence-based knowledge of the underlying mechanisms, and even less of the best management of post-concussive symptoms.

Every head trauma is likely to cause brain injury to some extent (2). In fact, 10 % of MTBI presenting with GCS 15 may show relevant lesions on brain tomography (CT). That percentage might be greater if brain magnetic resonance imaging (MRI) was available at admission, as it can show mild pathological changes. These mild structural lesions could explain the different symptoms comprising post-concussive syndrome, for example, vestibular and visual defects. However, it is likely that small functional changes in biochemical, synaptic, and neuronal membrane processes are involved in persistent symptom complexes, particularly those considered psychological in nature (1).

Physiopathology

Mild head injury is likely to have at least a temporary adverse effect on brain function, mostly in the form of microscopic diffuse axonal injury (Fig. 21.1). However, macroscopic injury such as intracerebral or epi- or subdural bleeds may also occur. Minor head trauma related to neural damage has been documented both in animal (4) and

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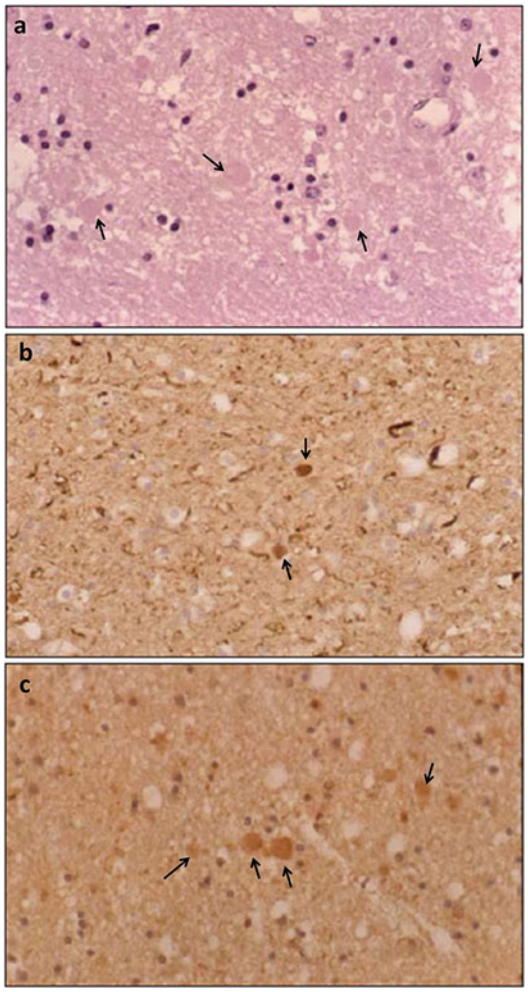

Fig. 21.1 Histopathological changes in diffuse axonal injury: espheroids or retraction balls in injured axons in white matter. (**a**) hematoxylin eosin staining (×75). (**b**) immunohystochemistry for neurofilaments (68 KD). (**c**) antibodies against ubiquitin (×150) (Modified with permission from Jose Luis Palomo Rando. From Lafuente JV. Diffuse axonal injury. Diagnostic importance in forensic neuropathology. Cuadernos de Medicina Forense. 2005;11(41):173–82.) (3)

human studies. First, there are histological studies that have confirmed neuronal changes in patients dying from other causes. Second, functional imaging studies using MRI have shown hypometabolism and hypoperfusion, even beyond 3 months after injury (5). These changes can be seen mostly in prefrontal and temporal brain regions (6).

Impact forces generated by head trauma are of high magnitude and of relatively short duration (5–200 ms). They produce acceleration forces on the head and brain. During the impact, the head's center moves along a straight line (translation) and around its center of gravity (rotation), being the last yet the most important. Macroscopic lesions such as fractures, hematomas, or edema are hardly ever seen (less than 1 %) (7). However, at a later time, that is, after several hours to days/weeks, microscopic and metabolic changes can be observed (Fig. 21.2). These changes underlie both biochemical events that cause neuronal damage and biomechanical events resulting in axonal injury (4).

Biochemical Events

Neuronal damage and death associated with MTBI have been widely studied in the past decades (8,9). However, little has been documented about remote and diffuse neuronal changes after trauma (10,11). Studies have shown evidence of neuronal death, bilaterally, in cerebral sites remote from the impact, primarily within the neocortex, hippocampus (pyramidal layers C1, C2 and C3) (10, 11), and diencephalon, in addition to the striatum, both inferior and superior colliculli and the cerebellum. These cells show ultrastructural changes of both necrosis and apoptosis (10).

Despite evidence of neuronal death far from the impact site, the involved mechanisms are not understood. It is likely that other potentially important factors and mechanisms associated with direct mechanical perturbation and its sequelae may be at work in the pathogenesis of this diffuse necrotic neuronal death. As such, they should be taken into account (10).

At the present time, apoptotic cell death is believed to play a role in delayed neuronal death occurring several hours to weeks after diffuse traumatic brain injury (DTBI). The dominance of proapoptotic factors in the presence of a persistent energy supply results in the activation of cysteine proteases such as caspases that are regulators and effectors of apoptotic cell death (10).

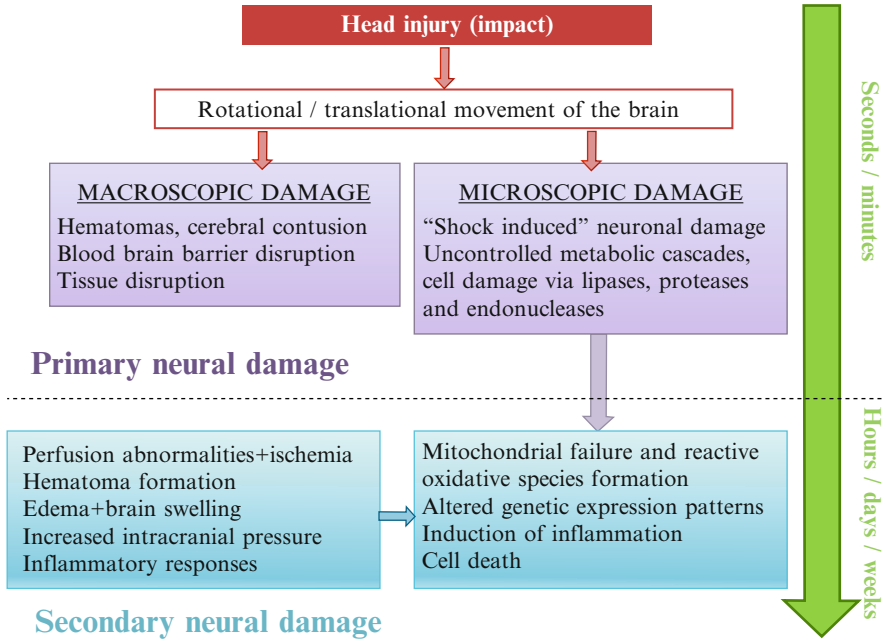

Fig. 21.2 Sequence of different mechanisms that lead to neural damage after traumatic brain injury (Adapted by permission from BMJ Publishing Group Ltd. Anderson T, Heitger M, Macleod AD. From Concussion and mild head injury. Pract Neurol. 2006;6:342–57) (1)

Changes include altered expression of DNA transcription factors and apoptotic proteins, uncontrolled release of proteases, lipases, and endonucleases, with the subsequent oxidative stress. Collectively, these changes cause degradation of cell membrane and cytoskeletal proteins, mitochondrial failure, and production of free radicals. Although both necrosis and apoptosis mechanisms have been well described and different pathways are known to take part in some non-traumatic central nervous system (CNS) pathologies, their specific role and pathways in TBI need still to be studied (10).

Neuronal death in localized areas leads to the release of cytokines and other proinflammatory molecules. This fact induces an inflammatory response, which is believed to be responsible for secondary brain injury. It increases permeability of the blood brain barrier and activates microglia. Enough is not known about these changes occurring after minor injury. There is some evidence suggesting that cellular signaling after neuronal injury and the resultant cytokine cascade, microglial response and cytopathological alterations might lead to amyloidogenesis and accumulation of amyloid beta-peptide (A-beta). A-beta is toxic to neuroglia and plays a major role in neurodegenerative changes following some types of brain injuries such as trauma and stroke. Indeed, damaged axons can become a reservoir for A-beta, contributing to A-beta plaque formation after MTBI. These quiet changes can add up after several concussions and produce brain dysfunction (12). This could for instance, explain cognitive impairment and dementia seen after repeated sports concussion.

Alterations in neurotransmission, both in excitatory and inhibitory systems, may also play a role in long-term deficits in memory and cognition. Among others, long-potentiation, an N-methyl-D-aspartate (NMDA)-dependent measure of plasticity seems to be persistently impaired in the hippocampus after concussion. It also produces changes in choline acetyltranspherase activity and loss of cholinergic neurons in the forebrain. In addition, there is a loss of GABAergic neurons, which can compromise normal inhibition of hippocampal dentate granule cells, facilitating seizures (2).

Finally, as seen with different animal models of brain injury, blood brain barrier (BBB) and its disruption play an important role in secondary neural damage. Deep changes in BBB permeability have been seen around areas of micronecrosis, partially mediated by the release of vascular endothelial growth factor (13, 14). These alterations may accompany other mechanisms occurring after MTBI.

Biomechanical Events

After a brief review of neurochemical cascades triggered by the forces of injury, we shall consider the mechanically induced neuronal death cascades led by direct perturbation of the neuronal cell membrane or its prolongations.

Although ***axotomy-related neuronal somatic changes*** occurring adjacent to sites of traumatic axonal injury had been previously recognized, the relation to any subsequent injury cascades has not yet been appreciated. A recent study using immunohistochemical techniques to recognize amyloid precursor protein (APP), a well-accepted marker of traumatic axonal injury (TAI), identified some neuronal somata directly linked to it in the cerebral cortex, hippocampus, and thalamus. It showed that despite contemporary thought, even in immediate proximity to the neuronal soma, TAI did not result in acute neuronal death. Instead, those neurons revealed reactive changes. These changes included loss and degranulation of the rough endoplasmic reticulum, disaggregation of polysomes, and dispersal of the Golgi apparatus, as well as transient suppression of protein synthesis. Furthermore, there was no cytoskeletal or mitochondrial alteration. In combination these transient reactive changes suggest a potential neuronal attempt at reorganization and repair, rather than the initiation of any prenecrotic/apoptotic change.

Using extracellular tracer infusion techniques, multiple in vitro and in vivo studies have demonstrated that immediately following DTBI, non-axotomized neurons can take up high molecular weight tracers. For example, horseradish peroxidase or other molecular weight dextrans and other molecules normally excluded from the neuronal cytoplasm by the intact cell membrane, are taken up. This immediate tracer uptake suggests that the mechanical force of the injury itself evoked neuronal cell membrane disruption (***mechanoporation***). It is probable that this phenomenon allowed the influx of damaging ions through the compromised cell membrane (11).

Following these initial descriptions, subsequent studies revealed a rather heterogeneous neuronal response to axonal disruption. Some of them showed ultrastructural signs of necrosis while others did not. Furthermore, at the same time and in the same brain foci, different populations of injured neurons did not reveal evidence of membrane disruption. Instead, some of them showed perisomatic axonal injury resulting in increased neuronal somatic APP-positivity. Others exhibited non-axotomy-related induction of heat shock protein expression. After this, the potential resealing of the neuronal membrane leading to its recovery became a possibility to be taken into account (11). By administrating different extracellular tracers at varied times post-injury, in vitro studies revealed that the majority of neurons sustaining mechanoporation could reseal their disrupted membranes in the first few minutes post-TBI. However, in vivo studies did not confirm these observations. Rather, the results suggested that independent of mechanoporation resealing, areas of enduring membrane permeability were present. To further complicate things, recent findings show not only occurrence of potentially delayed membrane resealing, but also of the delayed membrane disruption (10). To date, there are no data that support neuronal death caused only by mechanical disruption.

Although the underlying mechanisms of this delayed membrane disruption are unknown, a sustained elevated intracranial pressure persisting for several hours after TBI could probably contribute to this phenomenon.

In addition to these changes, ***diffuse axonal injury*** has also been a distinguishing feature of traumatic head injury, even MTBI, for some decades (3,8). Contemporary studies have shown that in large part, the traditionally accepted premise of transection and retraction of the

axon is not correct. Rather, it has been seen that the forces of injury diffusely alter focal axonal segments. This results in a local impairment of axonal transport, with progressive local axonal swelling followed by detachment over a post-traumatic course ranging from several hours up to a day. Given the fact that these reactive axonal changes were found in scattered axons related to other intact axons, this precluded the potential for direct mechanical renting. It suggested that more subtle intraaxonal changes were at work in the pathogenesis of progressive axonal disconnection.

Emphasis has been directed on identifying the ***initiating intraaxonal cellular and subcellular factors*** for a better understanding of pathobiology of axonal injury and to develop treatment therapies. While immediate physical transection of the axon cylinder has been ruled out, the potential for focal disturbances in the axolemma leading to local ionic dysregulation was proposed. It was then demonstrated that damage in the mitochondria released cytochrome C. In turn, caspase-mediated spectrin degradation is activated (13). Additionally, degradation of the axonal cytoskeleton associated with concomitant neurofilament side-arm cleavage, neurofilament compaction, and microtubular loss were activated. Therefore, it is generally assumed that these intraaxonal mechanisms lead to an upstream impairment of axonal transport, which results in swelling and disconnection. However, additional recent studies trying to find spatial relation between transport impairment and axonal permeability disturbances have shown no correlation (14).

Therefore, all this research evidence shows the neurofilament-compacted axonal segments and swollen axonal segments demonstrating impaired axonal transport. It suggests that current findings are most likely evidencing the existence of two different populations of injured axons. These populations would respond differently to the traumatic episode. For those segments showing focally altered axolemmal permeability it appears that local calcium dysregulation activates cystine protease and causes local degradation of the axonal cytoskeleton (14). The reason why these sites of axonal injury do not reveal impaired axonal transport and axonal swelling is still unclear. It is conceivable that the suprathreshold calcium uptake occurring at these sites most likely converts anterograde to retrograde transport. Thereby it precludes the development of reactive axonal swelling. This theory is supported by the use of various therapeutic strategies targeting calpain inhibition. Thus, calpain inhibition significantly reduces the number of axonal profiles showing the above described cysteine protease activation and cytoskeletal collapse (15).

In contrast, it appears that those axons showing impaired axonal transport and local swelling do not sustain any alteration of those described previously. Rather, it is posited that other mechanisms are at work and that these are linked to more subtle forms of calcium dysregulation. Activation of micromolar calpains which trigger the activation of calcineurin may be involved. In turn, calcineurin alters the microtubular network to disrupt local axonal transport kinetics and thereby elicits the swellings described above (16). Limited direct evidence exists to support this pathway. However, the use of calcineurin antagonists such as FK506 directly attenuates the number of axons showing impaired axonal transport and swelling. Moreover, these antagonists have no effect on those axons showing neurofilament compaction and disconnection. This supports the premise that calcineurin is integral to the pathogenesis of impaired axonal transport and swelling.

Collectively, these studies illustrate the complexity of the pathogenesis of diffuse axonal injury, suggesting at least two differing types of initiating mechanisms. There is the caveat that both populations of injured axons will likely not be amenable to one form of therapeutic intervention.

Finally, ***other mechanisms*** that might participate in DTBI are being studied. All the changes we have discussed above take into consideration only the myelinated axons. Unmyelinated axons have not received the same attention in the studied CNS pathology. Recently, however, electrophysiological studies have demonstrated damage and dysfunction in unmyelinated nerve fibers within the corpus callosum of traumatically brain-injured animals. They have shown significant and sustained depression of the compound

action potentials (17). It has been seen in microscopical studies that in vitro, non-myelinated neurites subjected to mechanical strains ultimately led to local axonal swelling and disconnection. It occurred without any evidence of axolemmal change or disruption. Rather, the depolarization associated with this injury evoked sodium influx, the activation of voltage gated calcium channels and the concomitant activation of sodium/calcium exchangers, all of which contributed to local intraaxonal calcium overloading. These calcium-mediated changes were linked with the activation of proteases, which in turn contributed to subsequent proteolysis of its NaCh subunit, promoting a persistent elevation in intracellular calcium. This finally resulted in pathological changes through many of the pathways described before. Although this remains to be confirmed in vivo, it would involve a potential channelopathy as major player in the ensuing unmyelinated axonal perturbation.

Additional evidence suggests that altered dendrite structure may underlie the cognitive deficits observed after MTBI. At the cellular level, changes in dendrite structural proteins such as microtubule-associated protein 2 (MAP2) and neurofilament proteins are present in animal models and in autopsy specimens. Some studies show that stretch-induced axonal injury causes transient dendritic swelling, which was sodium-dependent, exacerbated by extracellular calcium removal and blocked by NMDA receptor antagonists. This finding may help us understand the mechanisms of cognitive symptoms after MTBI (18).

Post-concussive Syndrome

Early Symptoms

Post-concussive symptoms sometimes resolve spontaneously and completely within 2–3 weeks after impact. They can also sometimes persist and become disabling, thus requiring further investigation and treatment (19).

Both early intervention by a specialist service and the brief educational intervention have been shown to reduce social restriction and post-concussion symptoms in two randomized controlled trials (4). Additionally, bed rest for several days was no more effective than normal activity immediately after injury in preventing post-concussive syndrome. Taking these results into consideration, we should offer patients at least some education about the expected sequelae and coping strategies after a head injury (1).

Early post-traumatic ***headache*** is common and should be treated with simple analgesics, although these are not always as effective as time (20). Headaches can appear even after 2–3 weeks. Daily analgesia should be avoided to prevent the emergence of rebound headaches. Persisting focal head pain beyond 2 weeks may suggest localized traumatic injury (i.e., fracture, neuralgia, infection) and demand specific investigation or referral. Persistent or later onset post-traumatic tension-type headaches are often associated with neck muscle tenderness on palpation, commonly associated with whiplash, and usually settle spontaneously (1).

Dizziness and unsteadiness with associated ***nausea*** are frequent in the early stage and are usually rather non-specific (1). The origin of these symptoms is uncertain, but they may represent as either peripheral or perhaps more likely as central vestibular pathway dysfunction. Medical treatment of dizziness and vertigo is futile, it possible to treat nausea with antiemetics.

Minor visual complaints such as ***blurred vision*** or difficulties with focusing are common. Diplopia is non-comitant (that is, it changes with gaze direction) and suggests an oculomotor palsy from orbital fracture. If comitant, it can reflect temporary dysfunction of the vergence systems.

Irritability, in the manner of intolerance to light, noise, conversation, and socializing typically occurs in the early post-concussive period. Treatment of irritability should include reduction of exposure to the aggravating stimuli, in addition to rest and avoiding the premature return to full educational or employment activities. ***Insomnia*** with paradoxical daytime somnolence is usual. Benzodiazepines or zopiclone can be used in the short term, avoiding use in the long term because of tolerance (1).

Persisting Symptoms

Persisting post-concussional symptoms 3–6 months following injury may have different causes from the acute symptoms, although the initiating event is the same.

It must be said that the real prevalence of PCS is difficult to determine, as its symptoms may appear in a large percentage of the general population (21) (i.e., patients with depression or chronic pain). Moreover, they could have been present in the traumatized patient prior to the head trauma. Among MTBI patients, 75 % are symptom-free by 3 months after injury, while 5–15 % are still symptomatic at 12 months (21). Over time, there is a tendency to change from physiological or somatic symptoms (headache, dizziness, etc.) to more psychopathological symptoms (anxiety, dysthymia, irritability, etc.). There are some predisposing features that make PCS more likely (1) (Fig. 21.3).

For over a century there has been controversy over an organic or psychogenic origin of PCS (7). It is currently believed that a double insult to the limbic circuitry of the hippocampus could be responsible for the syndrome. First, there is a mechanical insult and second, a maladaptive neuroendocrine stress response reinforces disability. There is an obvious analogy between PCS and chronic pain syndromes (19).

With ***affective symptoms*** patients can experience nervousness and worry, which are inevitable factors in PCS. The anxiety may be reactive or symptomatic of an underlying anxiety disorder. Generalized anxiety, panic attacks, travel phobia, and states of post-traumatic stress can be induced in as many as 20–30 % patients after MTBI (22). Combined cognitive-behavioral psychological interventions, relaxation techniques, and medications may be indicated. Benzodiazepines must be avoided because they enhance cognitive deficits. Overt physiological, cognitive, and affective symptoms of depression are less common than a grumbling dysthymic state, which is more typical and might encourage a 3-month trial on an antidepressant.

Ensuring ***sleep*** is important. Insomnia, accompanied by diurnal fatigue and apathy or hypersomnia may appear. In some cases, medication is needed to treat it.

Another important factor to take into account is the impact of litigation and ***compensation***. Such factors can make patients present with subjective distress following MTBI that seems out of proportion considering the usual severity indicators. However, most studies have failed to find any significant causal link between compensation or litigation and PCS (23).

We usually refer to athletes and sports concussions when discussing the ***neurocognitive consequences*** of MTBI as they are the most common population to suffer from repeated MTBI. There are many studies indicating that there is no or low risk for long-lasting neurocognitive consequence after a single MTBI event (24). Studies show recovery within 7–10 days after injury in most cases despite presenting mild to moderate effects of concussion in the first 24 h on global measures of functioning (i.e., in speed and reaction time), and larger deficits on memory. However, it is noteworthy that the concussed group performed 'less well' than controls on verbal fluency 7 days and 90 days post-concussion and that 10 % of players needed more than a week for symptoms to resolve. Importantly, there was no evidence of 'lingering symptoms' or cognitive impairments at 90 days.

There is, however, preliminary but not confirmed evidence (6) of risk if cumulative damage is exerted by repeated injury (25). Indeed, a history of multiple concussions was associated with lowered performance for divided attention and visuomotor speed, as well as lower efficiency on tasks involving executive functions and attention (24). Another fact to be taken into consideration is age. If injury is exerted in a young person, it has a worst prognostic. Additionally, if trauma is exerted by a great repeated injury within a shorter span of time, it will affect recovery.

There is emerging evidence linking neurocognitive dysfunction to neuroimaging findings post-MTBI. In fact, complicated MTBI showing any intracranial lesion on admission CT scan was associated with worse performance, especially in executive and attentional functions. Patients also performed worse on memory and verbal learning,

	Predisposing Factors	Precipitating Factors	Prepetuating Factors
ORGANIC INFLUENCES	- Prior hrad injury - Female - Age>40 - Low IQ, dementia - Poor education - APOE-4 allele	Severity of head injury: - GCS 13−14 - PTA>1 hour - Intracranial abnormality on imaging - Acute headache, dizziness, nausea - Anosmia - "Double" trauma (intoxication, re-injury prior to recovery) - Serum S-100B	- Psychotropic medication - Analgesic use - Alcohol use - Cannabis use
PSYCHIATRIC INFLUENCES	- Anxiety - Major depression	- Traumatic memories of the event (traumatic memory)	- Anxiety state - Post-traumatic stress disorder - Major depression
PSYCHOLOGICAL INFLUENCES		- Expectation of disability - "Stress" - Inadequate information - Iatrogenic secondary to faild interventions (eg, return to work)	- Deactivation - "Fear" avoidance - Secondary gain (personal, social, or financial gain from the impairments) - Litigation

Fig. 21.3 Predisposing features that facilitate the onset of postconcussional syndrome (Adapted by permission from BMJ Publishing Group Ltd. Anderson T, Heitger M, Macleod AD. From Concussion and mild head injury. Pract Neurol. 2006;6:342–57)

being poorer with speed, attention, and executive functions at 1 month post-injury. However, at 3 months, speed and divided attention were much improved, although not fully recovered. No correlation between neurocognitive symptoms and MRI or single photon emission computed tomography (SPECT) studies has been found (26). The recently developed diffuse tensor imaging MRI, which provides measurements of the integrity of white-matter tracts, might provide interesting information for linking early neurological scan data, neurocognitive dysfunction, and delayed recovery. In addition, functional imaging studies have indicated that there may be differential patterns of activity following concussion (5).

In conclusion, as we have stated previously, biological factors are linked to the outcome of PCS after MTBI, but psychological variables may have a key role to play in genesis and/or maintenance of symptoms.

Assessment, Management, and Outcome

When dealing with MTBI, we must sift through all the possible influences, sorting out the ongoing symptoms, and therapeutically targeting each influence for each of the symptoms. Thus, the clinical history is obviously the best source of information, but a competent biopsychosocial assessment is also necessary. The neurologist's focus should be on identifying organic factors, particularly those that are potentially treatable. The involvement of occupational therapy, physiotherapy, psychology, and psychiatry may all be required. Although there are no systematic guidelines on neuropsychological evaluation after MTBI, it may be of value in those patients showing persistent complaints, being the most relevant cognitive functions to assess attention, concentration, speed of information processing and memory (19).

The particular features of the injury, pre-injury level of functioning, expectations, and anxieties all influence impairment and illness behaviors. The "self" may be shaken. The characteristics of the person and their head are relevant as is the quality of the recuperative environment enjoyed by the patient. Medications (particularly major tranquillizers and benzodiazepines) have been shown in animal studies to impair recovery. The people that "use their brains" for complex and sophisticated functions are more likely to recognize subtle impairments on their brain functions. The rate of spontaneous recovery depends on the individual and on "brain reserve". Not only is each brain injury unique, so too is the patient.

Traditionally, initial GCS score, post-traumatic amnesia, imaging, and neuropsychological testing have been the tools used to test injury severity. However, new approaches have emerged. Biochemical markers in the serum such as S-100 (27), the electroencefalography (EEG) (28), and the presence of functional abnormalities by way of advanced oculomotor screening are currently used, although their value has yet to be proved (29).

Proper evaluation of symptoms is the basis of management. Persisting acute symptoms and subtle cognitive impairment need careful consideration. Medication-induced fatigue, headache or migraines of cervical origin, benign paroxysmal positional vertigo, deteriorated sleep hygiene, alcohol use, and depression may all account for persisting symptoms.

Despite the lack of specificity to PCS, it seems a clinical phenomenon sensitive to measurement as there is considerable consistency in symptoms across a range of PCS checklists and questionnaires. Furthermore, the structure of symptoms in cognitive, emotional, and physical domains is relatively consistent across a variety of studies using different questionnaires and in different populations. The severity of PCS can be measured by using questionnaires such as the Rivermead Post Concussion Symptoms Questionnaire (RPCSQ) (30) or the Standardized Assessment of Concussion (SAC), which addresses orientation, balance and coordination, neurological signs, and delayed memory (30).

The patient can use PCS as a guide to return to everyday life. Symptoms usually increase after physical and cognitive exertion, but settle with rest. Thus, relieving the brain of some of its load may diminish symptom intensity in the early stages, and forcing return to normal functioning can cause the explosive resurgence of acute symptoms (particularly fatigue, irritability, and headache) with adverse psychological consequences. This unpleasant experience sensitizes the individual and creates fear and hesitation for future similar situations. The assistance of occupational therapy may be helpful in gauging the appropriate grade of the resumption of activity (1). Education about the effects, cognitive restructuring techniques, as well as cognitive rehabilitation can also all be helpful in relieving the symptoms .

Assessing outcome is difficult. The resolution of newly acquired motion sickness, the ability to be able to shop in a busy supermarket, and to take the escalator without exacerbation of symptoms usually indicates that spontaneous recovery has occurred. Of course, some PCS-like symptoms may have been present before the head injury. Those who have to use their cognitive abilities to a high level in their employment may take longer to full recovery but, on the other hand, innate intelligence may confound and disguise the recognition of subtle impairments. If symptoms persist at 12 months they may be permanent and influencing them is difficult, and perhaps not possible. The returning of retrograde memory is to be expected in recovery (1).

It is important to remember that "organic" and "psychological" factors tend to coalesce. Unraveling them is difficult and demanding. Patients are often resistant to any consideration of non-organic influences. If PCS persists, psychological and psychiatric factors, whether primary or secondary, are clinically relevant. Referral for assistance to occupational therapy, physiotherapy, psychology, and psychiatric colleagues is generally advisable, ideally around 3–6 months after the injury (1).

When talking about recovery of neurocognitive functions, it may be helpful to think about MTBI as a spectrum disorder with the "dosage" of injury depending on biomechanical factors being important to settle a context for recovery (30).

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Animal Models of Psychopathology and Its Relation to Clinical Practice

22

Amauri Gouveia and Thiago Marques de Brito

Since the mid 1950s, neuroscience has thrived as a set of theories and techniques intending to provide answers regarding the functioning of the nervous system. Anchored to great progress in experimental pharmacology, which at that time aimed to understand the central and peripheral neurochemical functioning, in addition to the major mechanisms of the action of drugs used in the clinical treatment of several psychopathologies (anxiety, depression, schizophrenia), neuroscience has developed and improved many animal models.

These models have contributed and still play a determining role in the comprehension of the major mechanisms involved in developing and maintaining most of the psychopathologies that were identified in clinical reports. Clinical professionals, however, are not always able to understand and make appropriate usage of the data provided by experimental studies to support their patients.

Thus, this chapter has two main goals: (1) To present the main basis underlying the theory of experimental models in psychopathology; (2) To provide a checklist to simplify the understanding of the models by clinical professionals.

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Animal Models in Psychopathology

The understanding of the concept of model is imperative to the science and it is based on a logical construction that differs from the research with an empirical basis from the theory itself, adopting its own steps and ways of generating knowledge. Thus, the concept of a model can be understood as the reduction of a complex fact into an ideal form, a paradigm, which enables being reproduced out of a simplified form, and is also comprised by its major defining elements.

A theoretical model can be better understood using constructs that account for clarifying or reproducing a phenomenon from reality [1]. Hence, a model picks up some variables out of reality and manipulates them so as to explain the variables with solid reproducibility. Therefore, the development of new models is a central activity in science.

In the study of health sciences, the scenario is not diverse because much of the research is conducted based on a complex fact, reproduced under a controlled situation which simulates conditions that are appropriate to the onset of the main elements that define the model, using some model organisms such as rats, mice, fish, dogs, as well as many others. During the process of

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construction of a model, three main elements can be indentified: the *Zeitgeist* (historical context), the punctuality, and the reducibility [2, 3].

The selection of a certain model in a given historical moment is influenced by demands related to the period when this model is developed, in addition to the group of researchers involved with it, restricting their contributions into a particular social focus of observation; this is so called ***Zeitgeist*** (spirit of the period of history). We can similarly assume that a certain model meets the criteria for defining itself as a model of something specific, namely, it is characterized as being the representation of a singular phenomenon in the world. We call this element **punctuality**. During the process of building a model, the choice for some elements that better define the model, ends up implying in a theoretical option for certain variables which are valued and identified as relevant, in detriment to the others. We call this element **reducibility**.

Furthermore, a particular model can also be valued or classified as a "good" or "bad" model, depending on the criteria. Such measurement is generally based on three main criteria: (1) its value in practical use, also known as predictive value; (2) its ability of generating new knowledge, also named construct value; and (3) its similarity with the proposed phenomenon, also called face value.

For our propose, we consider the set of disciplines in which behavior is the object of study as behavioral sciences. Along with psychology, they comprise sociology, anthropology, ecology, a considerable part of zoology, and the information sciences. In these sciences, the use of models for studying several kinds of natural phenomena or conditions simulating a natural phenomena has been widely used. However, for the study of disruptive behavior alterations, or psychopathologies, there is a displacement in the concept of model if compared withother biological and health sciences.

The concept of an animal model in psychopathology is not defined as the way the organism behaves. It is rather the manipulation of a set of variables that may generate a particular state in an organism, which in turn can be useful for studying a particular pathology. This displacement occurs as a result of the difficulty in defining a behavioral disease as such.

The concept of pathology is present with a set of criteria (a syndrome, a predictable time course, or an anatomopathological basis) met by few psychiatric disorders [4–6]. In general, the diagnosis of pathologies in behavioral sciences is restricted to the presented syndrome, given our relative unfamiliarity with anatomopathological alterations and with the time course of the nosological categories used for pathology classification. The Diagnostic and Statistical Manual of Mental Disorders - version IV (DSM-IV) [5, 6] extensively illustrates this.

The seeming fragility depicted by the displacement of a concept of a model based on the animal, to a concept based on the procedure, has allowed and still allows us to use several species in order to research the deviant behavior.

McKinney [7] suggests four questions that should be asked by a researcher, or even by a clinician who wants to evaluate an animal model of psychopathology: (1) Does this model describe the pathology causes and treatments? (2) Are the presented "symptoms" similar to what is observed in the symptomatology of the pathology? (3) Does the pathology and the model share a similar biological substrate? (4) Is it a specific model for a disorder or is it a modeling of human psychopathology aspects?

Mckinney's proposal meets the general requirements for the construction of a model, presenting predictive value and face value, and, furthermore, he introduces a new question regarding the range of the model; namely, does the proposed model meet the criteria for a model of psychopathology, emotional reactivity, or even for a specific pathology?

In order to answer these questions, we need to list which behaviors (responses) of the animals exposed to the model are related to responses emitted by humans. Afterward, it will be necessary to evaluate which stimuli or dimensions of the stimuli are relevant to that animal, exposed to that particular situation.

Such considerations could be associated in a particular acquired knowledge, regarding the basic assumptions that define the psychopathology to be studied, as well as an extensive investigation regarding the ecology and ethology of the animal used in the model for psychopathology.

It is evident that understanding the study of experimental models as it has been approached in this text, implies assuming a biological substrate related to topography and function of the responses emitted by animals when they are exposed to certain situations. This substrate probably has the evolutionary basis relatively preserved along the changes undergone by species during the evolutionary process, so that certain features are similarly exhibited by different species [8, 9].

In general, a model of psychopathology can be defined as a set of stimuli aiming to mimic and measure the aspects considered essential in a determined psychopathological entity. These aspects may be topographic, of process, or even regarding sensitivity to drugs [2, 3, 10, 11], and in general, its description is made using a mixed vocabulary, combining words from different psychological approaches with words from research areas that have an interest in the model, such as pharmacology, ecology, physiology, among others [11].

Thus, before a model is accepted by the scientific community, a validation process occurs. This process consists of (1) describing similarities with the pathologies to be mimicked; (2) evaluating its capacity to react to drugs and other therapeutic manipulations commonly used to treat disorders; (3) evaluating its effectiveness in generating new treatments and management possibilities. The three abovementioned processes of validation are commonly known as face validity, pharmacological validity, and predictive validity, respectively [2]. Finally, apart from the abovecited characteristics of validity, the construction of a model is also deeply affected by social and historical conditions, the *Zeitgeist* [2].

Limits of the Concept of Model

The limits of the models are diverse and widely described in literature [12–14] However, researchers who propose to develop them, or even use existing models, frequently face many problems that end up affecting the validity and applicability of the models. Factors associated with costs of acquiring and keeping the organisms; differences in strains of the same species; sex differences; problems with breeding subjects in captivity; and the need for generating a considerable amount of data for publication (performing research in a shorter time) are some of the reasons why some models are not widespread.

Most of the clinical essays produce statistically fragile data because they have low levels of randomizing and blinding, reduced number of animals, and low ecologic importance. Such aspects lead to generating models that don not reflect realistic conditions and cannot be correlated to pathologies identified and treated in clinics.

A good example of these limits can be shown through two experimental models of depression: (1) The Porsolt **forced swim** test [15], which is a model in which the animal is placed in a tank filled with water and avoid reaching the bottom, so the time until the animal stops swimming and starts to float is recorded (latency). This could be a correlate of anhedonia, displayed by individuals suffering from depressive symptoms. (2) The **tail suspension** test [16], in which the subject is suspended by its tail at an altitude of at least 50 cm, and the immobility time is recorded, which could be also a correlate of anhedonia.

Both tests have a good predictive validity and are sensitive to acute and chronic administration of antidepressants, such as tricyclic antidepressants and selective serotonin (5-HT) reuptake inhibitors that are commonly used in clinics and have increased the latency for floating (forced swim) and decreased immobility time (tail suspension). However, when we refer to any kind of mood disorders, it should be noted that the DSM-IV [5] points out several criteria to be met that are difficult to replicate in the laboratory, particularly in species other than humans.

Such factors end up hindering the detection of behaviors that are presented by animals exposed to these models, and that could be somehow correlated to behaviors presented by a depressive subject. This difficulty ends up affecting its similarity with the pathologic framework of depression, weakening the face validity of these models, and impairing the development of new knowledge regarding the main mechanisms related to evolution and time course of the pathology, as well as the best ways to treat it.

Thus, just as it is necessary to understand and properly interpret an experimental model of a particular psychopathological entity, it is also important to attach great importance to limitations in the model being studied in order to avoid unreal statements.

A Guide to the Reading of Models for Clinical Professionals

What details must a clinical professional notice so as to fully understand a study regarding models? We believe that the clinician must keep in mind the following elements:

(1) A model is not the pathology
Models are theoretical tools used for researching. Although models such as the elevated plus-maze [17] are useful for the study of anxiety, in this model the rat does not suffer from generalized anxiety disorder. However, in this situation, the rat is very sensitive to drugs that act on this pathology.

(2) Models are not determined by theoretical choices
The choice for a model based on operant or respondent behavior, in a naturalistic situation or related to pharmacologic manipulation reflects the knowledge of the person performing the experiment regarding the pathology to be studied. Although this choice provides insight, it is impossible to reduce the pathology into this very same mechanism. This element is an extension of the former element.

(3) Organisms have variations among themselves, according to strain, sex ,or species, so the effect of a drug may vary among them.
The antidepressant drugs used in clinics are mainly tricyclics and selective serotonin reuptake inhibitors. In rodents, they generally produce proactive effects over depressive-like behaviors, when administered in acute doses (a single dose) in several experimental models related to these kinds of psychopathological entities. In contrast, such and effect cannot be found in humans suffering from depressive symptoms, a chronic treatment for at least 3 weeks being necessary in order to obtain positive effects. Therefore, it is necessary to know the physiology of each animal to avoid distortions and mistaken generalizations.

(4) Similarities in form of behavior (topography) do not mean similarities in biological basis.
Whenever threatened, chimpanzees frequently exhibit a behavior of laughing [18]. The same topography is exhibited by humans, however, most of the time it is unrelated to a response toward a threatening stimulus. In our species, laughing often indicates an expression of joy. Thus, the ecology of an animal can favor similar behaviors, but with diverse functions and contexts.

(5) Every scientific study points toward a central tendency for the studied phenomenon
It is not possible to deduce that all organisms will have the very same reaction when exposed to a certain situation. It depends on the ecology, species, or even animals of the same species, but dwelling in different environments may show diverse responses related to learning history or even to inherited genes, expressed or silenced.

(6) Be aware to the characteristics of the proposed method
The same model can be proposed with procedural variations and will provoke changes on its meaning. A good example is given by the elevated plus-maze model. Some studies use this test to measure anxiety-like behaviors with only a single exposure of the animal to the apparatus. However, there are other studies using this test to measure fear, or for some, aversive learning, using a method in which the animals must undergo testing for two consecutive days with the same apparatus [19]. Therefore, understanding the method is essential for the study.

(7)Review the concept of the studied psychopathology in addition of possible biases by the authors toward it.
In general, the authors already refer to these aspects in the introduction. Read them carefully

in order to identify elements that indicate whether the author partially supports a point of view (and the work seeks to confirm it), or whether the study is less biased. Taking such care can improve the use of data from animal research by the clinical professional.

Conclusion

It follows that knowledge of major issues that guide the work of the researcher, as well as their own research, can be useful to the clinical professional, to the extent that they can generate new possibilities for treatments. However, care must be taken with certain generalizations related to experimental studies, because not always the experimental and clinical settings share the same characteristics, and such factor can be determinate for a proper research of the phenomena to be studied.

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Biological Markers in Psychiatry and Its Relation with Translational Approaches: Brief Historical Review

23

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Introduction

The conviction that humoral factors may influence behavior started with the name of these factors. Thus, the humors were conceived as biological elements that changed the mood. The Hippocratic theory of humors is significant at this point. The belief that a balanced presence of these humors was compatible with health (eucrasia) and that an imbalance of them generates disorders (dyscrasias) may appear to be naïve today, but it must be emphasized that these concepts are valid antecedents of modern neuroendocrinology, or, if you will, psychoneuroendocrinology [1, 2].

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This concept implies that there are somatic disorders underlying some psychic disturbances, mainly those known today as "psychosis", that have led to the assumption that these underlying biological disorders can be observed or measured via chemical or physical analysis. The search for biological markers in psychiatry is the main objective of biological psychiatry [3]. Biological markers may be used for detection and prevention of these types of disorders, as well as eventual targets for treatments [4]. The hope to find a perfect biomarker implies that it may lead to confirm the diagnosis, to assess the severity of the disorder, to objectively predict the therapeutic response, and to evaluate and determine a prognosis [5].

The concept of "biomarkers" has two meanings, the state markers and the trait markers. State markers are considered those that are only altered during pathological episode. State markers are indicative of the status of symptoms or clinical situation [6]. These markers are used for experimental approaches, to increase understanding of mechanisms, and, in this way, search for new therapeutics [7]. In some cases, trait and state markers have been applied associated to scales of clinical symptoms [8].

Trait markers are properties of biological and behavioral processes acting as antecedents with a possible causal role in the disease [6], representing a vulnerability index which remains throughout the life pathology [5]. They are used for screening or stratification [7].

The markers have a certain sensitivity and specificity of their own. Sensitivity is the percentage of "true positives" (i.e., the studied patients with the disorder in which the test is altered). In contrast, the specificity denotes the percentage of "true negatives" (i.e., percentage of healthy individuals or persons with another diagnostic in which the test is normal) [5].

Even with the risk of oversimplifying the exhibition, we can divide the recent history of these attempts to create "bookmarks" in three stages: level measurements of peripheral substances, neuroendocrine windows, and central physicochemical studies.

Measurement of Peripheral Chemical Substances

The measurement of peripheral chemical substances is the result of a conception of mental illnesses as a metabolic pathology. One of the first compounds involved was taraxeíne. The findings in blood of schizophrenic patients were discussed, and it was postulated that a schizophrenogenic effect generated from the endogenous substance. For various reasons the hypothesis failed to explain the idea of this illness, but there were still attempts throughout four decades to maintain it [9].

As a result of their structural relationship with LSD, the psychotogenic methylated compounds led to postulate its role in schizophrenic psychoses. The idea that there is a link between schizophrenia and methylated compounds, and the presence of methylated compounds peripherally measured, suggested a link between these high values with schizophrenic psychoses, mainly bufotenine [10–15]. These human experimental approaches led to the study of perceptual distortions [16] and to postulate them as animal models of schizophrenia [17]. The effects of glutamatergic *N*-Methyl-D-aspartate (NMDA) antagonists, psychotogenic drugs, and methylated compounds have subsequently been compared. NMDA antagonists have been linked more with primary symptoms, and the methylated substances with secondary symptoms [18]. Because the first group of these molecules acts on glutamate receptors and the other compounds on serotonin receptors, this relationship has been subject of several studies. Interaction between the two neurotransmitter systems has recently been postulated in a complex receptor which integrates both pathways [19].

The urinary phenylethylamine was also measured in the dawn of biological psychiatry [20–22]. It has recently been described in a family of mammalian trace amine receptors. It has motivated a renewed interest in the physiological role of these compounds [4]. In this area, Argentina made very transcendent contributions. The phenethylamine brain concentration effects

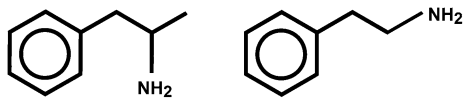

Fig. 23.1 Comparative graphic of the amphetamine and phenethylamine molecules. Note the similarity of the molecular structure

produced by depression inducing agents such as reserpine and antidepressants were experimentally studied [23]. This led to treat depressive symptoms with the precursor D-phenylalanine [24]. Subsequent studies extended the initial findings. They allowed therapeutic treatments that are even currently used to treat depressive disorders [25]. Initial studies in Argentina were replicated in Germany [26]. These evidences strongly suggest a treatment based on a dietary supplement. Recently, in the same direction, we have observed the antidepressant effect of phenylalanine in an animal depression model [27]. It is possible that, given the similarity between amphetamines and phenylethylamine (see Fig. 23.1), it explains the prevalent activator effect of these compounds. The existence of various neurotransmitter circuits associated with depression has recently been proposed, and it has been to use various drugs to mitigate residual symptoms [28, 29].

Other groups have reported a reduction in phenylacetic acid levels measured in urine, plasma, and cerebrospinal fluid (CSF). It has been proposed as a state marker. In the same way, changes in the levels of these compounds in addition to antidepressant treatment have also been suggested as state markers for depression. It has been proposed that an impaired p-tyramine conjugation after a tyramine challenge could be considered an acceptable depression trait marker [30].

Neuroendocrine Windows

Beginning from the idea that mental illness in a strict sense is due to nervous pathways disorders and the fact that it can be studied in the same form that neuroendocrine pathologies are, a neuroendocrine windows model was generated. It was proposed as a way of accessing what happens within the "black box", sending a stimulus and expecting a measurable response. Neuroendocrine research strategies in biological psychiatry began in the 1960s, but they shone in the 1980s.

These neuroendocrine studies started from various experimental observations and from diverse clinical and basic facts [2, 5, 31–33]. There is an overlap or co-activation of the formations involved in the regulation of emotions and hormonal secretion patterns at the level of brain structures. Relations between the limbic system and stress activation patterns are known. These reactions are mediated by the same neurotransmitters, and the relationship between the conventional neurotransmitters and hypothalamic releasing factors have been extensively studied by other teams as well as our group (see Fig. 23.2).

In many cases the hypothalamic releasing factors also have endocrine and behavioral functions and eventually, psychotropic effects [31–33]. In turn, hormones can have obvious psychotropic actions ([34, 35], see Figs. 23.3, 23.4, 23.5, and 23.6). On the other hand, psychotropic drugs can alter hormone levels [38–40].

Endocrine illnesses induce psychiatric disorders. Thus, hypothyroidism can generate a symptomatic depression [41, 42] and, in turn, patients with major depression have a higher risk of hypo- or hyperthyroidism [43]. Finally, psychiatric disorders such as depression, characterized by high levels of cortisol [44], may trigger endocrine disorders such as Cushing disease [45, 46]. In some cases, neuroendocrine tests have been associated with immunological markers, aiming to increase sensitivity using them as state markers [47].

The scope of the relationship between the neurotransmitters and hormone release was one of the biggest contributions of our country. It decisively contributed to correlate the behavior to endocrinology and neurotransmission [48]. From these premises, we postulated some examples about the possibility of scrutinizing the efferent of central circuits involving hormones, using the concept of "windows" [1, 2]. Thus began the study of mental disorders through neuroendocrine tests. After numerous initial attempts, some of them reached the stage of clinical use. They

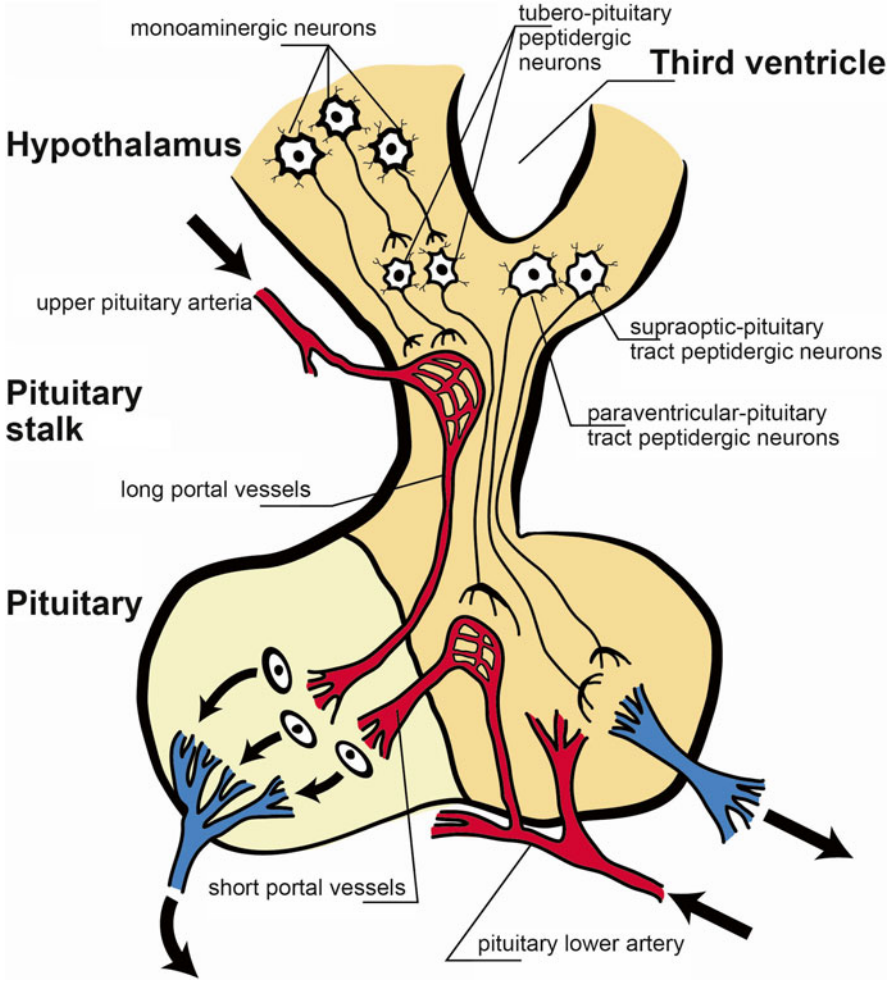

Fig. 23.2 Anatomical and functional scheme of the hypothalamic-pituitary axis. The pituitary stalk stem connects the hypothalamus to the pituitary. Monoaminergic neurons activate releasing factors peptidergic neurons (tubero-pituitary peptidergic neurons), and they exert its action on the adenohypophysis. Peptidergic neurons from supraoptic-pituitary and paraventricular-pituitary tracts project to neuro-pituitary. Note the pituitary lower artery and the vessels emerging form it, the short portal vessels. The long portal vessels are released form the upper pituitary artery [Modified from the Ph.D. Thesis of Prof. Dr. Pascual Angel Gargiulo [1],. and Gargiulo, P.A.: "Psiconeuroendocrinología". En: Vidal, G.; Alarcón, R.D.; Lolas Stepke, F. (Eds.): Enciclopedia Iberoamericana de Psiquiatría. Vol. III: 1376-1386. Editorial Médica Panamericana. ISBN: 950-06-5054-1. Buenos Aires. 1995 [2]. (With permission from Editorial Médica Panamericana)]

were considered "windows" to study some relevant circuitries of the brain, which were the dexamethasone suppression test (DST), thyrotropin releasing hormone (TRH)/ thyroid stimulating hormone (TSH) test, luteinizing hormone releasing hormone (LHRH) and luteinizing hormone (LH) test, and the clonidine test [44, 49–52]. A brief commentary is made at the end about cortisolemia and schizophrenia [8].

Dexamethasone Suppression Test

Among the biological changes in depressive disorder, an overactivity of the hypothalamo-pituitary-adrenocortical (HPA) axis has been signaled. The DST, as a reflection of HPA axis activity, has been the most thoroughly investigated "biological test" in psychiatry to date [53], and it has been proposed as a state marker for

endogenous depression. There are some variables involved in the present interpretation of DST results and its relation to clinical symptoms [54].

The DST was first used in the 1980s beginning with the idea that corticoadrenal hyperfunction is the hormonal profile that better characterizes the depressed patients. It consists of an increase of cortisol in plasma and CSF, alterations in the circadian cycle, and increased urinary excretion of its metabolites [44]. To sensitize the diagnosis the DST was used, it was initially proposed to diagnose Cushing illness [55, 56], but the methodology was modified to adapt to psychiatric illness. Carroll applied a variation of the test to psychiatric patients, finding that a significant percentage of depressed patients had an early cortisol escape to dexametsone [44, 49].

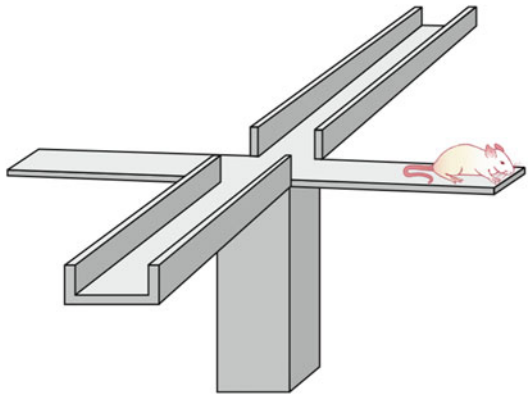

Fig. 23.3 Schematic representation of the plus maze test. The rat is placed on the extreme of the open arm extreme [Modified from the Ph.D. Thesis of Prof. Dr. Pascual Angel Gargiulo. [1], and Gargiulo, P.A.: "Psiconeuroendocrinología". En: Vidal, G.; Alarcón, R.D.; Lolas Stepke, F. (Eds.): Enciclopedia Iberoamericana de Psiquiatría. Vol. III: 1376-1386. Editorial Médica Panamericana. ISBN: 950-06-5054-1. Buenos Aires. 1995 [2]. (With permission from Editorial Médica Panamericana)]

The standardized form of the test consists of an oral intake of dexamethasone (1 mg) at 23 h, and performing two measurements of plasma cortisol at 16 and 23 h the next day [44, 49]. A cortisol level higher than 5 g/dl in one of the samples in this scheme was considered abnormal. According to some studies, it has been proposed in various publications that there should be a degree of specificity of 96 % and sensitivity between 40 and 60 %. Later studies have demonstrated a significant sensitivity in other psychiatric patients, with significant stress factors such as hospitalization, age, and weight loss [57].

Beyond the agreements and disagreements in the interpretation of clinical significance, the positivity of this test suggests a biological treatment, and it is an indicator of poor response to psychotherapy, and lack of response to placebos [58]. In

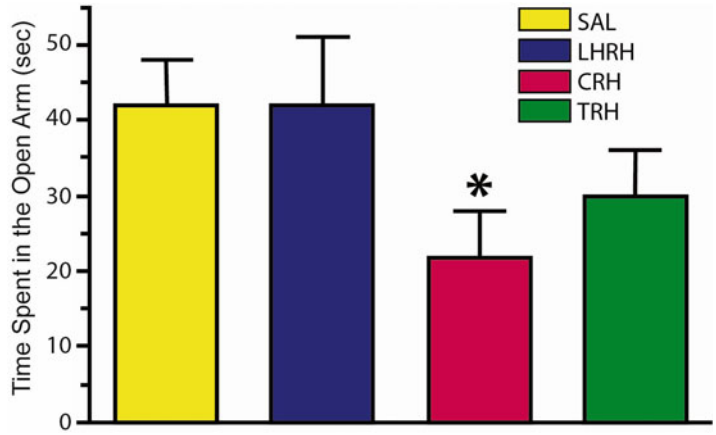

Fig. 23.4 Time spent in the open arm in the plus maze test injecting saline, luteinizing hormone releasing hormone (LHRH), corticotrophin releasing hormone (CRH) and thyrotrophic-releasing hormone (TRH) via the intracerebroventricular (ICV) method. Note the significant decrease in time spent in the open arm induced by CRH [Modified from the Phd Thesis of Prof. Dr. Pascual Angel Gargiulo [1], and from Gargiulo and Donoso [36]. (With permission from The Brazilian Journal of Medical and Biological Research)]

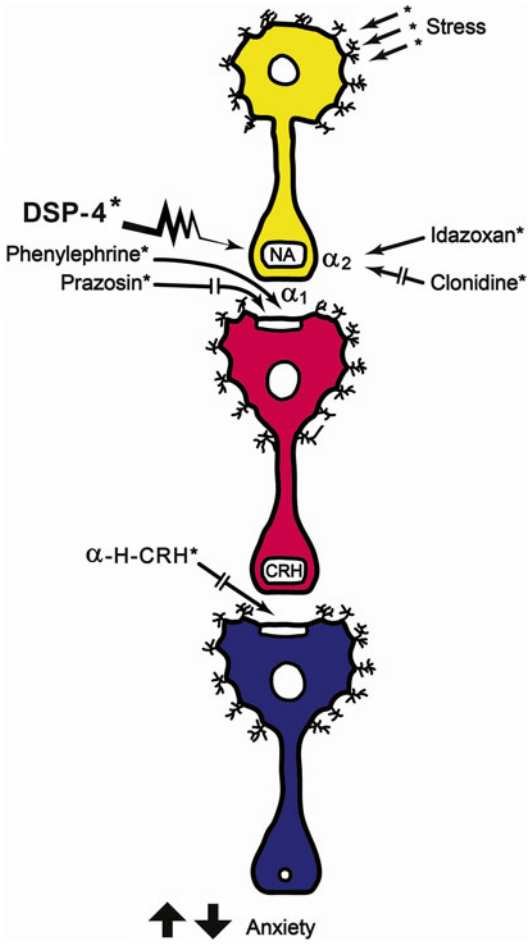

Fig. 23.5 Schematic representation of the findings of Berridge and Dunn [37]. A decrease in exploratory behavior can be seen when stress activates noradrenergic neurons, which could be considered as an anxiety index. The α-2 auto-receptor blockade using idazoxan potentiates the stress response. The stress response was decreased by clonidine (α-2 agonist) and DSP-4 (a noradrenergic selective neurotoxin). It can be concluded that facilitation of noradrenergic response increases stress responses, and the antagonisms decrease them. Postsinaptically, and taking into account the postsynaptic neuron, stimulation of the α-1 receptor of the CRH neuron, the stress response can be elicited. The prazosin blockade of this receptor interfere the stress response. The postsynaptic blockade of CRH actions by the antagonist α–helical-CRH decreases the parameters related to stress response [Modified from the Ph.D. Thesis of Prof. Dr. Pascual Angel Gargiulo [1],. and Gargiulo, P.A.: "Psiconeuroendocrinología". En: Vidal, G.; Alarcón, R.D.; Lolas Stepke, F. (Eds.): Enciclopedia Iberoamericana de Psiquiatría. Vol. III: 1376-1386. Editorial Médica Panamericana. ISBN: 950-06-5054-1. Buenos Aires. 1995 [2]. (With permission from Editorial Médica Panamericana)]

turn, the normalization of the test can be observed before or after clinical improvement, and the continuation of a positive result is considered a factor of poor prognosis linked to suicide risk [59].

It was observed during a clinical trial that transient suppression of HPA function using dexamethasone suppression appears to reduce the exaggerated fear prevalent in patients with posttraumatic stress disorder (PTSD), showing a significant treatment effect in those subjects that was not observed in the control group [60]. It may be related to a negative feed back decreasing corticotrophin releasing hormone (CRH) activity, an anxiogenic factor [36] that can be overactive in this population.

An alternative test has been proposed using a 5 mg dose of prednisolone. The dose generates a partial HPA suppression, proposing assessment of salivary cortisol as a tool to use it in wide samples of psychiatric patients [61]. Prednisolone has additional effects on mineralocorticoid that seems to induce different effects in the same depressed patients and to give different biological and clinical information when compared with dexamethasone in depressed patients groups [62]. It has been proposed that severe treatment resistance in depressed patients is associated with a dysfunctional feedback response when glucocorticoid and mineralocorticoid receptors are simultaneously activated by prednisolone [63].

Another procedure that was derived from several recent studies, is the combined dexamethasone (DEX)/CRH test [64]. In its classic technique or method of implementing the combined dexamethasone (DEX)/CRH test [65] consists of an oral pretreatment with dexamethasone (1.5 mg, 11:00 p.m.), and on the next day, a CRH intravenous bolus (100 μg, 03:00 p.m.). A cortisol response curve is obtained, and it is possible to recognize mood disorder vulnerability and response to stressors [64]. It has been proposed as a marker in depressive disorders, but it has not been adequately studied, and new studies are necessary for this purpose [66]. This test has been proposed as a marker of antidepressant effects [67].

Using this test in normal people, the function of the HPA axis has been studied in conditions of

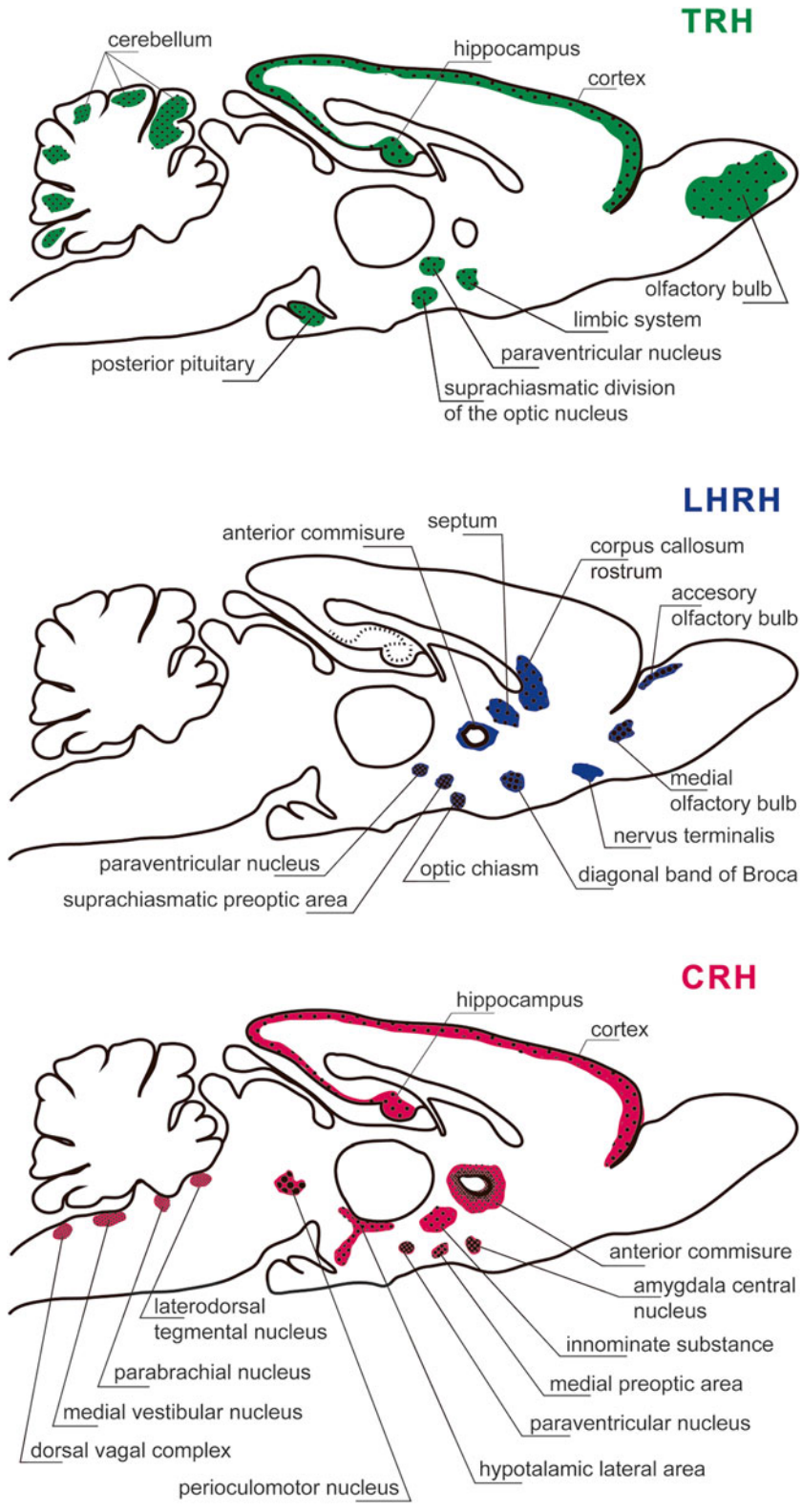

Fig. 23.6 Extrahypothalmic localization of the peptidergic-releasing hormones thyrotrophic releasing hormone (TRH), luteinizing hormone-releasing hormone (LHRH), corticotrophin-releasing hormone (CRH) in hypothalamic and extra-hypothalamic regions. It should be appreciated that TRH neurons are present in the olfactory bulb, cerebral cortex, hippocampus, limbic system, suprachiasmatic division of the optic nucleus, paraventricular

psychological distress and coping styles [68] as well as during sleep. In this case poor sleep is accompanied by increased cortisol response [69]. In other study using the temperament and character inventory (TCI), and evaluating the HPA axis reactivity using the DEX/CRH test, it was observed that some psychological features diverged between incomplete suppressors and enhanced suppressors. This late group showed lower cooperativeness and higher reward dependence as significant predictors of an enhanced suppression [70]. These findings suggest that personality subtypes condition different patterns of cortisol reactivity, turning relative and downplaying a purely biologically interpretation of this test, leading to a detailed consideration of the importance of individual psychological factors in biological findings [70].

TRH/TSH Test

Similar to the HPA axis, the hypothalamo-pituitary-thyroid (HPT) axis has been also investigated in depression. The TRH is a hypothalamic tripeptide, which in humans stimulates the release of prolactin and TSH, and in some cases growth hormone [71]. It was observed that depressed patients had decreased or absent TSH response to TRH [72], making it a new "window" for the study of psychiatric conditions. In its most widespread form, it is performed in the morning, fasting, by the intravenous injection of 200 to 500 mg of TRH. A blood draw is done prior to the injection (T0), and then blood is taken again at 15 and 30 min. On another schedule, periodic blood draws are done completing a 3 h curve. The expected response is a hormonal TSH peak at 30 min. It is considered a pathological response when this peak has a maximum below TSH 5 mUI/ml [50] or 7 mUI/ml [73]. A sensitivity of 40–50 % has been postulated according to the study and the diagnostic criteria used in them [74, 75]. After various studies, this test doesn't allow differentiation between uni- or bipolar depression [51, 76]. The general status, the hormonal status, stress factors, addictions, and psychiatric drugs may have a significant impact on the results.

Most studies have focused on the TSH response to exogenously administered TRH. In those studies, blunted TSH responses have been found in depressives compared with normal controls. However, the frequency of blunted responses in other types of psychiatric patients has made this test marginally useful for differential diagnosis [53]. Additionally, in some cases an exaggerated response of TSH to TRH stimulation in a group of depressed patients with normal basal TSH level has been reported. The value of this test is relative, and limits its validity to some subgroups that must be determined [77]. When a comparison was made between normal controls, agoraphobics, and depressive and panic patients, a significantly higher response was observed in TSH response to TRH in the first two groups, suggesting a lower biological component regarding HPT axis in agoraphobia [78]. Responses to TRH in females appear to be higher when compared with males in both TSH and prolactin (PRL) levels [79].

There is no consensus on whether this test is a marker of status or rank, and it appears that no relationship exists between the abnormality and treatment response [51], although some studies suggest that a persistent alteration appears to be a predictor of relapse and suicide risk [80, 81]. Its use in suicide risk has been a matter of numerous studies with different conclusions.

Fig. 23.6 (continued) nucleus of the hypothalamus, posterior pituitary, and cerebellum. There are LHRH neurons distributed in accessory olfactory bulb, medial olfactory tract, nervus terminalis, diagonal band of Broca, rostrum of the corpus callosum, septum, anterior commissure, optic chiasm, hypothalamic paraventricular, and suprachiasmatic preoptic areas. The CRH neurons are present in the cerebral cortex, hippocampus, anterior commissure, innominate substance, amygdala central nucleus, medial preoptic area, hypothalamic paraventricular nucleus, lateral hypothalamic area, perioculomotor nucleus, laterodorsal tegmental nucleus, parabrachial nucleus, medial vestibular nucleus, and dorsal vagal complex [Modified from the Ph.D. Thesis of Prof. Dr. Pascual Angel Gargiulo [1],. and Gargiulo, P.A.: "Psiconeuroendocrinología". En: Vidal, G.; Alarcón, R.D.; Lolas Stepke, F. (Eds.): Enciclopedia Iberoamericana de Psiquiatría. Vol. III: 1376-1386. Editorial Médica Panamericana. ISBN: 950-06-5054-1. Buenos Aires. 1995 [2]. (With permission from Editorial Médica Panamericana)]

Early studies suggested that neurochemical measures and neuroendocrine tests, independent of clinical diagnoses, may be used with the goal for exploring human aggression and suicide, including the TRH/TSH test [82]. Later, in a larger patient population, studying the possible predictive value of a reduced TSH response to TRH in patients followed along 6 years, it was observed that a smaller group that felt in suicide. They had a lower response than those that did not commit suicide. However, the difference did not reach statistical values, suggesting that this test cannot be used as a predictive parameter for suicide [83]. The hypothesis of a reduced TSH response to TRH was studied in a smaller group of euthyroid primary unipolar depressed female patients. They were tested using the TRH/TSH test while receiving psychiatric treatment. The study showed that the simultaneous presence of a symptomatic constellation integrated by panic, agitation, and suicidality in a depressive state may be correlated with the greatest reduction in TSH response [84].

Recently, the search on the use of this test in suicide risk was continued. In a study performed in a reduced group of male suicide attempters compared with healthy volunteers, no correlation was observed between TSH response to TRH and violent suicidality or a subsequent suicide. However, cerebrospinal homovanillic acid levels were related to TSH response in the suicide attempters group, suggesting a role for a failure of dopaminergic regulatory mechanisms in suicide attempters. Negative correlations were observed between T3 levels and suicide and depression scales used [85]. An association was established between history of suicide and the degrees of HPT axis dysregulation in a large sample of depressed patients [86]. Controversial findings give substance to this topic.

Using a combined registration system, and considering nocturnal hormones secretion and electroencephalogram (EEG), it has been observed that thyrotropin was decreased, and adrenocorticotropin hormone (ACTH) was elevated at initial time of sleep compared with controls, suggesting a negative correlation between HPT and HPA axes. These findings allow using these parameters as a ratio between TSH/ACTH in the first half of the night [87]. This related disturbance has been attributed to an impaired effect of TRH-related corticotropin-release-inhibiting-factor, trying to explain the negative correlation between both systems [87]. However, other groups, using other schedules, did not observe a clear interrelation of abnormalities between the axes (HPT and HPA) in depression [79], and some clinical experimental evidence strongly suggests that different biological dysfunctions could be underlying different markers, such as sleep EEG or hormonal disturbances in depressive patients [88, 89]. Technical differences may explain this contradiction and the phenomenon could be present only in the early state of sleep. Some clinical experimental evidence strongly suggests that different biological dysfunctions could be underlying different markers, such as sleep EEG or hormonal disturbances in depressive patients [88, 89].

This test has been used to evaluate treatment responses. The therapeutic action of electroconvulsive therapy (ECT) in depression appears not to be directly related to its effects on the HPT axis because ECT in depressive patients did not modify the response of the TRH/TSH test. Independent of the acute effect, a delayed effect of ECT on the HPT axis function cannot be excluded [90]. Finally, studies have been done regarding potentiation of antidepressants, and recently, selective serotonin reuptake inhibitors (SSRI) have been included in these clinical trials. In this way, concomitant administration of T3 to nonresponders, SSRI treated depressed patients improved the mood scores, starting from normal values in TSH and TRH/TSH test before T3 addition [91].

The endocrine tests were used also in schizophrenia, where higher basal TSH and GH levels appear to be a predictor of a poor treatment response [92]. However, some parameters could be considered markers of a good response, such as higher T3 levels, blunted TSH response to TRH stimulation, and positive DST [92].

LHRH/LH Test

It has been postulated that major depressive disorder is associated with abnormal regulation of

LH secretion, and it has been argued that this LHRH abnormality can explain the fertility decline in depressive patients [50]. LHRH antagonists induce major depressive and panic attacks in women without depression [93]. However, other studies suggest a preserved normality on the hypothalamic pituitary gonadal (HPG) axis in endogenous depressed patients [94] and in depressed patients in general [95]. This evidence generates reasonable doubts about the exploration of this axis in depressed patients.

Clonidine/Growth Hormone Test

The clonidine test explores the release of growth hormone (GH). GH release is stimulated by dopamine, noradrenaline (α-2 receptors), and possibly serotonin. It has been theorized that noradrenergic inhibition is mediated by β receptors and gamma amino butiric acid (GABA) [96]. Clonidine stimulates GH secretion in normal subjects through α-2 receptors [97]. This test evaluates the response of GH to clonidine, a central agonist of α-2 adrenoceptor agonist. The most widespread technique involves the intravenous injection of 150 mg in 10 min, and GH is measured over 120 min. In normal people a GH peak between 30 and 60 min is expected. A pathological response to GH is considered when this procedure originates a peak below 5 ng/ml. The criterion also includes baseline rates below this level [52, 98]. A decrease of GH response to clonidine in patients with major depression has been reported compared with healthy subjects or minor depression [99–102]. To our knowledge, there are no studies of large groups. Its sensitivity is questioned [103–105]. Nonspecific factors also appear to play into their results [106, 107].

The proposed underlying disorder is reduced by postsynaptic α-2-adrenoceptor sensitivity and responsiveness [100, 108], and it may be accompanied by an increased presynaptic noradrenergic availability [100]. Blunted GH responses to clonidine observed in depressive patients are not attributable to pituitary GH secretion defects [109]. A consensus can be considered in the sense that noradrenergic and dopaminergic neurotransmitter disturbances are present in major depression, and an individual variability appears to be present regarding biochemical anomalies [102]. A study of a large group of patients showed that patients with affective disorders such as major depressive disorder or schizoaffective disorder, showed a blunted response when compared with controls or schizophrenic patients [110].

A decreased response in patients with major depression has been reported[52]. Some findings also support the hypothesis of decreased noradrenergic receptor sensitivity in unmedicated male patients with a nonendogenous major depressive episode [111]. A reduced GH response has been reported in depressive patients when compared with neurotic depressives, schizophrenics, and controls. It has been interpreted as a trait marker or vulnerability factor in endogenous depression, and it would be the case for unipolar or bipolar illnesses.

It has also been proposed as a tool to evaluate differential diagnostics in psychiatry. In this way, it has been reported a study in which normality of HPA axis responses to the Trier Social Stress and GH responses to clonidine were found exclusively in depressed patients. Patients with only anxiety showed a profile with normal HPA responses but the GH response was blunted. An elevated HPA and blunted GH response were observed when comorbidity was present [112]. Concurrent use of biological markers such as rapid eye movements (REM) sleep latency and endocrine tests may improve accuracy of diagnosis [113].

Endocrine status appears to have some influence. Hypercortisolemia, evaluated by DST, appears to not inhibit GH response to apomorphine or clonidine, suggesting that HPA axis in overactive depressed patients does not explain GH abnormal responses in major depressed patients [114]. Additionally, a lower GH response was observed in DST nonsuppressors, suggesting a correlation between positive DST and α-2-adrenoreceptor dysfunction [115]. Evidence suggests that gender and menopausal status are very important in GH test interpretation. In male patients diagnostic groups differed in the GH response to clonidine, but in female patients an additional difference was observed between the premenopausal and postmenopausal state, as was evident in different diagnostic groups [116].

This test is considered as a trait marker of depression because the alteration is maintained after healing [117, 118]. A pathological response does not predict a better outcome by treatment with adrenergic or serotonergic antidepressants, whereas a normal response has been regarded as a good argument for choosing a serotonergic antidepressant [119].

Therapeutics do not modify results. Postsynaptic α-2-adrenergic responsiveness is not enhanced after chronic antidepressant treatment because GH response is not modified [120]. It has been postulated that the effect of antidepressant treatments is mediated by a decrease of sensitivity of the α-2 adrenergic autoreceptor. It was observed in a clinical study using amytriptiline that some related parameters such as plasma levels of norepinephrine metabolite 3-methoxy-4-hydroxyphenethyleneglycol, standing systolic blood pressure, and sedation were induced by treatment. These findings may indicate a subsensitization of α-2 adrenergic autoreceptors. However, no effects were observed in the GH response to clonidine [121].

Cortisolemia in Schizophrenia

While searching for biological markers useable as predictors of therapy efficacy in schizophrenia, it was reported that cortisolemia or its changes using dexamethasone and structural computed tomography parameters may have a high value in this sense. Furthermore, these parameters can be used as tools to measure variables related to vulnerability-stress model of schizophrenia [8].

Central Chemical Studies

In the 1990s, the investigations were focused on the central study of the behavioral effects of pharmacological manipulation of delimited nuclei through experimental stereotactic procedures and its correlation with the findings of brain images. In this way new advances were made using translational and electrophysiological studies, starting in the clinic. The clinical counterpart has made an important number of new techniques that allowed the receptor concentration in brain nuclei and its modification by treatment.

Translational Approaches

Our team, which had projected to behavioral and neuroendocrine research using stereotactic techniques (see above), focused in the decade of the 1990s and the following years on the study of the neural basis of perception, cognition, and anxiety with a translational criteria based mainly in stereotactic access to various brain nuclei. This methodology had significantly lower costs when compared with the imaging studies. It allowed accessing and manipulating neurotransmitter systems in discrete brain areas, assessing its impact on the behavioral and neuroendocrine level. In some cases translational studies were designed beginning with previous clinical studies detecting the area involved in psychiatric disorders, to then generate a model of translational approach, which in turn brought additional pharmacological details to the imaging study. Therefore, we worked in translational models of anxiety, depression, and schizophrenia.

In our translational approaches to anxiety, our lines gave relevant data about various neurotransmitter systems involved in anxiety in different brain areas, including amygdala [33, 122, 123]. Nucleus acumbens septi (NAS) [124, 125] and the NAS-projecting structures such as the amygdala, medial prefrontal cortex, and hippocampus [123] established potential interactions between these structures in modulating anxiety. In that paper we compared our findings with data from neuroimaging in anxiety disorders [123].

We have experimentally addressed both the schizophrenic and the affective psychoses in the psychoses. In our approaches to depression we mainly studied the role of tracer amine precursors as antidepressants, as referred to above [27], with different lines and ongoing clinical translational approaches. In the area of schizophrenic psychoses, we started our clinical studies of perceptual disturbances in schizophrenic patients [126, 127].

This led us to propose schizophrenic psychoses translational models such as models inducing primary symptoms [128, 129], secondary or negative symptoms [125], and cognitive symptoms of schizophrenia [124, 130, 131] acting prevalently on glutamatergic transmission of NAS.

EEG Mapping and Event-Related Potentials

Cerebral biophysics made its most significant and stimulating contributions in the decade of the 1990s from a clinical standpoint [132, 133]. The introduction of evoked potentials (EP) and quantified EEG mapping began a continuing road with emission tomography. The start was linked predominantly to biophysics, but studies of receptors led to a remarkable biochemical refinement. After 35 years of images in psychiatry, it has achieved a remarkable level of spatial resolution, both temporal and molecular [134].

Recent advances in EEG processing and analysis may provide high resolution functional brain imaging with interesting spatial and temporal detailed information [135–137]. These techniques may incorporate relevant data for the study of psychiatric disorders [136]. Because of several clear advantages such as simple use, low cost, and noninvasive acquisition characteristics, it became an interesting research and clinical psychiatric tool, increasingly used as neuroimages, constituting a way to search for future diagnostic biomarkers and not merely an oscilloscope or a spike-counter [135–137]. It has been said that as magnetic encephalography (MEG) is an instrument designed for recording magnetic brain fields, EEG measures electric potential fields [135]. These EEG techniques have been concurrently used with functional magnetic resonance imaging (fMRI) and MEG aiming to obtain a multimodal functional neuroimaging [137]. Some strategies have been proposed with the goal of improving images in a probabilistic mode and delineating algorithms in neurological and psychiatric diseases such as Alzheimer and schizophrenia [138].

The quantified EEG (QEEG) mapping, a sophistication of the EEG, was one of the first techniques used largely in the 1990s in this newly initiated development, starting the imaging studies in psychiatry [139]. This new method was ahead of conventional polygraph allowing the anatomic correlation. Soon numerous studies appeared showing markers in anxiety disorders, affective disorders, schizophrenia, dementia, and other disease entities, and continues at present [140, 141]. Another important study emerged from that initial stage of EP linked to an event (event related potentials, ERP). It's most well known is the detection of auditory stimuli [142]. Cognitive EP and mapping QEEG have enabled interesting and accessible approaches in professional practice [132].

Anxiety Disorders

In anxiety disorders, the QEEG has shown to have utility as a marker, and has shown instability in the levels of cortical "arousal", perceptible in the register [143]. Also common to most anxiety disorders are specific difficulties in the sensory input conditions and the allocation and utilization of attention, which is evident in EP and ERP [144]. These alterations can be observed in all manifestations of anxiety and distress, such as obsessive-compulsive disorder, generalized anxiety disorder, panic attack, phobias, and PTSD as prevalent anxiety manifestations [143].

Affective Disorders

In affective disorders, the QEEG has been proposed as a selection criterion for selecting the antidepressant drug [144–146]. Comparing antidepressant drugs, it has been proposed that it is important to consider gender in the studies of drug treatments. It was proposed in the same study that the higher sigma frequency range of non-REM sleep and REM density, registered during the sleep EEG, could be used as markers of drug efficacy [147].

A combined approach using sleep EEG and hormonal secretion patterns has been tried, measuring testosterone and cortisol in depressive patients. The patients were diagnosed with major depressive disorder, and values were considered during the illness and after remission. A blunted testosterone level and an increase in cortisol was observed, suggesting an interaction between both axes involved in this interaction (hypothalamic-pituitary-gonadal and limbic-hypothalamic-pituitary-adrenocortical axis) [148]. The overactivity of the HPA axis is a well-known fact [53]. This phenomenon could be explained starting from previous basic experiments [149, 150]. CRH injected within medial preoptic area (mPOA) decreases plasma LH levels in rats, not modifying follicle-stimulating hormone levels [149]. The mPOA and the hypothalamic arcuate nucleus are involved in this regulation [148, 149]. Stressors may activate HPA axis and suppress the HPG axis, inducing an antireproductive effect [148, 149].

Some reports have focused on cognitive functions for both schizophrenia and bipolar disorder, aiming to identify phenotypes and even common markers [151, 152]. It has been reported that relatives of people with depressive disorder showed an increased activation of brain-related zones during a verbal working memory task, registered by functional MRI (lateral occipital cortex, superior temporal cortex, and superior parietal cortex) [153].

Schizophrenia

Anatomical Images

Anatomical images have been used as markers in schizophrenia. Hippocampal volume loss has been reported in early schizophrenia, is not shared by healthy siblings, and appears to be related to the course of the illness, as an important intermediate phenotype of the illness, and proposed as trait markers [154]. Using a mix of images and neurological checking, smaller left dorsolateral prefrontal cortex volume and some exaltation of primitive reflexes at baseline may be useful tools to predict enhanced negative symptoms, suggesting that neurological soft signs could be clinically used as a mean to evaluate prognosis of schizophrenic patients [155].

Cognitive Functions

From our earlier work on the subject the importance of perceptual distortions in schizophrenia has been increasingly valued [126]. Particular deficits in masking tasks in schizophrenic patients have been reported in experimental approaches. These deficits consist of short-term visual stimuli followed by masking stimuli. This latter stimulus interferes with the perception, and this difficulty is higher in schizophrenic patients. This problem has been attributed to deficits in cholinergic transmission center [156]. It is also correlated to auditory perception deficits of schizophrenic patients with deficits in the consistency of high and low bands of gamma activity, postulating that disconnection would relate to the processing of auditory stimuli [157]. Auditory hallucinations have been studied with various strategies [158], mainly by combining data from EEG and MEG. Research has shown a difficulty with interregional connections between the activity of the frontal and temporal lobe, among other phenomena [158]. Perception performance has also been used in schizophrenia as a functional marker. It has been suggested that certain visual deficient functions in schizophrenia, can be used in this manner. The goal is to establish trait or state markers for schizophrenia. It has been reported that motion integration is dysfunctional only in schizophrenics, and not in their relatives or bipolar patients. However, motion discrimination appears to be dysfunctional in schizophrenics and in their relatives, as well as being normal in bipolar patients [6]. These findings have relevant value because they allow the distinguishing of trait markers from transient state markers in schizophrenic patients using visual processes as markers [6]. Because abnormalities in visual scan paths have been reported in schizophrenic patients, scan path measures have been postulated as trait markers for schizophrenia [159].

Other groups have proposed relatively specific olfactory identification and spatial working memory deficits as markers that existed previously to illness onset. It has been suggested that they may be more potent as trait markers for psychosis than other cognitively tasks (i.e., verbal memory). A progressive declination could be expected with illness, the progressive steps of the illness, and have been proposed as states relative to trait factors [160].

With the goal of establishing stable trait markers for schizophrenia, an important number of studies have been displayed searching for neurocognitive deficits. It has been proposed that they may be detected before the initial manifestations of the illness, and could be present in the relatives of schizophrenic patients [161]. These premorbid cognitive deficits may be conditioned by brain abnormalities, giving the necessary basis for presentation of the illness, and the possibility of an early detection of the problem, mainly during adolescence, which is considered the age of maximal vulnerability for schizophrenia [161]. Studies pointing to other functions such as measures of attention regulation, working memory, episodic memory, and emotion processing have been proposed as tools to identify phenotypes with cognitive disturbances related to schizophrenia and bipolar disorder [151]. There has been reported evidence about attentional and executive impairments in patients with schizophrenia and in their unaffected first-degree relatives. It has also been theorized that the performance of bipolar patients does not significantly differ from that of schizophrenic patients. Using a neuropsychological battery, it has been observed that both groups and their corresponding relatives showed a marked deficit in time execution in the Stroop test when compared with healthy controls. These findings suggest the possibility of transnosographical markers for a shared familial vulnerability common to schizophrenia and bipolar disorders [152].

EEG Mapping and ERP

Interest in the biomarkers for abnormal parameters detection using QEEG is a current issue in schizophrenic psychoses, and the idea is that the modification of these biomarkers is a "target" for possible treatments [162]. In the same sense, and given the relationship between cannabinoids and psychosis, some approaches have tried to establish links and relationships. These psychoses-inducting effects exerted by cannabinoids, and the relationship between cannabinoids and schizophrenic psychoses, have inspired these studies. These drugs exacerbate the positive, negative ,and cognitive symptoms of schizophrenia [163]. Following these ideas, some studies have explored the effects of acute and chronic use of cannabinoids using EP and related techniques [163].

Other studies, using numerous electrophysiological variables such as QEEG and ERP, have attempted to establish markers of positive and negative symptoms of schizophrenia, evaluated in scales and specific neuropsychological profiles [164]. This has led to suggest, even at the risk of oversimplifying the proposition, a relationship between negative symptoms and frontostriatal dysfunction or deficit. On the other hand, positive symptoms would be related to the dominant temporal lobe [164].

ERP are techniques that has also been used in psychiatry. First, it has been observed that aging leads to an increase in latency of P-300 [165]. In major depression, cognitive EP have been proposed as markers of suicide risk [166]. Attentional and cognitive problems in major depressive disorder appear to have a translation through a difficulty to discern significant stimuli from the environment. The mechanism by which this occurs has been associated with disorders of sensory processing (P200) and additionally with impaired context processing (N200/p300 complex). This could be an explanation for the attentional impairment observed in cases of more severe depression [167].

One of the most robust findings, reproducible and consistent in biological psychiatry, is the amplitude reduction of the P-300 wave in schizophrenic patients which allows it to sustain as the more trustworthy biomarker of disease [168, 169]. The fact that different brain generators can contribute to EEG findings has led to studies of correlation between hemodynamic changes studied by fMRI and electrophysiological studies [169].

While searching for trait markers in schizophrenia, it was observed that a P300 reduction is present in schizophrenics and non-psychotic siblings in different schedules [170]. Temporoparietal P300 amplitude reduction and frontal P300 amplitude increase seem to be quantitative phenotypes associated with increased risk of schizophrenia. Both measures can be useful for increasing the statistical power of genetic studies of schizophrenia [171]. Other approaches related to P300 wave have been used in schizophrenic patients. It has been postulated that when it is induced by a crying-face, it may be used as state marker. When it is induced by a smiling-face, it may be used as trait marker during recovery [172]. It has been studied in schizophrenic patients that auditory P300 is affected by both arousal and emotion, and a significant negative correlation was found between P300 amplitude and the values of negative symptom scores. It was concluded that P300 amplitude and area can be considered state markers with an aim to measure parameters modified by recovery in schizophrenic patients. It was concluded that the observed effects were mediated by attention and emotion levels [173]. Using a frontotemporal event-related EEG coherence as a measure of functional connectivity, an impaired interaction of the frontotemporal macro-circuit indirectly reflects genetically determined abnormalities of frontal and temporoparietal microcircuits, and these abnormalities in frontotemporal connectivity have been proposed as trait markers of genetic risk for schizophrenia [174].

The constancy of the P300 reduction in schizophrenia has served to give consistency to diagnose it and to sustain its biological condition, starting from the reality of the marker [168]. It has been stated that this finding is a state and trait marker, and appears to be sensitive to the course of the disease, interacting with persistent negative symptoms, decreased attention, and underlying brain changes [168]. In experimental studies of perception of own movements associated with visual event-related potential, it has been observed that schizophrenic patients have difficulty in recognizing their own movements, and that this is accompanied by changes in the ERP [175]. The risk of suffering schizophrenia has been also attempted to be measured using ERP [176, 177]. The electrophysiological patterns have been used to study acute and chronic effects of ketamine, linking in these schemes the first with acute psychoses and the second with cognitive deficits; they have been related to electrophysiological disorders in translational models [178]. Other approaches, carried also using EP, allowed to find multisensorial processing deficits in schizophrenic patients, suggesting an important role for this alteration in schizophrenia [179].

Dementia

Quantitative EEG coherence, established between brain areas, mainly linked by fascicles (fascicle coherence), and establishing connections through cortico-cortical fibers, appears to be differentially compromised in subjects with Alzheimer disease. Oppositely, the so called "visual coherence" established by short cortico-cortical and cortico-subcortical fibers, mainly in postcentral areas, appears to be compromised in patients with multi-infarct dementia [180]. Visual coherence demonstrated stability in both demented groups, but the so-called "fascicle coherence" appears to show stability in patients with multi-infarct dementia and even in control subjects. A relevant variability appears to be present in patients with Alzheimer disease. It strongly suggests that both state and trait factors can be involved. These findings strongly suggest that decreases in coherence can be considered as diagnostic trait markers for both dementia states [180].

New Techniques

Positron Emission Tomography, Single Positron Emission Computed Tomography, and Magnetoencephalography

Many findings have pointed out the utility of positron emission tomography (PET) and single positron emission computed tomography (SPECT) in

psychiatric disorders being of particular use in studying the pathophysiology underlying schizophrenia, depression and dementia, and the mechanism of action of drugs used in the first two illnesses [181]. In dementia, important progress has been made in the development of ligands that bind to amyloid [181]. In depressions, searching markers determining treatment-resistant depression, the interest has been focused on MRI and PET [3].

MEG has emerged as an interesting method in psychiatry [182–184]. The registered elements of MEG are extracranial magnetic fields produced by the brain arising from intraneuronal ionic current flows originating in cortical pyramidal cells. The possibility of identifying frequency band patterns and topographic organizations has been proposed with this method [184]. This technique may complement other electrophysiological recordings. The record of brain surface sources predominates, and is remarkable because it captures very high frequencies [183]. In psychiatry, these studies have improved understanding of the lateralization abnormalities in psychotic patients, correlating changes in gamma band abnormalities in the P-50, and have provided evidence of impaired hemispheric abnormalities in auditory short-term memory [183]. Temporal lobe theta activity and schizophrenic symptoms have also been correlated [184].

Finally, as previously anticipated, neuroimaging has demonstrated a remarkable impact in the explanation of the causes of psychoses, particularly in the underlying biochemical disorders [185]. Thus, in the field of schizophrenia, it has been possible to provide highly relevant findings in the determination of receptors and neurotransmitters in the brain of patients with schizophrenia using PET and SPECT, confirming previous studies in post-mortem tissue, ruling out the possibility that these effects were merely artifacts caused by medication or processing of samples [185]. The PET and SPECT studies have helped to confirm, via additional methods, the role of dopamine in the schizophrenic disorder and its relevance in therapy [185, 186].

Conclusions About the Clinical Use of Markers in Psychiatry

A variety of approaches to the study of psychiatric diseases have appeared in recent years. The translational research has produced important contributions to the treatment of these illnesses and the interpretation of the markers. New markers initially emerged from biophysical and electrophysiological techniques, later introducing new techniques that have allowed registering brain receptors in vivo and opening the field to biochemical research. In all individual cases, as we previously mentioned when we developed corresponding ideas describing neuroendocrine windows, personality in relation to biological findings must be taken into account. The practical application of the findings of biological psychiatry markers often has an individual meaning which requires adjusting the interpretation.

The use of concomitant psychological scales can be a tool for correlation between desirable and promising objective variables. The Minnesota Multiphasic Personality Inventory is an interesting tool used to detect psychopathological symptoms and its scores, which can be correlated with neuropsychological functions, psychopathological scales, or biological parameters as a promissory line for the future [187–190].

Recent findings have shown potential correlation between functions such as semantic processing brain areas against different types of distractors [191]. Human communication has also been the subject of studies using last generation methodology [192], and empathy has been studied in schizophrenic patients in a similar way [193]. We have come a long way since our first experimental approaches [1]. All these new findings promise surprising changes in our knowledge of normal and pathological central nervous system functions.

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24 'Two Hit' Neurodevelopmental Mechanisms in Schizophrenia: Focus on Animal Models and the Role of BDNF

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Schizophrenia

Schizophrenia is a severe mental illness that affects about 1 % of the population worldwide with the annual incidence rate around 0.2–0.4 per 1,000 [1]. Rates are similar across countries, cultures and sexes, although women tend to have a later age of onset and a better outcome prognosis [2–4]. The age of onset is typically between the ages of 16 and 30 years and, once schizophrenia has developed, impairments are usually present throughout most of life [1]. When looking at costs of care per patient, psychotic disorders are considered to be the most expensive mental illnesses accounting for 1.5–3 % of total national health care expenditures in many countries [5]. According to the World Health Report (2001), schizophrenia is the eighth leading cause of disability worldwide in the age group of 15–44 years [6]. In addition to the financial burden, schizophrenia has a large impact on families and communities, and of course the schizophrenic individual.

The symptoms of schizophrenia can be classified into three broad categories: positive symptoms, negative symptoms, and cognitive symptoms. Positive symptoms reflect the presence of distinctly abnormal behaviors that are not seen in healthy people. They include hallucinations (mostly auditory), where people hear, see, smell, or feel something that is not real; delusions, which are false beliefs that are strongly held in spite of invalidating evidence; and disturbed thought, where the patient can not organize his or her thoughts or connect them logically, leading to disorganized speech [7, 8]. Negative symptoms include avolition, the lack of motivation; anhedonia, the inability to experience pleasure; blunted affect; asociality; and alogia, poverty of speech. These symptoms often occur during times when positive symptoms are absent or low and are the least likely symptoms to improve over the course of the illness because treatment efficacy is limited [7–9].

Cognitive symptoms are not used to diagnose schizophrenia but are nonetheless very common

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and have strong adverse effects on daily life and are usually associated with poorer outcome of the disease [10]. Cognitive symptoms include memory problems, slow thinking, difficulty in understanding, poor concentration and poor executive functioning [7, 11] and they appear to be present during all stages of the illness [12] and seem to remain stable over time [13]. While antipsychotics are usually able to treat the positive symptoms of schizophrenia, they only seem to have a modest effect on the cognitive symptoms [14].

Underlying Brain Mechanisms in Schizophrenia

One of the earliest findings that has often been replicated, showed that individuals with schizophrenia have enlarged ventricles when compared to healthy controls [15]. Magnetic resonance imaging (MRI) studies have replicated these findings and revealed additional changes such as decreased frontal lobe, thalamus and whole brain volume [16–18]. Furthermore, one of the most affected regions in schizophrenia is the hippocampus which has been shown to be smaller in patients with schizophrenia [19, 20]. Most of these deficits are already seen in patients with first-episode psychosis [21, 22] indicating that these brain changes are not caused by a chronic disease state but may precede the onset of schizophrenia.

In addition to structural changes, functional abnormalities have also been observed in schizophrenia. One of the most consistent findings is a reduction in dorsolateral prefrontal cortex (DLPFC) activity especially during the performance of working memory tasks [23]. Similarly, reduced activation in the hippocampus during recognition tasks has been observed in schizophrenic patients when compared with healthy controls [24, 25]. However, despite a number of gross structural and functional abnormalities seen in schizophrenia, none of these are specific to the schizophrenic brain or even present in all schizophrenic patients and cannot be used to diagnose or predict schizophrenia. Attention has therefore shifted to the cellular level.

Post-mortem studies have found a number of abnormalities including increased neuronal density and smaller neuronal size as well as reduction of synaptic markers in brain areas such as the hippocampus, DLPFC, thalamus, and striatum [26]. Overall, studies have shown that a number of brain abnormalities are involved in the development of schizophrenia linking the disease not only to one brain region but rather to altered neural circuits within the brain.

The neurotransmitter most prominently implicated in schizophrenia is dopamine. The classical dopamine hypothesis proposed that hyperactivity in the dopaminergic system was responsible for schizophrenic symptoms. This was based on two findings: 1) drugs that increase dopamine activity (such as amphetamine) induce symptoms that resemble psychotic episodes and worsen psychotic symptoms in schizophrenic patients, and 2) antipsychotic drugs used to reduce symptoms of schizophrenia all block dopamine D2 receptors to some extent [27]. However, over the years it was shown that this hypothesis did not account for the negative and cognitive deficits observed in schizophrenia and rather that these were associated with low dopamine activity in the prefrontal cortex (PFC) [28]. As a result, the reformulated dopamine hypothesis proposes an imbalance in dopamine activity with hyperactivity in the subcortical mesolimbic regions (accounting for positive symptoms) and hypoactive dopaminergic mesocortical projections to the PFC (accounting for negative and cognitive symptoms) [28]. Post-mortem studies using functional MRI or single-photon emission computed tomography have further yielded evidence that implicates the dopaminergic system in schizophrenia. In patients with schizophrenia, dopamine transmission is enhanced to a greater extent than in healthy controls after a challenge with amphetamine [29–31] and baseline occupancy of D2 receptors is higher than in control subjects [32]. These changes are all associated with the positive symptoms of schizophrenia while cognitive symptoms have been associated with changes in prefrontal dopamine transmission and the D1 receptor [33].

A role for glutamate in schizophrenia is suggested by the observation that drugs such as phencyclidine and ketamine can induce schizophrenia-like symptoms in normal controls. These drugs function by blocking the N-methyl-D-aspartate (NMDA) receptor, one of the main glutamate receptors [34]. These findings have led to the suggestion of a glutamatergic dysfunction in schizophrenia and indeed investigators have found abnormalities in schizophrenia brains that could lead to hypofunctioning of the NMDA receptor [35, 36]. Importantly, the glutamatergic system interacts with the dopaminergic system [37] indicating that a role of NMDA receptors in schizophrenia does not negate the dopamine hypothesis but rather implies a combined hypothesis.

GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter in the brain and multiple studies have shown alterations in the GABA system of individuals with schizophrenia (for review see [38]). For example, decreased expression of the mRNA for the 67 kDa isoform of the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD67), has been found in the prefrontal cortex as well as in the hippocampus of patients with schizophrenia [39–41] and this seems to be particularly pronounced in a subset of GABA neurons that contain the calcium-binding protein parvalbumin [42]. Other neurotransmitters that have been implicated in schizophrenia include serotonin [43] and acetylcholine [44].

Treatment of Schizophrenia

Despite years of research, there is currently no treatment for schizophrenia that is 100 % effective. Antipsychotic medication was first introduced in the 1950s and can be divided into two classes: typical (known as first generation) and atypical (known as second generation) antipsychotics. All of the drugs reduce dopamine activity by blocking dopamine receptors but differ in their side-effect profiles. Typical antipsychotics such as chlorpromazine and haloperidol are effective in treating mainly the positive symptoms but induce unfavorable side effects such as extrapyramidal symptoms and tardive dyskinesia [7, 8, 45]. Atypical antipsychotics also act on dopamine receptors but additionally affect multiple other systems including serotonin and cholinergic and muscarinic receptors [46]. These newer atypical antipsychotics have mainly replaced the older typical antipsychotics because they have a reduced risk of causing extrapyramidal symptoms or tardive dyskinesia. However, these drugs are not without side effects either as they can lead to weight gain, hyperglycemia, and in the case of clozapine, to agranulocytosis [7, 45, 46]. Compared with typical antipsychotics, atypical antipsychotics are believed to be more effective in the treatment of cognitive deficits [14, 47–49] but these effects seem to be minimal and not all studies could replicate these findings [50–52]. Because cognitive dysfunction is a major predictor for clinical outcome, it is therefore important to find better treatment strategies that can target the cognitive deficits as well as the other symptoms associated with schizophrenia. However, while developing new drugs and treatment strategies is important, the main goal would be to prevent the disease in the first place. This can only be done by gaining as much information as possible about mechanisms involved in schizophrenia development which may lead to the discovery of new treatment targets.

Etiology of Schizophrenia

There is substantial evidence from family, adoption, and twin studies that a genetic component plays a major role in the development of schizophrenia. The risk of developing schizophrenia is higher among relatives of patients than in the general population. This risk increases with the number of family members affected to nearly 50 % when both parents are affected [53] and to around 80 % when a monozygotic twin is affected [54]. Numerous genes such as catechol-O-methyl transferase (COMT), neuregulin 1 (NRG1), and disrupted-in-schizophrenia (DISC)1 have been associated with schizophrenia (reviewed in [55]). However, it is clear that the genetic transmission

does not follow a simple single-gene Mendelian pattern. To date, none of the genes associated with schizophrenia have been found to be the single cause for the disease and it is more likely that multiple genes are affected and that these interact with environmental factors.

Environmental risk factors can be of biological or psychosocial background. However, it is not clear how and when these risk factors affect the disease process. Some of the events may occur during the early stages of life (prenatal or early childhood) while others may occur later on in life (e.g., during adolescence). Among the suggested early risk factors are obstetric complications, viral infection during pregnancy [56, 57], prenatal exposure to famine [58], or stressful events such as the loss of a parent [59] or separation from the mother [60]. Several studies have shown that childhood abuse can significantly increase the risk for psychosis and schizophrenia and in particular hallucinations [61–64]. In addition to early environmental risk factors that can increase the risk for schizophrenia in later life, social factors also seem to play a role [65].

Individuals with schizophrenia may not experience higher levels of stressful events than the general population but they seem to be more vulnerable toward them [66–68]. Stress exposure worsens schizophrenic symptoms and can lead to a relapse in schizophrenic patients [66, 67]. Furthermore, there is evidence which implies that the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the stress response, can be altered in patients with schizophrenia [66, 67]. People with first-episode psychosis have been shown to have an enlarged volume of the pituitary which is associated with heightened HPA activity [69]. Furthermore, larger pituitary volume in a group of individuals with ultra-high risk of developing schizophrenia was significantly associated with transition to psychosis indicating that HPA hyperactivity may play a role in the development of schizophrenia [70]. Another risk factor is substance abuse. Multiple studies have shown that people with schizophrenia commonly use drugs and that consumption is more frequent than in the rest of the population [71–73].

The 'Two Hit' Hypothesis of Schizophrenia

The interplay of multiple developmental factors in schizophrenia development has led to the introduction of the 'two hit' hypothesis which proposes that two or more major disruptions at specific time points during development are responsible for the illness. A first 'hit' during early brain development increases the vulnerability to develop a mental illness and a second 'hit' later on in life will then trigger the onset. Epidemiological studies have provided evidence that individuals who later on develop schizophrenia have problems in motor development, social skill and language expression during childhood [75–77]. However, these early neurodevelopmental insults do not by themselves cause the illness as shown by the long delay until the onset of first symptoms, occurring most often at an adolescent age [78]. Thus, the disruption at early age increases the risk of developing schizophrenia but does not necessarily lead to it. In this scenario, factors causing the abnormal childhood behavior are risk factors which, together with social adversity or drug abuse later on in life, can trigger the onset of psychosis [79]. This model has been described in the literature as the 'two hit' hypothesis or 'two hit' model [80–82] (See Fig. 24.1).

Bayer and colleagues proposed that the first 'hit' is genetic predisposition, with the affected individual carrying a mutant candidate gene, which is involved in brain development and maturation. The second 'hit' is environmental and will then modulate effects of the mutant gene inducing schizophrenia [82, 83]. Evidence for this hypothesis comes from a study by Caspi and colleagues [84], where they were able to show that individuals homozygous for the COMT valine (Val) allele (leading to increased COMT activity) were ten times more likely to exhibit psychotic symptoms if they used cannabis during adolescence while there was no such increased risk for carriers of the methionine (Met) allele. Similarly, carriers of the Val allele showed an increase in hallucinations after exposure to cannabis [85] and unrelated to cannabis exposure were more prone

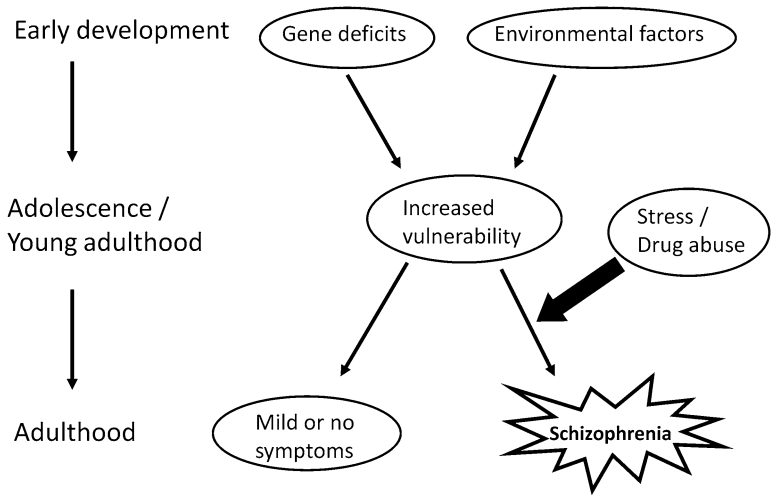

Fig. 24.1 Depiction of the 'two hit' hypothesis of schizophrenia: Early developmental disruptions such as gene deficits and/or environmental factors can increase the vulnerability of an individual to develop schizophrenia. Late environmental disruptions such as stressful life events or drug abuse during adolescence or young adulthood can then trigger the onset of schizophrenia in these at-risk individuals while in the absence of these factors the disease will not develop [74]

toward the effects of stress on psychotic symptoms [86]. However, not all studies could show an interaction between COMT genotype and environmental factors such as cannabis abuse [87, 88] and further larger studies are needed. In addition to the COMT polymorphism, other genes have been implicated in gene-environment interactions associated with schizophrenia. One of these is the BDNF Val66Met and it has recently been shown that the age at onset of psychotic disorder (AOP) is significantly (7 years difference) reduced in female BDNF Met carriers exposed to cannabis while cannabis use did not affect AOP in Val/Val genotypes [89]. Those studies showed that the development of schizophrenia is influenced by complex interaction between gene deficits and environmental factors and that they can additionally be influenced by the sex of the patient.

Animal Models of the 'Two Hit' Hypothesis

Most previous animal model studies have only assessed one developmental 'hit', either during early development (e.g. maternal deprivation or neonatal lesions) or during adolescence/young adulthood (e.g. cannabinoid treatment). Similarly, most of the studies using genetically-modified mice have previously assessed baseline behavior only without a second 'hit' [90, 91].

In recent years, more groups have begun to generate models that integrate multiple development disruptions. The first 'hit' is usually during early development (in the prenatal or early postnatal phase) while the second 'hit' is sometimes also during the early developmental phase and in other studies during adolescence/young adulthood. Some studies investigated behavior during the application of the second 'hit' but usually behavioral testing is done in adulthood some time after the 'hits' have been applied to test for long-term and not acute effects of the stressors. However, the time span between the end of the second 'hit' and beginning of behavioral testing can vary extensively among studies with some leaving only a few hours in between, and others weeks. An early study combining two different methods that are individually used to induce schizophrenia-like behavior was done by Hori and colleagues [92]. They used neonatal ventral hippocampus lesions and combined it with 14 days of repeated phencyclidine treatment starting at postnatal day 42 and showed that the phencyclidine-induced locomotor hyperactivity was more pronounced in lesioned rats [92]. However, behavioral testing was only assessed during the phencyclidine treatment phase and the study did not investigate the long-term effects of both 'hits' combined. In 2007, Schneider and Koch used neonatal medial prefrontal cortex lesions as their first 'hit' and then treated the

animals with a cannabinoid receptor agonist during puberty [93]. Behavioral testing in adulthood revealed that both 'hits' individually impaired recognition memory but that the combination exacerbated this effect, showing that two 'hits' can be more detrimental than one individual 'hit' [93]. In a different study, social isolation was used as the first 'hit' and repeated MK-801 treatment during young adulthood represented the second 'hit' [94]. After a 1-week washout period, animals were tested for amphetamine-induced locomotor hyperactivity and while both treatments significantly enhanced the response to amphetamine, no synergistic or additive effects were found for the 'two hit' group [94].

A few studies have combined maternal deprivation/separation with a second stressor. The first study to show that the postweaning environment can influence the behavioral outcome of maternal deprivation was by Ellenbroek and Cools [95]. Animals were maternally-deprived for 24 h on pnd 9 and at weaning age they were either housed in groups or in social isolation. Paradoxically, in both single 'hit' groups, prepulse inhibition (PPI), a measure of sensorimotor gating, was decreased in adulthood while the combination of both insults brought PPI levels back to normal [95], although similar 'hits' in mice showed different effects [96]. This study looked at the effects of early separation combined with social isolation after weaning and found that early separation induced deficits in a variety of tasks including social interaction, Y-maze, novel-object recognition (NOR), fear conditioning and PPI and these effects were usually more pronounced in animals that were additionally single-housed after weaning [96]. This shows that the combination of two 'hits' can produce stronger effects than one 'hit' alone.

In previous studies from our laboratory, a 'two hit' rat model was developed that included maternal deprivation at pnd 9 and corticosterone (CORT) treatment during young adulthood from 8 to 10 weeks of age [97–100]. Behavioral testing from 12 weeks of age showed that the combination of the two 'hits' induced a marked deficit in short-term spatial memory in the Y-maze but no such changes were seen after one 'hit' only [98]. Baseline PPI was disrupted after maternal deprivation but CORT treatment had no additional effects [97]. Furthermore, acute apomorphine treatment disrupted PPI in all control groups but not in the 'two hit' group indicating differences in dopaminergic regulation of PPI even though no differences in dopamine receptor levels were observed [99, 100]. A more recent study used a similar method but replaced the chronic CORT treatment with a chronic unpredictable stress paradigm [101]. Both 'hits' induced a deficit in the NOR in both sexes but no additive or synergistic effects were found for the 'two hit' group [101].

Other studies have investigated the effects of chronic cannabinoid treatment in maternally deprived animals. Treatment with the cannabinoid receptor agonist, CP55,940, during adolescence significantly disrupted PPI in adult female animals but maternal deprivation did not further influence this [102]. Maternal deprivation impaired recognition memory in female rats but Δ9-tetrahydrocannabinol (THC) treatment paradoxically restored this deficit [103]. Both treatments were accompanied by changes in NMDA and dopamine receptors but none of these changes were exclusively seen in 'two hit' animals indicating that there were no synergistic effects after the combination of maternal deprivation and THC treatment [103].

We recently examined the combined effect in rats of a combination of maternal separation stress and chronic young-adult treatment with CP55,940 from 8 to 10 weeks of age [104]. Here, the combination of maternal separation and cannabinoid exposure induced anhedonia-like behavior in males, expressed as a significant decrease in sucrose preference. Additionally, in male rats both maternal separation and cannabinoid receptor stimulation induced an anxiety-like phenotype in the plus maze and expressed as center time in the open field. These effects were additive and most pronounced after a combination of these 'hits'. In both males and females, PPI was reduced by maternal separation but there was no effect of CP55,940 treatment. Moreover, memory performance in the Y-maze and novel object recognition test was not affected by either of the two 'hits' [104].

Studies utilizing genetically-modified mice and exposing them to an environmental stressor

differ in their protocols in regard to stressors used, as well as timing of these stressors. While some apply the second 'hit' in the early developmental phase, others use the adolescent phase. NRG1 mutant mouse models have been used to study schizophrenia-like behavior but have yielded mixed results. A recent study investigated the behavior of adult heterozygous transmembrane-domain NRG1 mutant mice that were exposed to chronic social defeat stress during adolescence [105]. NRG1 mice exhibited impaired PPI and a reduction in social novelty preference which were independent of the second 'hit'. Chronic stress during adolescence reduced the hyperactivity seen in unstressed NRG1 but induced a memory deficit in the mutant but not wild-type mice. Furthermore, BDNF levels in the striatum were significantly decreased in the 'two hit' animals only [105]. A few studies have investigated the effects of cannabinoids in NRG1 mice but most investigated the acute effects of cannabinoids immediately after a chronic cannabinoid treatment schedule [106–108]. Only one study additionally investigated the effects of cannabinoid treatment after withdrawal [109]. Animals were tested for locomotor activity, PPI, and anxiety at 48 h after the last injection of a 21-day long chronic THC treatment regimen. NRG1 mice displayed higher locomotor activity than wild-type controls but this was more pronounced in vehicle-treated animals. PPI was not affected by either genotype or cannabinoid treatment and this was similar for anxiety-related behavior [109]. Overall, these results indicate that THC treatment does not significantly alter behavior in NRG1 mice, although more studies are necessary to investigate a larger battery of behavioral tasks. Similar studies have investigated the effects of cannabinoid treatment in COMT mice. Animals were treated with a cannabinoid agonist for 20 consecutive days and behavioral testing started 21 days after treatment had ceased. Cannabinoid treatment impaired PPI, enhanced locomotor activity, impaired spatial memory, and decreased anxiety-like behavior in male COMT knock-out mice [110, 111]. Interestingly, some of these behavioral deficits were only seen after adolescence and not adult cannabinoid treatment [110].

Other studies have investigated the effects of two 'hits' during the early developmental phase. For example, one study looked at the effects of maternal separation in heterozygous reeler mice [112]. Maternal separation decreased levels of BDNF but this was less marked in heterozygous reeler mice. Similar effects were seen in the social novelty task indicating that heterozygous reeler mice are less vulnerable toward an environmental stressor [112].

Brain-Derived Neurotrophic Factor

BDNF belongs to the family of neurotrophins which also includes nerve growth factor and neurotrophin 3 and 4 [113]. Neurotrophins are necessary for the normal development of the central nervous system and have important roles in the regulation of neural development, maintenance, survival, and activity-dependent synaptic plasticity [113–115].

The BDNF gene contains nine promoters, and through alternative splicing, a number of different transcripts can be produced which all encode the BDNF protein [116]. BDNF is initially synthesized as the precursor protein pre-pro-BDNF which is then cleaved into pro-BDNF. Pro-BDNF is either secreted and then extracellularly cleaved to mature BDNF (mBDNF) or less commonly converted intracellularly and then secreted as mBDNF [117]. mBDNF binds to the high-affinity tropomyosin-related kinase B (TrkB) receptor which is associated with promoting cell survival and long-term potentiation (LTP) [113, 118]. Major pathways that can be activated through BDNF-TrkB signaling include the phospholipase C-γ pathway, important in synaptic plasticity, which leads to an increase in intracellular levels of Ca^{2+}; the mitogen-activated protein kinase pathway, which leads to the activation of several transcription factors resulting in neuronal differentiation and growth; and the phosphatidylinositol 3-kinase pathway, which leads to the activation of the protein kinase Akt which is involved in cell survival and protein translation [113, 119].

In addition to its role during development, BDNF also plays an important role in learning

and memory in adulthood. LTP is considered to be the cellular mechanism of learning and memory and is defined as a stimulation-induced persistent increase in synaptic strength [120]. Neuronal activity that leads to LTP increases the transcription of BDNF [121] and BDNF seems to be necessary for early phase LTP as well as late phase LTP (L-LTP) [122]. L-LTP has been shown to be impaired in mice with a deletion of the BDNF gene [123] and these impairments could be rescued by exogenous BDNF administration [124, 125]. BDNF mRNA in the hippocampus was found to be increased after acquisition of memory tasks such as the radial arm maze [126] and the water maze [127]. Furthermore, administration of exogenous BDNF improved performance in spatial memory tasks [128] while inhibition of BDNF mRNA expression in the hippocampus impaired performance [129]. These studies all show that BDNF is strongly implicated in the process of learning and memory and that loss or low levels of BDNF can have functional consequences for cognition. In addition to its implication in cognitive deficits, altered BDNF expression has been associated with a range of diseases including depression, addiction, eating disorders and schizophrenia [130].

BDNF and Schizophrenia

Due to its role in development, decreased BDNF functionality could alter normal neurodevelopment leading to dysfunctional neural networks, thereby contributing to the development of schizophrenia. Indeed, post-mortem studies comparing tissue from schizophrenic patients and healthy controls have revealed changes in BDNF levels in schizophrenia. However, while some studies have found decreased protein levels of BDNF in the hippocampus of patients diagnosed with schizophrenia [131], others reported an increase of BDNF but a decrease of TrkB [132]. Protein as well as mRNA levels of BDNF were shown to be significantly decreased in the prefrontal cortex of schizophrenic patients and this was accompanied by a significant reduction of TrkB expression [133–135]. Most of the data derived from these studies came from the brains of schizophrenic patients treated with various medications. If is therefore not entirely clear whether the changes seen are indeed a result of the pathology of schizophrenia or whether they are induced by antipsychotic treatment. BDNF can cross the blood-brain-barrier [136] and several studies have therefore assessed BDNF serum levels in patients with schizophrenia with or without medication. In 2011, Green et al. conducted a meta-analysis of these studies and concluded that there is a moderate reduction of BDNF levels in both medicated and drug-naïve patients with schizophrenia [137].

In addition to altered levels of BDNF, a single nucleotide polymorphism in the BDNF gene which leads to a substitution from valine to methionine at codon 66 (Val66Met) has been implicated in schizophrenia [89, 138–141]. The Val66Met polymorphism leads to altered trafficking of BDNF and subsequently to impaired activity-dependent secretion of BDNF [142, 143]. Healthy Met allele carriers have been shown to perform worse in hippocampus-dependent tasks [143, 144] and to have smaller hippocampal volume compared with homozygous Val/Val controls [145]. Data regarding schizophrenic patients seem to be more complex. While some studies found that the Val allele was associated with schizophrenia [139] and psychosis [140], a meta-analysis from 2007 reported that Met/Met carriers had a higher risk of developing schizophrenia [138] while further studies found no interactions [146, 147]. Age of onset seems to be associated with the Val66Met polymorphism with Met carriers showing an earlier AOP [89, 141, 147]. However, not all studies were able to replicate these findings and a recent study suggested that cannabis use might contribute to the genotype-dependent age of onset [89]. Age of onset was significantly associated with cannabis use in male subjects independent of genotype while in female subjects earlier age of onset as a result of cannabis use was only seen in Met carriers [89]. Overall, despite some conflicting results, it seems that BDNF is associated with schizophrenia and further studies are needed to clarify its role in the disease process. Animal models investigating the role of BDNF can help elucidate some of the findings.

BDNF Animal Models in Schizophrenia-Like Behavior

Because of its important role during development, complete deletion of BDNF from birth leads to severe brain abnormalities and BDNF knockout mice die soon after they are born [148]. Therefore, other models have been produced which include BDNF heterozygous mice, conditional and region-specific deletion of BDNF, as well as BDNF Val66Met knock-in mice.

BDNF heterozygous mice (BDNF HET) have about 50 % reduction of normal BDNF expression [149] which is similar to what has been observed in schizophrenic patients [134]. In previous studies, BDNF HET mice showed enhanced amphetamine-induced locomotor hyperactivity [150, 151] and while one study showed enhanced baseline activity [152] this was not replicated by other studies [151, 153, 154]. Studies investigating spatial learning and memory in the water maze have provided conflicting results with one reporting significant impairment in BDNF HET mice [155], although this was not replicated in another study [156]. BDNF HET mice display impaired contextual fear conditioning and this deficit could partially be restored by infusion of BDNF into the hippocampus [157]. No differences between wild-type and BDNF HET mice were found for sucrose preference indicating that animals were normal when tested for anhedonia-like behavior [154]. Overall, the phenotype of BDNF HETs seems to be very subtle. It may be that partial loss of BDNF does not in itself lead to schizophrenia but that it is rather a risk factor and that further 'hits' are needed to induce behavioral impairments.

Mice with region-specific deletions of BDNF are used to assess the role of BDNF in specific brain regions that have implications in schizophrenia. Forebrain-specific deletion of BDNF during early development resulted in impaired spatial memory in the water maze but did not alter sensorimotor gating [158]. A similar mouse model with forebrain-specific deletion of BDNF during early development resulted in hyperactivity and severe deficits in fear conditioning while loss of BDNF during adulthood resulted in a less profound outcome [159]. A specific deletion of BDNF only in the dorsal hippocampus during adult age produced impairments in the water maze and novel object recognition task but had no effect on baseline locomotor activity [160]

To study the role of the Val66Met polymorphism in animals, knock-in mice carrying the human Met allele were generated by Chen and colleagues [161]. These mice showed smaller hippocampal volume and demonstrated deficits in context-dependent memory compared to wild-type mice [161]. The study was conducted in male animals and when female mice were used, results were less clear as impairments in novel object recognition were dependent on the phase of the oestrous cycle, indicating that BDNF genotype might interact with oestradiol to regulate memory function [162].

'Two Hit' Studies in BDNF Animal Models

As discussed above, previous preclinical studies show that the combination of two adverse events (either genetic and/or environmental) may lead to additive or synergistic effects, however, the results strongly depend on the stressors used and differences between protocols regarding timing of stressors and time span between application and behavioral testing. We recently conducted a series of studies to assess the involvement of BDNF in the combined effects of early-life stress and a young-adult second 'hit'.

As mentioned above, our initial studies included a 'two hit' model with maternal deprivation at postnatal day 9 and CORT treatment during young adulthood from 8 to 10 weeks of age [97–100]. Behavioral testing from 12 weeks of age showed that the combination of the two 'hits' induced a marked deficit in the Y-maze which was accompanied by a decrease of BDNF mRNA in the hippocampus. No such changes were seen after one 'hit' only [98]. Baseline PPI was disrupted after maternal deprivation but CORT treatment had no additional effects [97]. Furthermore, acute apomorphine treatment disrupted PPI in all control groups but not in the 'two hit' group

indicating differences in dopaminergic regulation of PPI even though no differences in dopamine receptor levels were observed [99, 100].

In subsequent studies, we used BDNF heterozygous mice treated from 6 to 9 weeks of age either with CORT to simulate chronic stress, or with the cannabinoid receptor agonist, CP55,940. The stress hormone, CORT, was administered to female and male BDNF heterozygous mice and their wild-type controls through their drinking water [163]. When the animals reached 11 weeks of age, we observed a profound memory deficit in the Y-maze in male, but not female BDNF heterozygous mice treated with CORT. The groups displayed no differences in baseline PPI or its disruption by the NMDA receptor antagonist, MK-801. In addition to the expected reduced levels of BDNF in the mutant mice, there were no additional changes in the expression of this neurotrophin after CORT treatment. Protein levels of the NR2B subunit of the NMDA receptor were markedly increased in the dorsal, but not ventral hippocampus of male BDNF heterozygous mice treated with CORT, an effect which could be related to the spatial memory deficits in these mice, respectively. No significant changes in the levels of subunits NR1, NR2A, and NR2C were observed in males and there were no changes in any of the female groups [163].

We were then interested to see if the role of BDNF in this 'two hit' mouse model would generalize to other second 'hits'. Thus, we investigated whether a BDNF deficit would interact with chronic cannabis intake, a well-described risk factor for schizophrenia development. As with the previous study, BDNF heterozygous mice and wild-type controls were chronically treated during weeks 6–9 of life, this time with the cannabinoid receptor agonist, CP55,940 [164]. Behavioral testing again commenced at 11 weeks of age and revealed no CP55,940-induced deficits in short-term spatial memory in the Y-maze and no changes in novel object recognition memory either. In this study, baseline PPI was found to be reduced in BDNF heterozygous mice and chronic CP55,940 treatment did not alter this. However, acute CP55,940 administration caused a marked increase in PPI particularly in male BDNF heterozygous mice pre-treated with this same drug but not in any of the other male groups. All female groups showed small increases of PPI after acute CP55,940 administration. We then analyzed the levels of [^{3}H] CP55,940 binding by autoradiography and found a significant increase in the nucleus accumbens, but not caudate nucleus of male BDNF HET mice previously treated with this drug. There were no changes in binding in any of the other groups [164]. These results contrast with the effect of CORT treatment in the BDNF HET mice in that BDNF deficiency and chronic young-adult cannabinoid receptor stimulation did not interact on learning and memory later in life. Again in contrast to CORT, cannabinoid receptor stimulation elicited a hypersensitivity to the effect of acute CP55,940 on PPI, which could be related to up-regulation of cannabinoid receptor density in this region.

Clearly, 'two hit' effects in BDNF animal models appear to depend markedly on the nature of the second 'hit'. To investigate if this was unique to the BDNF heterozygous mouse model, we followed this up with a series of studies in rats that were subjected to a maternal-separation protocol as the first 'hit' and either CORT treatment or CP55,940 treatment as the second 'hit'.

Wistar rats were exposed to neonatal maternal separation for 3 h per day on postnatal day 2–14 and/or received CORT in their drinking water during 8–10 weeks of age [165]. Male, but not female 'two hit' rats showed marked disruptions in short-term spatial memory in the Y-maze. However, female 'two hit' rats showed signs of anhedonia in a sucrose preference test, which were not observed in males. Novel object recognition and anxiety measures in an elevated plus maze task were unchanged by either of the two 'hits'. We then obtained dorsal and ventral hippocampus regions and used quantitative polymerase chain reaction (qPCR) to assess exon-specific BDNF gene expression or Western blot to assess BDNF protein expression and downstream signaling. In the dorsal hippocampus, maternal separation caused a male-specific increase in BDNF exons I, II, IV, VII, and IX mRNA but a decrease in mBDNF and phosphorylated TrkB (pTrkB) protein expression

in adulthood. These effects were not seen in the male ventral hippocampus. However, in female rats only, maternal separation caused a significant decrease in mBDNF and pTrkB protein expression in the ventral hippocampus in adulthood. Thus, in this maternal separation model, long-lasting, region-specific, and sex-specific effects on BDNF expression and signaling were observed, which could be involved in the sex-specific qualitative differences in the behavioral profile of these animals, particularly with respect to spatial memory and anhedonic behaviors.

Together with the above-mentioned studies on the effects of CORT or cannabinoid receptor stimulation in BDNF HET mice [163, 164], these results in rats confirm that early developmental disruptions and young-adult stress or cannabis use [104] on their own or in combination can differentially affect behaviors related to neuropsychiatric disorders and that BDNF is likely to play a central role in this interaction. However, different 'two hit' combinations produce markedly and qualitatively different behavioral phenotypes in male vs. female animals.

Conclusions

There is ample evidence that the etiology of schizophrenia and other neuropsychiatric disorders involves complex gene-environment interactions where altered expression of brain factors relevant to plasticity and development produces a vulnerability to other 'hits' later in life, such as adolescent young-adult stress or drug abuse. Adding to substantial, but incomplete and often inconsistent clinical literature, animal model studies are beginning to unravel the complex multifactorial mechanisms in the brain which mediate these interactions. Our work has focused on BDNF but it is clear that several other early neuromodulators, including immune factors and neuregulin, COMT and DISC1, could be involved and may synergize with different second 'hits' to induce their own unique profile of behavioral effects in adulthood. Further pre-clinical studies will be of paramount value to elucidate the brain mechanisms involved in these various combinations. This may be relevant to recognize targets for early intervention which appear more promising to reduce the burden of complex psychiatric illnesses like schizophrenia than symptomatic pharmacotherapy which is often associated with severe side-effects.

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25 Translating the Glutamatergic Hypothesis of Schizophrenia Through Homeostatic Regulation of Brain Glycine

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Schizophrenia is a major psychotic disorder characterized by profound disturbances in cognitive function, emotion, and behavior. The disease has a lifetime prevalence of 1 % worldwide, and is generally considered as a neurodevelopmental brain disorder determined by both genetic and environmental factors. The onset of the first presentation of florid psychotic symptoms is preceded by the prodromal phase where signs of cognitive disturbances may be detectable. The complex symptoms of schizophrenia are typically divided into clusters. Positive symptoms are characterized by an excess of functions normally not experienced by healthy people and include hallucinations and delusions that are typically seen in acute psychosis. On the other hand, negative symptoms refer to the loss or diminution of normal functions in the affective and cognitive domains. Cognitive symptoms such as deficiency in working memory and executive function severely undermine a patient's intellectual capability to lead a normal independent life (e.g., to earn a living), whereas other negative symptoms concerning affect and motivation diminish the desire or drive to pursue a normal productive life (e.g., neglect with regard to basic personal hygiene, social dysfunction). Negative and cognitive symptoms are not responsive to medication currently available [1], and are therefore major roadblocks to successful rehabilitation. This pressing medical need for effective treatment for negative and cognitive symptoms is therefore high on the public health agenda. The US Food and Drug Administration has recently accepted in principle negative symptoms and cognitive impairment in schizophrenia as legitimate drug targets [2]. This chapter traces the development of a novel pharmacological strategy that may fulfill this urgent medical need.

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Glutamate Deficiency in Schizophrenia

The realization that dopamine receptor agonists and antagonists are associated with the induction and suppression of positive psychotic symptoms, respectively, has given birth to the enduring *dopamine hypothesis* of schizophrenia [3]. However, decades of clinical experience have shown that effective treatment of negative and cognitive symptoms is unlikely to be achieved by blockade of the appropriate dopamine receptors alone [4, 5].

Attention to the glutamate neurotransmission system was initially prompted by early clinical

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data suggesting that schizophrenia may be associated with a deficiency in brain glutamate [6]. Subsequently, the emphasis on a deficiency in glutamatergic signaling via *N*-methyl-D-aspartate (NMDA) receptor was prompted by the revelation that psychoactive substances such as phencyclidine and ketamine are potent antagonists of the NMDA receptor. Similar to the dopamine releasing agent, amphetamine, blockade of NMDA receptors has been shown to precipitate schizophrenic-like symptoms in healthy subjects and aggravate symptoms in schizophrenics [7–12].

The current consensus is that deficiency in glutamatergic neurotransmission via NMDA receptors contribute directly to the negative and/or cognitive symptoms of schizophrenia and may additionally lead to a functional imbalance of dopamine transmission implicated in the genesis of positive symptoms [13, 14]. In fact, dopaminergic alterations might be secondary to NMDA receptor dysfunction in schizophrenia [15, 16].

The activation of NMDA receptors has been linked to numerous forms of neuroplasticity that underlies the induction and maintenance of memory. It is therefore not surprising that a deficiency in NMDA receptor function could impair learning and memory. Furthermore, animal models also show that NMDA receptor blockade can lead to social withdrawal which resembles aspects of the negative symptoms [17]. Disturbances of glutamate homeostasis and neurotransmission have also been implicated in major depressive disorders, although the precise mechanisms whereby NMDA receptor dysregulation may lead to avolition and the emergence of depressive symptoms are still unclear [18].

The search for a viable pharmacological strategy to boost NMDA receptor function, however, has been met with difficulties because activation of NMDA receptors by direct agonists is associated with toxic side effects [19]. To avoid excessive stimulation of NMDA receptors, an alternative approach is to target the positive allosteric sites of the receptor (Fig. 25.1). In particular, the allosteric glycine-B site may hold promise for the first translation of the glutamate hypothesis into a new generation of antischizophrenia drugs. This concept is central to early attempts of glycine augmentation therapy, and more recently the development of glycine reuptake inhibitors by a number of pharmaceutical companies.

Glycine as a Modulator of NMDA Receptor

The amino acid, glycine, is an important signalling molecule in the central nervous system (CNS). As an inhibitory neurotransmitter, glycine binds to the strychnine-sensitive glycine-A site on ionotropic glycine receptors and activates an inward chloride current through the integral anion channel of the glycine receptor complex which leads to the hyperpolarization of the postsynaptic membrane. This fast neuronal inhibition is the predominant function of glycine receptor found predominantly at inhibitory synapses in the spinal cord, brainstem, and retina in the adult brain. The existence of non-strychnine-sensitive high affinity binding sites was revealed by [^{3}H] glycine autoradiography because such sites were not labeled by [^{3}H] strychnine [20]. The distribution pattern of the non-strychnine-sensitive, glycine-B sites, corresponds well to the binding profile of NMDA receptors [21]. The physiological interaction between glycine and NMDA receptors was demonstrated by Johnson and Ascher [22]. They showed that glycine could augment NMDA receptor-mediated electrophysiological responses. Kleckner and Dingledine [23] subsequently demonstrated that glycine is a prerequisite for the activation of the NMDA receptor and coined the term "co-agonist" to describe its essential role at the NMDA receptor. It is known that glycine is not the only endogenous ligand at the glycine-B site. The amino acid D-serine can also bind to the glycine-B site and serves the function of an obligatory co-agonist [24–26]. Hence, the concomitant binding of glycine or D-serine at the glycine-B site is a prerequisite for the activation of postsynaptic NMDA receptors by glutamate released from presynaptic terminals. The distinction and cooperation between the regulatory function of D-serine and glycine at NMDA receptors, however, remain to be fully delineated. One suggestion is that D-serine might be preferentially involved in the regulation of synaptic NMDA receptors, whereas glycine appears to be more critically involved in the regulation of extra-synaptic NMDA receptor excitability—a demarcation that can be linked to the differential distributions of synaptic versus

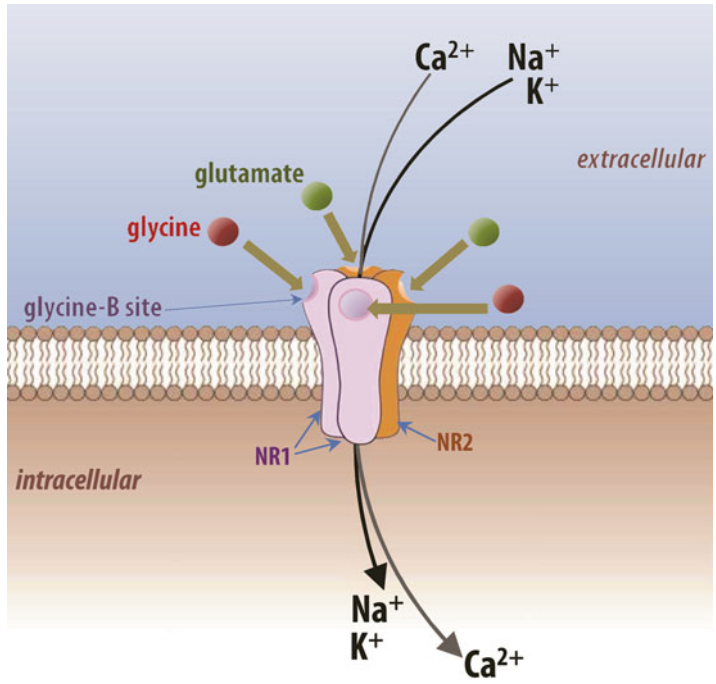

Fig. 25.1 The *N*-methyl-D-aspartate (NMDA) receptors are heterotetramic complexes composed of four subunits, typically two NR1 subunits (with eight variants by alternative splicing of the *GRIN1* gene) and two NR2 subunits (with four splicing variants) as depicted. The incorporation of NMDA receptor (NR) subunit, NR3, into conventional NMDA receptors forms glutamate-activated NR1/NR2/NR3 triheteromers, whereas the omission of the glutamate-binding NR2 subunits results in excitatory glycine-activated NR1/NR3 diheteromers. Combinations of different NR2 subunits determine the pharmacological and physiological profile of the receptor in terms of channel kinetics, and its affinity for agonists and antagonists for conventional NR1/NR2 NMDA receptors. L-glutamate binds to NR2 subunits, whereas glycine binds to the co-agonist, glycine-B site, of the two NR1 subunits. All four sites have to be activated to permit activation of the ion channel that permits the inward flow of Ca^{2+}, Na^{+} and K^{+} ions

extra-synaptic NMDA receptors, which can be distinguished by the presence of NMDA receptor (NR) subunit NR2A and NR2B, respectively.

Occupancy of the glycine-B site is not only required for initiating signaling through the NMDA receptor, but it also primes the NMDA receptor for internalization [27]. Saturation of the glycine-B sites is expected to trigger the internalization of NMDA receptors, and thereby would be expected to weaken the signals mediated by NMDA receptors. This could take place when the extra-cellular glycine concentration is sufficiently high. It has been shown that the magnitude of NMDA receptor-dependent long-term potentiation can be enhanced by increasing concentration of ambient glycine up to 10 μM, but signs of impairment can appear at ≥100 μM concentrations [28]. Therefore, disturbance of the homeostatic regulation of extracellular glycine concentration in the brain could have a profound effect not only on the excitability of individual NMDA receptors but also on their overall expression levels.

Modulation of NMDA Receptor Through Glycine Transporters

The ambient concentration of glycine in the extracellular space near NMDA receptors may be exploited as a new avenue to facilitate NMDA receptor neurotransmission [29]. One obvious means to increase extracellular glycine availability is the direct augmentation through dietary intake of glycine. However, this proves to be ineffective because of the poor penetration of glycine through the blood–brain-barrier (see section "Proof of principle I"). Alternatives include glycine-B site

partial agonist, D-cycloserine, which is able to penetrate into the CNS. A potential breakthrough hinges on the homeostasis (re-uptake, release, and metabolism) of glycine in the brain.

It was initially believed that the levels of glycine in the extracellular space were sufficiently high so that the glycine-B sites of the NMDA receptors would be saturated under physiological conditions [30, 31] and therefore not relevant in the normal regulation of NMDA receptor function. It soon became apparent that glycine transporters provide active removal of extracellular glycine such that the glycine concentration in the synaptic cleft is maintained at sub-saturating levels [32–34].

The two principal subtypes of glycine transporters are GlyT1 [35, 36] and GlyT2 [37]. They belong to the sodium-dependent intracelluar solute carrier family 6 of transporters but differ in terms of 1) regional and cellular expression patterns in the CNS, 2) Na^+:glycine stoichiometries, and 3) the ability to reverse-transport intracelluar glycine into the extracellular space [38–42]. Five variants of GlyT1 (a–e) and three variants of GlyT2 (a–c) as a result of alternative splicing and promotor usage have been identified [40, 43–45].

As illustrated in Fig. 25.2, GlyT2 expressed in glycinergic terminals assumes the critical role in the reuptake and recycling of glycine released by the pre-synaptic terminal into the synaptic cleft, and together with astrocytic GlyT1 contribute to the termination of the stimulation of glycinergic receptors in the post-synaptic membrane [40]. The coordinated activities of GlyT1 and GlyT2 are essential for the vital functions that depend on inhibitory neurotransmission such as respiratory regulation. The loss of pre-synaptic GlyT2 drastically curtails the refilling of glycine vesicles by the presynaptic cell and severely undermines inhibitory glycinergic neurotransmission [42, 46, 47]. On the other hand, the loss of astrocytic GlyT1 severely undermines the clearance of glycine released from the synaptic cleft leading to sustained neuronal inhibition over brain stem respiratory centers [48]. In fact, constitutive homozygous deletion of either the *GLYT1* or *GLYT2* gene is lethal in mice [46, 48].

In contrast, GlyT1 expressed in pre- and post-synaptic sites of glutamatergic synapses plays the pivotal role in preventing the saturation of the glycine-B site on NMDA receptors, with GlyT1 expressed in adjoining astrocytes as well as extra-synaptic sites playing a supplementary role [33, 34, 49–52]. GlyT1 is therefore the obvious target of choice to modify NMDA receptor function. Inhibition of GlyT1 should elevate baseline glycine-B site occupancy and thereby increase the probability of NMDA receptor activation by pre-synaptic release of glutamate (Fig. 25.1). The result is an 'on-demand' facilitation of NMDA receptor activation which amplifies the glutamatergic signals. This is unlike the elevation of the tonic activity of NMDA receptors produced by direct NMDA receptor agonists. GlyT1 inhibition is therefore expected to confer therapeutic potential for diseases such as schizophrenia, in which deficient signaling via forebrain NMDA receptors is implicated. We have shown that the deletion of neuronal GlyT1 in the forebrain alone is sufficient to enhance selectively the NMDA receptor current; and the knockout mice have exhibited antipsychotic-like as well as pro-cognitive phenotypes [53, 54].

Schizophrenia could be the first neuropsychiatric disorder to benefit from this novel pharmacological approach. The recent phase II clinical trials of the GlyT1 inhibiting drug, bitopertin, [4-(3-Fluoro-5-trifluoromethylpyridin-2-yl) piperazin-1-yl]-[5-methanesulfonyl-2-((S)-2,2,2-trifluoro-1-methylethoxy)phenyl]methanone] (a.k.a RO4917838 and RG1678) developed by Hoffmann-La Roche and its subsidiary Chugai Pharmaceutical have yielded promising outcomes for the treatment of schizophrenia negative and cognitive symptoms [55–58]. The compound had the potential to become the first successful translation of the glutamate hypofunction hypothesis of schizophrenia into a clinic-ready pharmacotherapy since the hypothesis's inception in the 1980s [59] before the hope was dashed finally with the release of the disappointing results. First, two trials were reported to have failed to meet the primary endpoint in improving negative symptoms (www.roche.com/med-cor-2014-01-21-e.pdf). Subsequently, more information were released in abstract form in three international conferences [60–62]. Out of the six phase III trials, only one treatment arm (10 mg/kg adjuvant biotopertin) in the study, which had targeted positive symptoms, had yielded significant improvement the primary endpoint relative to placebo adjuvant treatment.

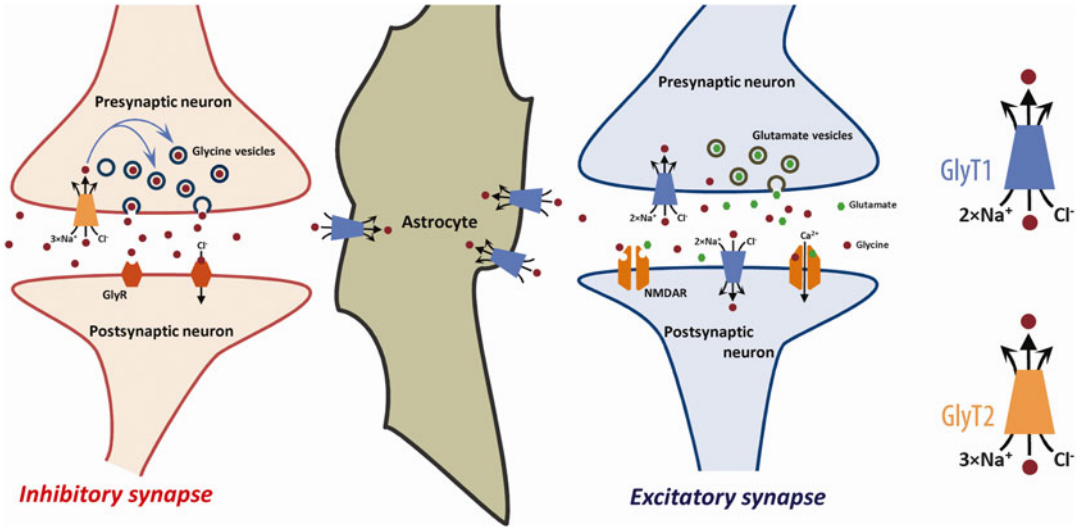

Fig. 25.2 Regulation of glycinergic neurotransmission at inhibitory and excitatory synapses by glycine transporters. At an inhibitory synapse (*left*) containing glycine receptors, GlyRs, the extracellular level of glycine is regulated by both GlyT1 and GlyT2. The activation of postsynaptic GlyRs by glycine released from the presynaptic bouton is terminated by the re-uptake of glycine by GlyT2 expressed in the presynaptic terminals and GlyT1 in adjoining astrocytes. Glycine transported back in the presynaptic terminals by GlyT2 is incorporated into presynaptic vesicles by the vesicular inhibitory amino acid transporter (not shown). At the excitatory synapse (*right*), GlyT1 is located on pre- and post-synaptic neurons, where it co-localizes with *N*-methyl-D-aspartate (NMDA) receptors, as well as on neighbouring astrocytes. GlyT1 regulates the extracellular glycine concentration at the synaptic cleft and keeps the glycine levels below what is required to saturate the glycine-B site. Inhibition of GlyT1-mediated glycine reuptake near glutamatergic synapses is highly effective in elevating the baseline occupancy of the glycine-B site and thereby increases the probability of NMDA receptor responses

The evidence was too weak to justify further commercial development of bitopertin, and Roche has since shelved its development as an anti-schizophrenia agent, although it is still being pursued as an adjuvant treatment for obsessive compulsive disorder (NCT01674361).

Pharmacological Models

The development of potent and highly selective GlyT1 inhibitors has been initiated with the synthesis of the sarcosine derivatives, Org 24598 and N[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl] sarcosine (NFPS; also known as ALX 5407 since 2001) [63, 64]. A variety of compounds have since been synthesized, including compounds that are not based on a sarcosine backbone such as SSR504734, SSR103800, and bitopertin [53, 65]. As proof of mechanism, GlyT1 inhibitors have been demonstrated to elevate brain glycine levels, potentiate NMDA receptor-mediated transmission and synaptic plasticity, and antagonize the behavioral effects of NMDA receptor blockade [53].

The first preclinical evidence of antipsychotic-like potential of glycine re-uptake inhibition can be traced back to the study by Toth et al. [66], which showed that the glycine derivative, glycyldodecylamide, was much more effective than glycine itself to reverse the hyperlocomotor response induced by the NMDA receptor antagonist, phencyclidine. However, it took over a decade until the connection with glycine transporter was identified by Javitt and colleagues. They demonstrated that behaviorally effective doses of glycyldodecylamide were highly effective in inhibiting forebrain glycine re-uptake [67, 68]. That early finding marked the beginning of the preclinical evaluation of many GlyT1 inhibitors in animal models of schizophrenia ranging from the readily available compound sarcosine to highly potent synthetic compounds developed by diverse pharmaceutical companies. As summarized in Table 25.1, the results obtained in

Table 25.1 Overview of major psychopharmacological findings of selective GlyT1 inhibitors in pre-clinical schizophrenia models

Compound	Locomotor activity	Prepulse inhibition (PPI)	Latent inhibition (LI)	Effects on memory	Others
Manufacturer					
ALX 5407 (a.k.a. NFPS)	• Attenuates PCP-induced hyperactivity • Fails to attenuate amphetamine-induced hyperactivity • Fails to attenuate apomorphine-induced hyperactivity	• Enhances PPI in DBA/2 mice • Attenuates PPI in C57BL/6 mice • Reverses MK801-induced PPI disruption	• Enhances LI expression • Normalizes MK801-induced LI abnormalities (namely, both disruption and persistent LI)	• Ameliorates MK801-induced spatial reference memory but not working memory • Enhances social recognition memory • Reverses MK801/PCP-induced memory deficits • Facilitates extinction learning of conditioned fear	
ASP2535 *Astellas*		• Reverses PCP-induced PPI disruption		• Reverses MK-801- and scopolamine-induced working memory deficit • Restores recognition memory impairment induced by neonatal PCP treatment	
Merck (S) *Merck*		• Enhances PPI in DBA/2 mice			
ORG 24461 *Organon*	• Attenuates PCP- and amphetamine-induced hyperactivity • Fails to attenuate apomorphine-induced stereotypy				
ORG 24598 *Organon*		• Restores PPI deficit in the rat model of neonatal ventral hippocampal lesions			

PF-3463275 *Pfizer*				• Reverses ketamine-induced memory deficits	
RG1678 *Hoffman-La Roche*	• Attenuates PCP-induced hyperactivity • Attenuates amphetamine-induced hyperactivity				
Roche-7 *Hoffman-La Roche*		• Enhances PPI in DBA/2 mice			
SSR103800 *Sanofi*	• Attenuates MK801-induced hyperactivity	• Enhances PPI in DBA/2 mice	• Enhances LI expression • Normalizes amphetamine- and MK801-induced LI abnormalities	• Reverses social memory deficit induced by chronic neonatal PCP exposure • Reverses object memory deficit in PCP-sensitized rats	• Antidepressant-like effect in the forced swim test
SSR504734 *Sanofi*	• Attenuates PCP- and MK801-induced hyperactivity • Exacerbates amphetamine -induced hyperactivity • Potentiate the *hypo*activity effect induced by low doses of apomorphine • Reverses hypersensitivity to amphetamine induced by neonatal chronic PCP exposure	• Enhances PPI in DBA/2 or C57BL/6 mice • Exacerbates the disruption of PPI induced by apomorphine	• Enhances LI expression • Normalizes amphetamine- and MK801-induced LI abnormalities	• Reverses social memory deficit induced by chronic neonatal PCP exposure • Enhances working memory performance in a continuous delayed alternation task	• Suppresses acquisition of contextual fear (anxiolytic effect?) • Facilitates extra-dimensional shift (improved cognitive flexibility?)

Key findings in three common in vivo animal models of schizophrenia are arranged according to preclinical paradigms: (1) spontaneous and psychostimulant-induced hyperactivity, (2) prepulse inhibition (PPI)—an operational measure of sensorimotor gating impaired in schizophrenia and thought to be related to sensory flooding and cognitive impairment, (3) latent inhibition (LI)—a form of selective attention whereby attention to irrelevant stimuli are tuned out, and (4) other tests linked to specific affective and cognitive symptoms. See relevant explanations and references in the text. NFPS: N[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine, which is also known as ALX 5407 in the literature; PCP: phencyclidine, a potent psychomimetic drug that blocks NMDA receptors; DBA/2 refers to an inbred mouse strain with intrinsic deficiency in prepulse inhibition (PPI)

preclinical animal models have been largely encouraging.

Reversal of the hyperlocomotor activity induced by NMDA receptor blockers such as phencyclidine and dizocilpine (MK-801) is most commonly used as antipsychotic potential test (Table 25.1). In an effort to more directly measure the in vivo efficacy of a given compound to enhance the occupancy of the NMDA receptor glycine-B sites, Alberati et al. [69] instead measured a given compound's ability to reverse the motor disturbance (head weaving, body rolling, hyperlocomotion) induced by L-687,414. As a partial agonist, L-687,414 is less efficacious than the endogenous agonists, glycine or D-serine, at the co-agonist glycine-B site; and therefore L-687,414 can induce behavioral effects resembling direct NMDA receptor antagonists. Hence, a drug that can effectively raise the levels of the endogenous co-agonist, glycine, could reverse the behavioral effects of L-687,414 because the increase in glycine directly competes with L-687,414 for binding to the glycine-B site. The test is uniquely suited for the detection of potential antipsychotic drugs that act to increase endogenous glycine-B site occupancy. At doses that were devoid of notable behavioral effects, glycine and five potent GlyT1 inhibitors (ALX 5407, ORG24598, RO4543338, RO4840700, and SSR504734) dose-dependently reversed L-687,414-induced hyperlocomotion. Additionally, the assay is sensitive to conventional antipsychotics, but not to other CNS drugs such as the analgesic morphine, the antidepressant fluoxetine, or the anxiolytic drug diazepam [69].

It is worth noting that the ability of GlyT1 inhibitors to antagonize the motor stimulant effect of the indirect dopamine agonist, amphetamine—a model of the positive symptoms associated with hyperdopaminergia [70, 71], is less consistent than their ability to reverse the hyperlocomotor effects effect of NMDA receptor blockade (see Table 25.2). One competitive

Table 25.2 Overview on clinical trials evaluating the therapeutic efficacy of GlyT1 inhibitors for the treatment of schizophrenia

	Compound	Study design and scope	Trial codes
	Manufacturer/institutions		
Phase 1	GSK1018921 *GlaxoSmithKline*	Repeat-dose study to assess safety, tolerability, pharmacokinetics, pharmacodynamics of GSK1018921 in healthy volunteers and patients with schizophrenia	NCT00929370 Terminated 2009
	PF-3463275 *Pfizer*	Evaluated the safety, tolerability, and efficacy of two-dose regimens of PF-3463275 compared with placebo added to ongoing atypical antipsychotic therapy for cognitive deficits in subjects with chronic symptoms of schizophrenia	NCT00567203 Completed 2008
	PF-04958242 *Pfizer*	Evaluated the safety and tolerability of multiple, ascending doses of PF-04958242 administered orally to psychiatrically stable subjects with schizophrenia receiving antipsychotic and adjunctive medication	NCT01518894 Completed 2012
	SSR504734 and SSR103800 *Sanofi*	No detailed information has been disclosed on the design or outcome of the Phase I study	*Not available*
	Org 25935 *Organon*	Investigated, in collaboration with Yale University, the effect of Org 25935 on ketamine-induced behavioural and cognitive effects in healthy male subjects. Early results suggested that Org 25935 might exacerbate some cognitive deficits induced by ketamine	NCT00700076 Completed 2008
	RO4917838 (*a.k.a.* RG1678, bitopertin) *Hoffmann-La Roche*	Single-center study will assess the effect on biomarkers measures of cognitive dysfunction, the clinical efficacy and, safety of RO4917383 (10 mg daily, orally) in patients with schizophrenia on stable antipsychotic medication	NCT01116830

(continued)

Table 25.2 (continued)

	Compound	Study design and scope	Trial codes
	Manufacturer/institutions		
Phase II	PF-02545920 Pfizer	Assessed the efficacy and safety of an investigational compound PF-02545920 for the treatment of schizophrenia. PF-02545920 is expected to be more effective than placebo in reducing symptoms associated with schizophrenia	NCT00570063 Terminated 2008
	PF-03463275 Pfizer	Examined the efficacy of PF-03463275 compared with placebo in treating negative symptoms of schizophrenia when added to ongoing antipsychotic treatment in stable outpatients with schizophrenia	NCT00977522 Terminated 2010
	SCH 900435 (*a.k.a.* Org 25935) Organon/ Schering-Plough	Tested whether SCH 900435 (16 mg twice daily) is more effective than placebo in the treatment of patients with schizophrenia, using 15 mg olanzapine once daily as active control	NCT00988728 Terminated 2012
	Org 25935 (*a.k.a.* SCH 900435) Organon/ Schering-Plough	Evaluated whether Org 25935 is more effective than placebo in improving negative symptoms in subjects with schizophrenia who are concurrently treated with a stable dose of a second-generation antipsychotic	NCT00725075 Completed 2008
	RO4917838 (*a.k.a.* RG1678, bitopertin) Hoffmann-La Roche	Evaluated the efficacy of RO4917838 (10, 30, or 60 mg) in patients with schizophrenia who are stable on current antipsychotic treatment (olanzapine, quetiapine, risperidone, paliperidone or aripiprazole) with prominent negative or disorganized thought symptoms	NCT00616798 Completed 2010
		Evaluated the efficacy of RO4917838 (bitopertin) in patients with acute exacerbation of schizophrenia. Patients receive either RO4917838 10 mg or RO4917838 30 mg or olanzapine 15 mg or placebo orally daily for 4 weeks as inpatients	NCT01234779 Completed 2014
	Sarcosine Medical University of Lodz	To evaluate whether dietary supplement of sarcosine is effective in the treatment of schizophrenia with a focus on quality of life and sexual functioning	NCT01503359
	Sarcosine Chang-Hua Hospital, Taiwan	Efficacy and safety study of sarcosine as an adjunctive therapy for schizophrenia	NCT01047592
	Unspecified compound China Medical University Hospital	The study will investigate the efficacy and safety of NMDA adjuvant therapy (GlyT1 inhibition) in refractory schizophrenia, and identify the predictors for treatment response to NMDA enhancers	NCT01251055
Phase III	RO4917838 (*a.k.a.* RG1678, bitopertin) Hoffmann-La Roche	Multi-center study assessing the efficacy of RO4917838 in schizophrenia patients with persistent negative symptoms on stable antipsychotic treatment. The study lasts for 52 weeks, followed by an optional treatment extension for up to 3 years	NCT01192867 NCT01192906 NCT01192880 discontinued/ completed 2014–5
		Multi-center study assessing the efficacy and safety of RO4917838 in patients with sub-optimally controlled symptoms of schizophrenia. Patients, on stable treatment with antipsychotics, will be randomized to receive daily oral doses of RO4917838 or matching placebo for 52 weeks, followed by an optional treatment extension for up to 3 years	NCT01235520 NCT01235559 NCT01235585 discontinued/ completed 2014–5

The compounds are listed arranged according to the phase of clinical development. The manufacturer that synthesizes the compound and/or academic institutions involved in the corresponding clinical trial is also provided. NMDA: N-methyl-d-aspartate. Glutamate receptors of the "NMDA" subtype are implicated in cognitive and negative symptoms (see text). *Sources*: www.clinicaltrials.gov

GlyT1 inhibitor, SSR504734, has even been reported to exacerbate the hyperlocomotor response of amphetamine [72] and the sensorimotor gating deficits in the prepulse inhibition paradigm induced by apomorphine (a direct dopamine receptor) [73]. These drug–drug interactions are consistent with a report that SSR50473 potentiated the release of dopamine in the nucleus accumbens triggered by direct electrical stimulation of the amygdala [74] and increased basal dopamine activity in the prefrontal cortices [75].

It is not certain, however, if similar interactions with the dopamine neurotransmission are shared by other GlyT1 inhibitors. If so, this may predict weak efficacy against positive symptoms and provides a rationale for GlyT1 inhibition as an add-on to be combined with conventional antipsychotic drugs that are known to block dopamine D_2 receptors [76–78]. On the other hand, the positive effects of SSR504734 on mesocortico-limbic dopamine may contribute to its efficacy to enhance working memory and problem solving in normal animals [79, 80] because stimulation of prefrontal dopamine activity could enhance cognitive performance in healthy humans and ameliorate cognitive deficits in schizophrenia patients [81–85]. However, this is unlikely the sole mechanism for the pro-cognitive potential of GlyT1 inhibitors. Several other GlyT1 inhibitors have also been shown to ameliorate the learning and memory deficits induced by NMDA receptor blockade across multiple tests (see Table 25.1).

The prepulse inhibition (PPI) and latent inhibition (LI) tests are two highly translational paradigms with face, construct, and predictive validity [86]. PPI measures a form of sensory gating that regulates stimulus access to higher cognitive resources. It is considered as a pre-attentional filtering mechanism whereby ongoing information processing is protected from intrusion by spurious environmental stimuli [87]. LI, on the other hand, refers to a specific form of selective attention whereby one learns to pay less attention to stimuli that are evidently irrelevant (i.e., lacking biological significance) in the past. This is an important component of associative learning, which suppresses the formation of spurious contingency between events in one's environment, and thereby favors the learning of reliable contingency between environmental events to guide future behavior [88]. Schizophrenic patients exhibit deficits in PPI as well as in LI [89, 90]; and antipsychotic drugs can strengthen both phenomena [91–100]. With few exceptions, GlyT1 inhibitors exhibited antipsychotic potential in the PPI test (Table 25.1). All three compounds (ALX 5407, SSR504734, and SSR103800) that have been evaluated in the LI paradigm are effective in enhancing LI in normal animals [101, 102], as well as antagonizing the LI impairment induced by the NMDA receptor antagonist MK801—a specific form of LI deficit suggested to mimic attentional dysfunction linked to negative symptoms [103].

Additional tests that might point to an efficacy against affective symptoms in schizophrenia include enhanced social recognition memory by ALX 5407 [104], and antidepressant-like property in the Porsolt forced swim test by SSR103800 [105]. Indications for potential anti-anxiety effects of GlyT1 inhibitors include the suppression of contextual fear learning by SSR504734 [106] and the facilitation of fear extinction by ALX 5407 [101].

Proof of Principle I: Glycine-B Site Agonists

One of the first clinical trials evaluating the antipsychotic potential of glycine augmentation therapy was an open-label pilot study of orally administered glycine (10.8 g/day) as an add-on medication to conventional antipsychotics drugs [107]. The study yielded inconclusive results, and another study done by the same group using milacimide, a prodrug for glycine, failed to reveal any benefits [108].

These early negative findings were followed by a series of placebo-controlled clinical trials of glycine add-on therapy with substantially higher doses (0.4–0.8 g/kg/day) designed to overcome poor brain penetration of orally administered glycine. At the highest dose, which was associated with a six-fold increase in the plasma levels of glycine, glycine add-on therapy significantly ameliorated the negative symptoms and improved global functions in the patients. However, the

required high doses are considered impractical for long-term clinical use [109] because of potential gastrointestinal disturbances [110, 111].

Subsequent add-on studies with the glycine-B site agonist, D-serine, revealed similar beneficial effects against the negative and cognitive symptoms at significantly lower doses (2–8 g/day) [112, 113]. Nephrotoxicity was a concern at the highest dose (8 g/day), but could be avoided at lower doses (≤4 g/day) that were still clinical effective [112]. As an alternative to overcome the poor brain penetrance of glycine, and to a lesser degree D-serine, the partial glycine-B site agonist, D-cycloserine, has also been evaluated as an add-on medication in clinical trials [114–118]. When added to conventional antipsychotics, D-cycloserine significantly reduced negative symptoms over a relatively small dose range with optimal therapeutic efficacy at 50 mg/day [114, 117, 119]. However, the clinical potential of D-cycloserine has been limited by the decrease in therapeutic efficacy over time, the modest effect size, and narrow effective dose range [116].

These studies have provided important evidence that adjunctive glycine augmentation therapy directed at increasing glycine-B site occupancy could improve negative and cognitive symptoms of schizophrenia. At the same time they have identified obvious limitations that justify the alternative approach to inhibit GlyT1. Targeting GlyT1 is an attractive strategy considering that the potential of designing new compounds that can selectively mimic the action of small molecules such as glycine and D-serine on the glycine-B site is limited because their simple backbone structures leave little room for possible structural modifications [113].

Proof of Principle II: GlyT1 Inhibition

The first proof-of-concept studies were carried out with the naturally occurring GlyT1 inhibitor, sarcosine (*N*-methyl glycine). Like glycine, sarcosine is a natural amino acid that is generated as an intermediate in the synthesis and degradation of glycine. Sarcosine is well tolerated and has no known toxicity as indicated by the lack of adverse effects in sarcosinemia, a rare congenital condition caused by dysfunction of sarcosine metabolism that leads to the accumulation of sarcosine in plasma and urine [120]. A number of randomized, double blind, placebo-controlled trials have shown that sarcosine add-on treatment improves positive and negative symptoms as well as general functions in patients stabilized on conventional antipsychotic medication [121–124]. The beneficial effects are not limited to patients with stable positive symptoms but are also seen in patients in the acute phase of the disease [122]. It should be emphasized that all these studies administered sarcosine as an add on, and so far only one small-scale non-placebo controlled trial had evaluated sarcosine as a monotherapy [124] and the tentative trend of negative symptoms reduction reported needs to be substantiated by standard controlled trials with larger sample size.

However, adjunctive sarcosine appeared ineffective when combined with clozapine [125], which is similar to what had been learned from combining glycine or D-serine with clozapine [126]. Adjunctive glycine treatment might worsen the positive symptoms in patients maintained on baseline clozapine [126], while a significant exacerbation of the negative symptoms has been observed when D-cycloserine is combined with clozapine [118]. The reason for the unique drug–drug interaction is not fully understood. It is suspected that clozapine may already increase synaptic glycine levels through as yet unknown mechanism [127]. Hence, baseline clozapine medication should be avoided; this has been recognized in all subsequent trials with synthetic GlyT1 inhibitors.

The encouraging outcomes with sarcosine have spearheaded the development of a variety of synthetic GlyT1 inhibitors. Many of them have been evaluated in clinical trials as a potential new class of antipsychotic drugs (Table 25.2). The first generation of synthetic compounds were substituted sarcosine derivatives characterized by irreversible and non-competitive inhibition of GlyT1 such as ALX 5407 and Org24589 [128]. They were associated with motor and respiratory side effects resulting from excessive glycinergic inhibition in the brain stem and cerebellum [128–130]. This has led to the development of a second

generation of non-sarcosine-based compounds exhibiting reversible and competitive inhibition of glycine transport such as SSR504734 [128] which have been associated with fewer side effects. However, this view has been challenged by Kopec and colleagues [131] who contended that the induction of motor side effects may instead be better predicted by the target residence time, which refers to the dissociative half-life of the compound–target complex.

In all but a few clinical trials, GlyT1 inhibitors were strictly tested as add-on medication to conventional (non-clozapine) antipsychotics primarily in subsets of patients with persistent negative symptoms. However, only limited amount of data have been published or made available to the public domain despite the completion of several trials. To date, following the withdrawal of bitopertin, no other synthetic GlyT1 inhibiting compounds are being actively pursued as anti-schizophrenia agents, for reasons that are often undisclosed. Concerns over limited therapeutic efficacy and potential side effects are suggested by published studies conducted in healthy individuals. GlyT1 inhibitor, GSK1018921-induced dose-dependent dizziness [132], and compound Org 25935 exacerbated ketamine-induced cognitive deficits [133]. Additionally, R213129 and R231857 failed to antagonize psychomotor and cognitive deficits induced by the muscarinic cholinergic receptor antagonist scopolamine [134, 135].

Bitopertin: The First GlyT1 Inhibitor Entering Phase III Trials

Bitopertin is a non-competitive GlyT1 inhibitor (Fig. 25.3), also known as RG1678 and RO4917838 in the literature. It is the first selective GlyT1 inhibitor that has entered into phase III clinical evaluation, with six large-scale, multicenter trials currently in progress (see Table 25.1). Hopes for positive results seem high. Estimated sales of bitopertin as a first-in-class agent have already been forecasted to reach $1.5 billion by 2022 (source: www.pharmatimes.com).

The outcomes of the latest phase II trial of bitopertin have been partly released in the form of conference abstracts [57, 58] and company news releases (www.roche.com/irp101209.pdf) with limited details until publication of the data in 2014 [136]. That key trial evaluated the therapeutic efficacy of adjuvant bitopertin treatment (10, 30, or 60 mg/day) in schizophrenia patients with prominent negative or disorganized thought symptoms (ClinicalTrials.gov identifier: NCT00616798). Eight weeks into treatment, bitopertin adjunctive therapy was better than placebo add-on in several key measures including negative symptom factor score, personal and social performance, as well as clinical global impression improvement. These supported the on-going phase III trials, although concerns regarding the low effect size (<0.4) and limited statistical support for the key claims have been raised [137]. The statistical issue centered on the fact that the superior reduction in the negative symptom factor score was only statistically significant when the analysis was restricted to "per protocol" subjects (i.e., patients who completed the entire study), but not when the analysis included all "intent-to-treat" subjects, which better approximate the clinical situation. This concern over small effect size turns out to be the Achilles heel of the phase III trials [60–62]. Indeed, the phase III data have casted further doubt on the reliability and robustness of the benefits of bitopertin adjuvant treatment as well as its expected preferential efficacy against negative symptoms. It remains to be seen whether there are identifiable subpopulations of patients that

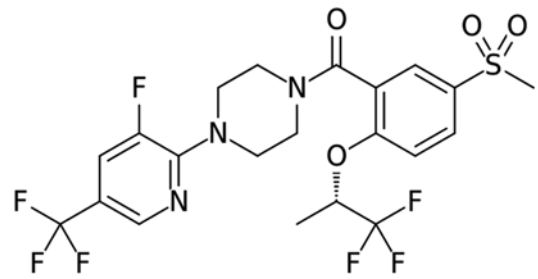

Fig. 25.3 The chemical structure of the selective GlyT1 inhibitor,bitopertin(4-[3-fluoro-5-(trifluoromethyl)pyridin-2-yl]piperazin-1-yl}{5-(methylsulfonyl)-2-[(1S)-2,2,2-trifluoro-1-methylethoxy]phenyl}methanone) also known as RO4917838 or RG1678, synthesized by Hoffman-La Roche

may benefit substantially from bitopertin after data from all six phase III are put under further scrutiny.

Critical dose-dependent data that were identified in the phase II study have been fully incorporated in the design of the phase III clincal trials. Specifically, the highest dose (60 mg/day) was devoid of any therapeutic effects beyond placebo, and was further associated with adverse effects that resulted in the withdrawal of 9 % of the patients from the phase II study [136]. All positive outcomes identified in the phase II trial were restricted to the two lower doses at 10 and 30 mg/day. The inverted U-shaped dose–response relationship indicates that optimal response should correspond to approximately 50 % target occupancy [55]. This implies that the effective dose range might be rather narrow (10–30 mg/day), and dose titration may be necessary to tailor individual patients using positron emission tomography [138].

The potential clinical efficacy of bitopertin when administered alone has also been pursued by Roche. The results of a phase II/III monotherapy study (NCT01234779) has yielded some interesting leads, suggesting again an efficacy against positive symptoms rather than negative symptoms [139]. It is largely in agreement with the reduction in positive symptoms observed in the phase II bitopertin adjuvant trial [136] with an effect size (−0.38) comparable with the reported improvement in negative symptoms (www.roche.com/irp101209.pdf). The drug may alone be sufficient to control both positive and negative symptoms, and preclinical evidence gathered from other synthetic GlyT1 inhibitors is pointing to a similar suggestion [53, 65]. Furthermore, more detailed neuropsychological assessments of cognitive function would be more instructive than relying on global functioning to index cognitive improvement. As a result, the magnitude of improvement in specific functions such as working memory, executive function, and selective attention in patients is still unclear and the on-going phase I study of bitopertin (NCT01116830) designed specifically to evaluate cognitive symptoms is timely.

Since the first introduction of clozapine nearly 40 years ago that marks the prototypical second generation antipsychotic drug [140, 141], bitopertin may define a new third generation of antipsychotic drugs contingent on favorable outcomes of the current phase III trials. In the event that bitopertin is approved as a new class of antipsychotic medication, guidance from the mechanistic perspective would facilitate the search and design of better "follow-on" compounds. Preclinical research will therefore continue to play an important role in the future development of GlyT1 inhibition therapy.

Mechanistic Considerations: Beyond the NMDA Receptors

The original concept behind GlyT1 inhibition therapy has solely emphasized the potentiation of NMDA receptor function as its goal, but the possible contribution of enhanced glycinergic inhibition should not be ignored. As explained in the beginning of this chapter, GlyT1 also assumes an important role in the termination of glycinergic neurotransmission (Fig. 25.2). Early hints that such a mechanism may be relevant can be traced back to the comparison between two specific mutant mouse models of GlyT1 disruption that our laboratory has extensively characterized. Mutant mouse models permit the localized disruption of GlyT1 confined to discrete brain areas or even cell types, and thereby they can achieve a level of specificity not readily realized by systemic pharmacological interventions. The divergent as well as convergent phenotypes observed in different gene disruption models are instructive in advancing our understanding of the brain circuitry mechanism involved in the modifications of higher brain function by GlyT1 inhibition [53].

Among existing genetic models, the mutant $Cre^{CaMKII\alpha}$:$GlyT1^{fl/fl}$ line with forebrain neuronal disruption of GlyT1 has yielded, by far, the clearest anti-psychotic and pro-cognitive phenotypes [53]. This mouse line exhibits multiple phenotypes that are opposite to the deficits observed in schizophrenia patients. Some of these "schizophrenia-resilient" phenotypes include resistance to psychomimetic drugs

(amphetamine and phencyclidine), faster reversal learning, and enhanced latent inhibition [54]. By contrast, these phenotypes are notably absent in the Cre^{Emx1}:$GlyT1^{fl/fl}$ mouse line, in which GlyT1 disruption is restricted to the telencephalon. The CaMKIIα promoter drives *Cre* expression in principal neurons of the entire forebrain that includes the striatum, whereas the Emx-1 promoter only allows *Cre*-mediated gene deletion in cells in the cortical mantle and limbic cortices (i.e., the telencephalon). The GlyT1 expression in the striatum is therefore preserved in the Cre^{Emx1}:$GlyT1^{fl/fl}$ mouse line but disrupted in the $Cre^{CaMKIIα}$:$GlyT1^{fl/fl}$ line. One speculation is that disruption of striatal GlyT1 may be critical for the schizophrenia-resilient phenotypes unique to the $Cre^{CaMKIIα}$:$GlyT1^{fl/fl}$ line. This is supported by the report that local GlyT1 disruption confined to the nucleus accumbens (the key component of the ventral striatum) is sufficient to confer resistance to psychomimetic drugs and to enhance latent inhibition [142]. This suggests that the mechanism underlying the antipsychotic potential of GlyT1 inhibition might in part stem from its ability to modulate striatal dopamine function, perhaps through regulation of the interaction between dopaminergic and glutamatergic input converging onto striatal principal neurons (Fig. 25.4) which has been predicted on theoretical grounds [143, 144].

Another possible link to striatal dopamine has been highlighted by a separate line of research into a new treatment against alcohol dependence that targets glycinergic inhibition within the striatal circuitry [145]. As illustrated in Fig. 25.4, release of dopamine in the nucleus accumbens (NAC) and prefrontal cortex (PFC) can be modulated by inhibitory glycine receptors located on

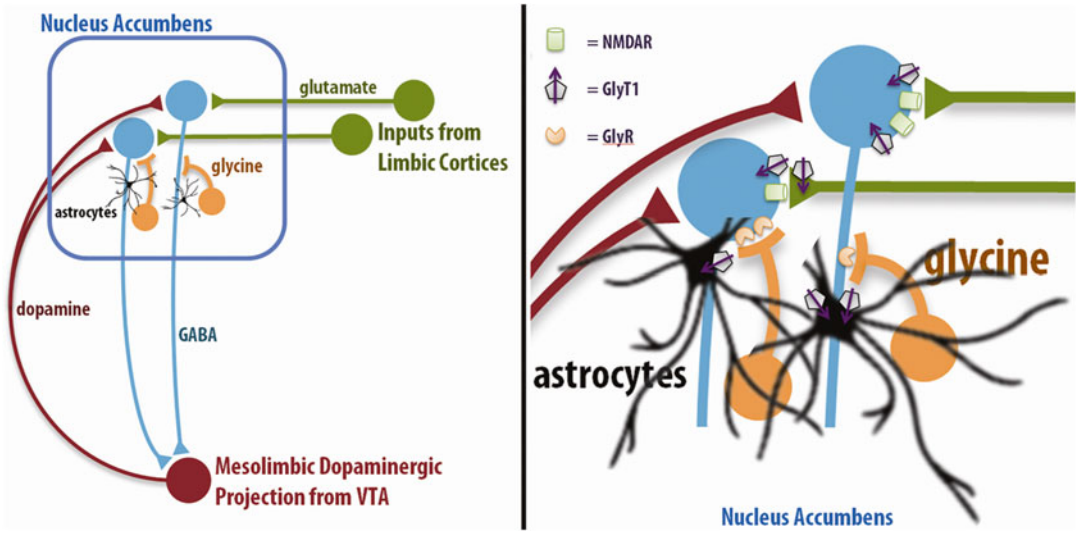

Fig. 25.4 GlyT1 inhibition and the modulation of striatal dopamine. The potentiation of *N*-methyl-D-aspartate (NMDA) receptor activation following neuronal GlyT1 inhibition is expected to strengthen the excitatory signals to the nucleus accumbens (in *green*) originating from limbic areas, e.g., the hippocampus, amygdala and entorhinal cortex. The limbic excitatory signals converge onto the same GABAergic medium spiny neurons (MSNs, depicted in blue) in nucleus accumbens that are also innervated by a dopaminergic projection (in *red*) originating from the ventral tegmental area (VTA) in the brain stem. Networks of accumbal MSNs carry out essential integration of the relevant glutamate and dopamine signals. Augmentation of the glutamatergic inputs via NMDA receptors by the inhibition of GlyT1 could restore the imbalance associated with excessive dopamine activity implicated in the production of positive symptoms and specific attentional dysfunction in schizophrenia. On the other hand, astrocytic GlyT1 inhibition is expected to potentiate glycinergic inhibitory neurotransmission (in *orange*) that regulate the GABA-ergic feedback projection (in *blue*) from the nucleus accumbens back to the VTA. This effectively disinhibits the tonic GABA-ergic suppression of VTA dopaminergic input to the nucleus accumbens. This may have an impact on the negative symptoms of schizophrenia (e.g., avolition and apathy) because a similar mechanism has been suggested to mediate rewarding effects associated with alcohol consumption

NAC medium spiny neurons that project back to the ventral tegmental area (VTA). Disruption of striatal GlyT1 is sufficient to potentiate glycinergic inhibition of the GABAergic NAC → VTA feedback projection, and thereby disinhibits the release of dopamine into the NAC from the VTA. This action of GlyT1 inhibition on the mesolimbic dopamine reward system has been hypothesized to underlie the observed reduction of relapse in animal models of alcohol dependence [145, 146]. This mechanism may explain the efficacy of systemic GlyT1 inhibition to facilitate the release of dopamine (originating from the VTA) into the NAC and PFC, which may be linked to promnesic effects of SSR504734 on working memory performance observed in wild type mice [79]. The same pathway, perhaps, may also contribute to the positive effects of the selective GlyT1 inhibitors, SSR504734 and SSR103800, in preclinical tests of anti-depressant action including resistance to stress [75]. It is therefore conceivable that GlyT1 inhibition is capable of regulating the activity of the mesocorticolimbic dopamine system in either direction through the modulation of neuronal inhibition via glycine receptors as well as neuronal excitation mediated via NMDA receptors (Fig. 25.4).

Another line of reasoning has led us to consider the relevance of glycinergic inhibition outside the striatum. Working memory is one of the cardinal features of schizophrenia cognitive impairment. Working memory performance was apparently enhanced in Cre^{Emx1}:$GlyT1^{fl/fl}$ mice [147] but there was no evidence that $Cre^{CaMKII\alpha}$:$GlyT1^{fl/fl}$ mice exhibited such a phenotype [148]. We reason that within the cortical mantle and limbic cortices, the additional loss of glial GlyT1 in Cre^{Emx1}:$GlyT1^{fl/fl}$ mice is expected to produce a stronger disruption of glycine uptake than in the $Cre^{CamKII\alpha}$:$GlyT1^{fl/fl}$ mice in which only neuronal GlyT1 is disrupted; and this has both quantitative and qualitative implications [53]. Not only was the overall blockade of glycine reuptake more severe in the Cre^{Emx1}:$GlyT1^{fl/fl}$ line, but the disruption of GlyT1 in astrocytes near glycinergic synapses may also delay the termination of the inhibitory glycinergic signals as the clearance of pre-synaptically released glycine becomes less efficient. Although the precise relevance of glycinergic inhibitory neurotransmission to higher brain functions remains unknown, neuronal inhibition mediated by glycine receptors as well as NMDA receptor excitation recorded in hippocampal slices is highly sensitive to the application of GlyT1 inhibitor [149]. Although GABAergic neurotransmission remains the major source of neuronal inhibition in the forebrain, glycinergic inhibition can readily influence network activity in the hippocampus and entorhinal cortex via direct inhibition of principal neurons or cross-inhibition with GABAergic activity [150]. Hence, it would be instructive to ascertain the extent to which glycinergic neurotransmission might be modified in the Cre^{Emx1}:$GlyT1^{fl/fl}$ mice and in wild type animals after administration of GlyT1 inhibitor at doses that are effective in enhancing working memory [79].

Conclusions

We end this chapter with a note on mechanisms because they are important considerations for the future development of GlyT1 and related drug targets (e.g., D-serine and its metabolic enzymes) for new and better treatments for schizophrenia. As the phase III trials of bitopertin has ended in failure, guidance from the mechanistic perspective would become even more critical for the continual race for the first-in-class compound. We have reviewed here some of the critical evidence indicating that GlyT1 inhibition may modulate multiple neurotransmitter pathways, including mesolimbic dopamine and brain glycinergic inhibition, even though the initial aim is solely to enhance NMDA receptor activity on the basis of the glutamate hypothesis of schizophrenia. The dopaminergic and glycinergic mechanisms may be critical to the anti-schizophrenia efficacy of GlyT1 inhibition as well as possible side effects that may be responsible for the disappointing outcomes of many candidate GlyT1 inhibitors. Therefore, one should not read the commercial decision by Roche to shelf the development of bitopertin as anti-schizophrenia drug as a failure of the glycine augmentation (through

inhibition of GlyT1) strategy to control schizophrenia symptoms or the refutation of the glutamate hypothesis. The recent excitement over bitopertin certainly will be remembered as an important landmark after years of stagnation in the field of anti-schizophrenia drug discovery.

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Adenosine in the Neurobiology of Schizophrenia: Potential Adenosine Receptor-Based Pharmacotherapy

26

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Introduction

In the late 1920s, Drury and Szent-Györgyi demonstrated that in addition to modulate kidney functioning in mammals, adenosine promoted profound hypotension and bradycardia [1]. Since that time, the physiological role and potential therapeutic use of adenosine have been largely studied [2, 3]. Indeed, a deficit in endogenous nucleosides, particularly adenosine, has been associated to multiple neurological diseases and neuropsychiatric conditions including epilepsy, chronic pain, and schizophrenia [4]. Therefore, increasing adenosinergic function either by inhibiting adenosine metabolism or by activating adenosine receptors appears to be a rational therapeutic strategy for these adenosine-associated diseases. Accordingly, in this chapter we will first highlight the role of adenosine function and dysfunction in physiological and pathophysiological conditions (i.e., schizophrenia), and then we will explore the potential use of adenosine-based drugs as new pharmacotherapeutic opportunities for schizophrenia.

Adenosine: An Overview

The purinergic neurotransmission system is a signaling system involving two main extracellular effectors, namely adenosine and adenosine 5′-triphosphate (ATP, Fig. 26.1a) [5]. Thus,

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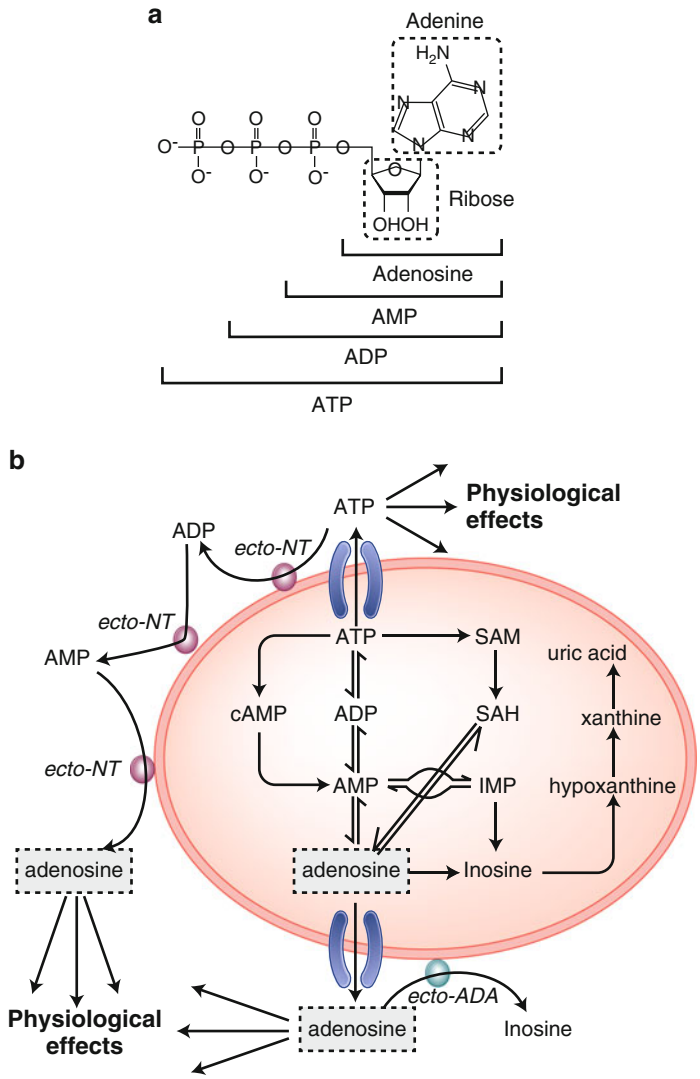

Fig. 26.1 (**a**) The structure of adenosine 5′-triphosphate (ATP) and ATP derivatives. (**b**) Schematic representation of purine metabolism from ATP to uric acid. ATP is degraded to hypoxanthine, which can be salvaged by hypoxanthine–guanine–phosphoribosyl–transferase (HGPRT) or further metabolized to xanthine and uric acid by xanthine oxidase. Enzymes are in italics. Guanine-based purines (GTP to guanosine, guanine and xanthine) are not illustrated. Allopurinol inhibits the enzyme xanthine oxidase, possibly favoring purine salvage. Purinergic system and sources of extracelular adenosine. ATP stored in presynaptic vesicles is released after depolarization, acting on P2 receptors and being dephosphorylated by ecto-NTPDases and ecto-5′nucleotidase (ecto-5′NT) into adenosine (ADO). ADO acts mainly on A_1R and A_2AR and can be converted into inosine extracellularly by ecto-adenosine deaminase (ecto-ADA) or uptaken by nucleoside transporters. Inside the cell, ADO preferentially forms AMP by adenosine kinase (AK) but also may be deaminated by ADA (not shown). The sources of extracellular adenosine are ATP, adenosine released as such by nucleoside transporters (in situation of energetic imbalance, such as brain damage), and cAMP, which is converted into AMP by an ecto- phosphodiesterase and later into ADO

adenosine acts as a neuromodulator in the central nervous system (CNS), while ATP is actually classified as a neurotransmitter. In such a way, despite the initial resistance to consider 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 the existence of a purinergic neurotransmission system [5]. ATP was then identified as a co-transmitter in peripheral nerves and subsequently as a co-transmitter with glutamate, noradrenaline, gamma aminobutyric acid (GABA), acetylcholine, and dopamine in the CNS [6]. Interestingly, extracellular ATP is quickly hydrolyzed into adenosine 5′-diphosphate, adenosine 5′-monophosphae (AMP), and adenosine plus inorganic phosphate, through the action of extracellular nucleotidases (ecto-NTs) (i.e., ecto- NTPDases and ecto-5′-nucleotidase, Fig. 26.1) [7].

Adenosine consists of a purine base (adenine) attached to the 1′ carbon atom of ribose (Fig. 26.1a). As mentioned above, this ribonucleoside is mostly produced by ATP catabolism, both at the intra- and extracellular level (Fig. 26.1b), although to a lesser extent it can also be generated by S-adenosyl-L-homocysteine (SAH) metabolism (Fig. 26.1b). Once formed, adenosine can be released either through Na^+-dependent transporters or intracellularly phosphorylated to form AMP by the action of adenosine kinase (Fig. 26.1b). In addition, adenosine can react with L-homocysteine to form SAH (Fig. 26.1b). Finally, adenosine can be deaminated to 6-1B. Interestingly, adenosine has been historically considered a retaliatory metabolite [8] that increases oxygen supply and decreases oxygen consumption, thus modulating a large array of physiological processes. In such a way, adenosine impinges on respiratory function [9], neural activity [10], platelet aggregation [11], neutrophil function [12], lymphocyte differentiation [13], and vascular tone [14]. Additionally, adenosine is able to provoke both coronary arteries dilatation and kidney blood vessels contraction, thus reducing renal filtration [15]. Adenosine also exerts a negative chronotropic and dromotropic effect on the heart [16] and mediates the inhibition of neurotransmitters release [17] and lipolysis [18]. Accordingly, it has been theorized that this purine nucleoside is a mediator of metabolic distress, thus having considerable impact on homeostatic cellular functioning.

On the other hand, adenosine has been shown to play a key regulatory role in the CNS, 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 are related 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 proposed 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. 26.1b). Overall, because 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 CNS

In 1972, it was shown that 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]. This phenomenon was also observed in other tissues such as the heart [26]. Collectively, these observations constituted the first piece of work suggesting that extracellular adenosine exerted its effects via specific plasma membrane receptors. It was later demonstrated that the adenosine-mediated antilipolytic effect on fat cells occurred with a concomitant reduction in cAMP [27]. This dual effect of adenosine on cAMP formation was further substantiated when it was demonstrated that adenosine could either inhibit or stimulate adenylyl cyclase. Overall, these observations concluded with the first subclassification of adenosine receptors into R_i and R_a [28], or alternatively, A_1 and A_2 adenosine receptors [29].

It is currently well established that adenosine mediates its actions by activating specific G protein-coupled receptors (GPCRs), for which

Table 26.1 Adenosine receptors

Receptor	Adenosine affinity	G protein	Transduction mechanisms[a]	Physiological actions
A_1	~70 nM	$G_{i/o}$[b] $G_{q/11}$ G_s	•*Inhibits*: AC[b] •*Activates*: PLC, AC	Vasoconstriction [31]; hypothermia and sedation [32]; analgesia [33]; neurotransmitter release [34, 35]; chemotaxis [36]; Neuroprotection [37]
A_{2A}	~150 nM	G_s[b] G_{olf} $G_{15,16}$ [§]	•*Activates*: AC[b], 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 nM	G_s[b] $G_{q/11}$	•*Activates*: AC[b], PLC	Vasodilation [43]; vasoconstriction [44]; cytokine production [45]; inhibition of cell proliferation [46]
A_3	~6,500 nM	$G_{i/o}$[b]	•*Inhibits*: AC[b] •*Activates*: PLC	Mast cell activation [47]; preconditioning [48]; coronary vasodilation [49]; regulation of intraocular pressure [50]; hypotension [51]

[a]*AC* adenylyl cyclase, *PLC* phospholipase C, *PLA2* phospholipase A2, *PLD* phospholipase D, *GIRKs* G protein-dependent inwardly rectifying K^+ channels
[b]Main mechanism of coupling

four subtypes (A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R) have been identified to date. These receptors have a distinguishable pharmacological profile, tissue distribution, and effector coupling [30], and its functioning has been largely studied in the CNS (Table 26.1). Adenosine receptors belong to the rhodopsin family, or class A of GPCRs [52], thus sharing some common molecular features. For instance, within their sequence, all adenosine receptors contain the widely conserved NPxxY(x)5,6 F and the DRY motifs [53, 54]. Thus, adenosine-mediated conformational rearrangement of adenosine receptors determines the binding and activation of specific G proteins (Table 26.1) which are responsible for activation of different intracellular signaling pathways associated to adenosine function (Table 26.1).

A_1Rs and $A_{2A}Rs$ are primarily responsible for the central effects of adenosine (Table 26.1) [55]. The most abundant and homogeneously distributed adenosine receptor within the brain is the A_1R, which is functionally coupled 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 (i.e., adenylyl cyclase, Ca^{2+} channels, K^+ channels, and phospholipase C; Table 26.1) [56]. Conversely, $A_{2A}R$ is expressed at high levels only in some specific brain regions (e.g., striatum, olfactory tubercle, and nucleus accumbens) [23, 57]. $A_{2A}Rs$ are mainly coupled to G_s/G_{olf} proteins [58], thus activating adenylyl cyclase and increasing intracellular cAMP levels (Table 26.1). Interestingly, $A_{2A}R$ may also signal through a G-protein independent pathway eventually associated to mitogen-activated protein kinase signaling cascade activation [59]. Next, the $A_{2B}R$ is positively coupled to adenylyl cyclase and phospholipase C (PLC) through G_s and G_q proteins, respectively (Table 26.1) [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 because the $A_{2B}R$-specific pharmacological tools are limited [60]. Finally, A_3R has been shown to be coupled to $G_{i/o}$ proteins, thus inhibiting adenylyl cyclase and also stimulating PLC (Table 26.1) [2].

The Adenosine Hypothesis of Schizophrenia

Schizophrenia comprises a heterogeneous group of syndromes of unknown etiology. It is a serious mental disorder affecting up to 1 % of the population worldwide that usually arises during late

adolescence and early adulthood (i.e., median age onset is about 23 years in men and 28 years in women). The definition of schizophrenia has evolved throughout the five editions of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) published by the American Psychiatric Association. 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: no one symptom was pathognomonic of the disorder. Additionally, in the fourth version the pathology had high diagnostic stability, with 80–90 % of individuals receiving an initial diagnosis of schizophrenia and retaining that diagnosis at 1–10 years [61, 62]. However, in the current DSM-5 version, five characteristic symptoms for the diagnosis of schizophrenia are established with the requirement that at least two of the symptoms be present for a month [63]. Three changes with regard to the previous version have been made and 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 [63]. Of note, the current 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 gained. Finally, the cognitive symptoms include alterations in attention, working memory, executive functions, and social cognition.

Several theories about the neurotransmission systems affected in schizophrenia have been presented. Thus, nearly all known neurotransmission systems (i.e., dopaminergic, glutamatergic, serotoninergic, GABAergic, and cholinergic) have been in one way or another involved in schizophrenia, although none of those theories fully explains the fundamental pathological process(es) associated with the disease. Interestingly, although they were initially proposed as separate hypothesis (i.e., the "glutamatergic" and the "dopaminergic" hypothesis) [64, 65], one of the most currently supported theories is based on a combined hyperdopaminergic-hypoglutamatergic phenomenon [66]. Indeed, current pharmacotherapy for schizophrenia is based on a "dopamine" and "glutamate" hypothesis, which is centered in a striatal dopamine D_2 receptor (D_2R) hyperfunction, a deficient stimulation of prefrontal cortex (PFC) dopamine D_1 receptors (D_1Rs) and a N-Methyl-D-aspartate (NMDA) receptor hypofunction in the PFC [67]. However, because negative symptoms, cognitive dysfunction, and decrements in psychosocial and vocational functioning often are still persistent on available pharmacotherapy, the development of a next generation of pharmacologic agents tackling these resilient symptoms is needed [68]. Overall, additional research in non-dopaminergic and non-glutamatergic alternatives is necessary to improve the caveats in schizophrenia treatment.

As mentioned above, 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 [69–71]. Thus, adenosine agonists and antagonists produce behavioral effects similar to those of dopamine antagonists and dopamine agonists, respectively [72]. Additionally, adenosine tone can also modulate glutamatergic neurotransmission [73, 74]. Hence, adenosine may play a unique role integrating glutamatergic and dopaminergic neurotransmission systems. Accordingly, a purinergic hypothesis of schizophrenia was proposed by

Lara and co-workers longer than a decade ago [75]. The authors theorized 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 schizophrenia phenotype [76]. Indeed, several experimental sources of evidence supported the adenosine hypothesis of schizophrenia, which will be highlighted in this chapter.

Experimental Evidence: Preclinical Models of Schizophrenia

In general, a considerable lack of knowledge still exists concerning psychiatric illnesses. Of note, this incomprehension is especially remarkable in the case of a highly intricate pathology as is schizophrenia. For that reason, animal models eventually mimicking some of the schizophrenia-associated symptoms may not precisely mirror what exactly occurs in a schizophrenic human patient. However, preclinical models can be valuable experimental tools to shed light on the mechanisms behind the etiopathology of schizophrenia. As mentioned above, the adenosinergic hypothesis of schizophrenia was proposed to interconnect both schizophrenia-associated dopaminergic hyperfunction and glutamatergic hypofunction. Indeed, some experimental sources of evidence obtained from experimental animal models supported the adenosine contribution to schizophrenia through the modulation of both dopaminergic and glutamatergic neurotransmission [77]. Hence, we will review the animal models supporting the glutamatergic hypofunction theory (e.g., phencyclidine model) and the hyperdopamergic hypothesis (e.g., amphetamine model) and its relationship with adenosinergic neurotransmission.

The formulation of the hypoglutamatergic-NMDA receptor hypothesis appeared in the late 1950s, when it was observed that phencyclidine (PCP) provoked a psychotic-like condition similar to that observed in schizophrenic patients [78]. However, no one suspected that NMDA receptors were behind this phenomenon until the 1980s when Lodge and colleagues [79] 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 [80], thus disrupting glutamatergic and dopaminergic neurotransmission in this brain region. It was then believed that the neurotransmitter imbalance could be well correlated with the cognitive and behavioral perturbations observed in schizophrenia [80–82]. Interestingly, the administration of NMDA receptor antagonists either in the late fetal or in the postnatal period of rats was shown to increase the neuronal death by apoptosis [83], a phenomenon that would be linked to adult schizophrenia-like behavior. On the other hand, administration of the same type of compounds in the adult animal increased the neuronal damage by necrosis with the subsequent gliosis [84], also associated with psychotic-like behavior. In conjunction, these experimental observations substantiate a neurodevelopmental link between NMDA receptor antagonists and schizophrenia. Thus, the hypoglutamatergic-NMDA receptor theory suggests the existence of disturbances in the pre- and perinatal brain development that could provoke clinical manifestations in early adult life [85]. 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, root of the glutamatergic hypothesis of schizophrenia [86], has been historically sustained by pharmacological animal models using NMDA receptor antagonists (i.e., PCP, ketamine, and dizocilpine). PCP is a dissociative drug first synthesized in 1926 as a surgical anesthetic. Despite its efficacy, the use of the drug was not prolonged because of its concomitant adverse effects (e.g., hallucinations, delusions, and agitation). Thus, because PCP mimics some schizophrenia symptoms in humans it has been extensively used in animals as a model for the illness. Indeed, acute administration of PCP produced hyperlocomotion [87], social withdrawal [88], and failures both in cognition [89] and in

sensorimotor gating [90] in rodents. On the other hand, chronic PCP treatment also promoted hyperlocomotion as indicative of positive symptoms. Furthermore, chronic treatment induced deficits in social behavior and reduced mobility in the forced swimming test, which corresponds to negative symptoms. As for the cognitive symptoms, PCP-treated animals displayed sensorimotor gating deficits and cognitive dysfunctions when subjected to learning and memory tests [91]. Interestingly, these PCP-mediated schizophrenic-like symptoms were maintained in humans for several weeks following chronic treatment [92, 93]. Thus, the PCP-induced model of schizophrenia seems to partially resemble the pathology, although some criticisms exist regarding this PCP-based animal model. For instance, sensorimotor deficit in the prepulse inhibition test does not last after PCP withdrawal in animals, which differs from the human disease; some discrepancies have been reported regarding negative symptoms between clinical features and PCP-treated animals [91].

Interestingly, adenosine receptors have been shown to be able 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 [94, 95], while $A_{2A}R$ blockade, but not A_1R, prompted exhacerbation of the motor-estimulant effects of the NMDA antagonist [96]. PCP-induced psychomotor activities were enhanced in a KO mouse specifically lacking the striatal neuronal $A_{2A}R$ [97]. However, an opposite effect was observed in a knockout mouse lacking the forebrain $A_{2A}R$ with the $A_{2A}R$ deleted in the neurons of the striatum and the cerebral cortex and hippocampus. Accordingly, a critical role for $A_{2A}Rs$ in extrastriatal neurons was described in providing a major excitatory effect on psychomotor activity [97]. 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 to the hypoglutamatergic-NMDA receptor hypothesis, the dopaminergic hypothesis of schizophrenia had several important conceptual changes throughout its history. It was initially based on a generalized hyperdopaminergic brain function, but quickly evolved into a combined subcortical hyperdopaminergic-prefrontal hypodopaminergic dysfunction. Nevertheless, Howes proposed an updated third version based on multiple changes of different neurotransmitters and neural systems, which 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 for schizophrenia and proposes that these interact to funnel through one final common pathway of presynaptic striatal hyperdopaminergia [98].

The hyperdopaminergic status of schizophrenia has been largely studied through means of pharmacological animal models. Thus, the administration of drugs (i.e., amphetamine) that increase the brain dopamine content is a classical experimental approach to study schizophrenia-associated positive symptoms. Amphetamine, first discovered in 1887 [99], is currently used as an attention deficit (i.e., attention deficit hyperactivity disorder) and narcolepsy treatment [100]. It is a drug that acts as a strong CNS stimulant by increasing dopamine concentration in the synaptic cleft, thus raising the response in the postsynaptic neuron. Apart from the well-known positive effects, its administration could also provoke long-term cognitive impairments [101, 102]. 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 [103–106], the studies reporting amphetamine-mediated negative symptoms are scarce.

Dopamine receptors on striatonigral and striatopallidal neurons (D_1R and D_2R, respectively) play a pivotal role in the control of motor responses [107], thus the efficacy of many antipsychotic drugs correlates well with their ability to block D_2Rs [108]. Because $A_{2A}Rs$ antagonistically interact with D_2Rs [109, 110], adenosine is

expected to exert a regulatory influence on psychomotor behavior, and indeed a role for $A_{2A}R$ regulating amphetamine-induced psychomotor behavior has been described [111]. Thus, $A_{2A}R$ activation restored responsiveness to amphetamine in adenosine-deficient mice [111]. The abovementioned preclinical data have 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 that the adenosinergic system can be altered in schizophrenia. The first striking piece of information pointing to this consists of the finding that $A_{2A}R$ expression was shown to be increased in necropsies from schizophrenic individuals [112]. Because the adenosinergic tone was shown to be reduced in schizophrenia, $A_{2A}R$ up-regulation could correspond to an adaptive physiological condition that in turn would be associated to a concomitant hyperdopaminergic state [76]. Interestingly, the genetic linkage of adenosine receptors to schizophrenia has been somehow evaluated. While an $A_{2A}R$ genetic variant (i.e., 1976 T>C) was not shown to confer susceptibility to schizophrenia [113], the involvement of some A_1R gene polymorphisms in the pathophysiological mechanisms of schizophrenia was suggested [114]. 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 [115]. Collectively, these results support the hypothesis of lower adenosinergic activity in schizophrenia.

Based on prior data, it seems feasible to think that the use of pro-adenosinergic drugs could be beneficial for the treatment of the pathology. However, this pharmacological proposal is still vague although some data exist regarding this hypothesis. Indeed, raising the endogenous pool of purines with allopurinol has been shown to produce promising results as add-on therapies for schizophrenia [116, 117]. Allopurinol is a well-known hypouricemic drug that inhibits xanthine oxidase (Fig. 26.1b) and was used as an add-on drug in the treatment of poorly responsive schizophrenic patients [116]. In a 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, general psychopathology symptoms, as well as the Positive and Negative Syndrome Scale (PANSS) total scores [116]. In a similar study, a double-blind, placebo-controlled, cross-over clinical trial of add-on allopurinol (300 mg/day) for poorly responsive schizophrenia or schizoaffective disorder (DSM-IV criteria), was conducted on 22 patients [117]. Allopurinol was an effective and well-tolerated adjuvant treatment, particularly for refractory positive symptoms [117]. Allopurinol also showed effectiveness as an adjunctive medication in schizophrenia outpatients ($N=59$) with persistent symptoms despite adequate pharmacotherapy [118]. In a recent case report, allopurinol prompted a rapid decrement of psychotic symptoms in a patient with schizophrenia [119]. Thus, within 2 weeks of allopurinol adjuvant therapy, the patient showed significant improvement with respect to positive and negative symptoms of schizophrenia (PANSS scores went from 88 to 41 within 2 weeks) [119]. Collectively, 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 a routinely used adjuvant therapy to antipsychotics [120]. 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 [121]. 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 [121], but it also

showed effectiveness when combined with lithium in the treatment of acute bipolar mania [122].

Although some clinical controversy has been established around allopurinol [123], the adenosine modulator adjuvant therapy was shown to be beneficial in overall psychopathology (especially positive symptoms) in schizophrenia and in treating mania episodes of bipolar disorder when compared with placebo [124]. However, larger and superior clinical trials are needed to undeniably sustain the use of these drugs in schizophrenia. Overall, all the abovementioned clinical data support the adenosine hypothesis of schizophrenia and highlight the potential pharmacological interest of combining antipsychotic drugs with a purinergic-based compound (i.e., allopurinol) 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 promoted mostly psychotic-like behavior 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 dichotomy exists when the adenosine receptor-based drugs are planned to be used in schizophrenia treatment. Nevertheless, based on the adenosinergic hypothesis, $A_{2A}R$ agonists would be selected. The antipsychotics that are currently under clinical use have D_2R antagonistic activity and because of the high level of expression of $A_{2A}Rs$ and the D_2Rs in the striatum [125] 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 [71]. The idea that $A_{2A}R$ agonists might be of interest for the treatment of schizophrenia initially derived from studies just showing the existence of the antagonistic intramembrane interaction between $A_{2A}R$-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 [71, 95]. 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 [95]. Furthermore, the $A_{2A}R$ agonist was more potent than haloperidol or clozapine at antagonizing the motor activity induced by PCP than the amphetamine-mediated one [95]. Overall, these results demonstrated an apparent "atypical" antipsychotic profile (i.e., low liability to induce 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 [126], $A_{2A}R$ agonists also showed detrimental effects at the central level in animal models for learning and memory [127]. These associated problems of direct $A_{2A}R$ activation with specific agonists would be a consequence of the lack of spatial anatomical resolution of these compounds, a common generalized problem in pharmacology. In addition to target striatal $A_{2A}Rs$ which would counteract the schizophrenia-associated D_2R hyperfunction, the compounds would also block extrastriatal (e.g., cortical) and peripheral $A_{2A}Rs$ with the concomitant detrimental effects discussed above. That 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 which can be achieved by targeting enzymes or nucleoside transporters that control the extracellular levels of adenosine [4]. However, the anatomical resolution of the adenosine raise might compromise its therapeutic efficacy, and a new adenosine-based drug for the treatment of the pathology has still not been developed.

Concluding Remarks

Since the first therapeutic consideration for adenosine in the 1930s [128], a remarkable wide range of diseases has been presented for treatment with adenosine-based drugs [2, 128]. Agonists and antagonists of adenosine receptors

[31–51] 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, pulmonary, and other inflammatory conditions, whereas antagonists are being explored for the treatment of Parkinson disease and congestive heart failure for which selective compounds are already in clinical trials. Furthermore, adenosine receptor-based drugs are under consideration for the management of dreadful and challenging diseases such as schizophrenia. However, the therapeutic proposal for schizophrenia is compromised by the anatomical distribution and functionallity 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 treatments 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 that are able to discriminate between subpopulations of $A_{2A}R$ in different brain regions.

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Mindfulness and Neuroimaging

27

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Introduction

Neuroimaging Techniques Based on Magnetic Resonance Imaging

In current research contexts, mindfulness is defined as nonjudgmental attention to experiences in the present moment [1]. Mindfulness is cultivated in formal meditation practices such as sitting meditation, walking meditation, and mindful movements [2]. Mindfulness meditation has beneficial effects on a number of psychiatric, somatic, and stress-related symptoms and therefore, has been increasingly incorporated into psychotherapeutic programs [3].

Aspects of human experience such as emotional responses, attention, or intention that meditators say can be changed with practice, are all defined as behaviors, and these behaviors are the result of brain activity, which in turn depends on the structure of the brain. Brain structure can be defined very loosely as anything related to how neurons communicate with each other, ranging from the number of connections between neurons to the amount of neurotransmitter that is released between them. It is generally believed that to have a lasting change in behavior, there must be a corresponding change in brain structure. This is called neuroplasticity.

We can hypothesize that in the course of the practice of meditation, the brain must show a specific pattern of brain activity that is different from normal brain activity. Repeated practice of this activity should lead to changes in brain structure that are related to the practice. Consistent with this idea, researchers have begun to identify specific neural networks activated in different forms of meditation.

The scientific basis of mindfulness involves attention, body awareness, the regulation of emotion, changes in self-perception, and the neural modulation of specific brain areas including the anterior cingulate cortex, posterior cingulate cortex, medial prefrontal cortex, insula, temporo-parietal

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junction, hippocampus, and amygdala [4]. Several neuroimaging studies support these data.

Functional magnetic resonance imaging (fMRI) is an imaging method for the mapping of activation patterns in various areas of the brain. This is an important technique to better understand brain function. When a brain region is activated, additional energy must be transported to this region, which leads to increased blood flow to that part of the brain. This can be imaged by repetitive magnetic resonance scans and detected by appropriate signal processing methods.

In studies using fMRI, changes can be observed when a task is performed or even when the subject is at rest. This technique has traditionally been used to study brain activation upon performance of a task; more recent studies have focused on the connectivity between brain areas (based on the co-activation of two areas).

Microstructural changes in grey matter and/or white matter can be revealed by specialized magnetic resonance imaging (MRI) techniques such as diffusion tensor imaging (DTI). This method analyzes the diffusion of protons in tissue, which is more restricted in white matter than in grey matter.

Proton magnetic resonance spectroscopy is one technique used to assess potential disruptions in neuronal integrity and associated neurochemical dysregulations. Magnetic resonance spectroscopy (MRS) is based on the differences in resonance obtained from hydrogen nuclei depending on the surrounding atoms (chemical shift). Each metabolite assessed produces a different hydrogen resonance frequency and appears in a different site in the spectrum. Each spectrum shows peaks corresponding to the different metabolite values: myo-inositol (mI), 3.56 and 4.06 ppm (part per million); choline compounds, 3.23 ppm; creatine (Cr), 3.03 and 3.94 ppm; N-acetylaspartate (NAA), 2.02; 2.5 and 2.6 ppm; glutamine and glutamate, 2.1–2.55 ppm and 3.8 ppm.

Default Mode Network

Despite the robustness of the default mode network (DMN) phenomenon in neuroimaging experiments, its precise meaning with respect to behavior is not well defined. Based on existing studies, the DMN has been associated indirectly with the pattern of evoked activity that is observed in tasks involving self-judgments, autobiographical memory recall, moral dilemma, and prospective thinking, among other functions.

Having identified the areas involved in each of the components of meditation, current research suggests that the practice of mindfulness could lead to changes in the DMN. The DMN comprises a set of brain regions that are co-activated during passive task states, 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 [5]. This network would be activated in states of rest, while the subject performs "mind wandering" (i.e., without focusing attention on specific external stimuli, activation is also associated with autobiographical memory and self-referential processing) [6]. In parallel, there would be another neural network called the "task positive network" (TPN) negatively correlated with the DMN and activated when the subject is exposed to external stimuli, such as when performing a cognitive task. TPN regions include the dorsolateral prefrontal cortex, medial temporal regions, the inferior parietal lobe and motor areas [7]. Some authors have studied the relationship between functional connectivity of the DMN and the practice of mindfulness in healthy subjects in both sleep states and during different types of exercise. One such investigation [8] addressed the functional connectivity of the DMN in subjects who commonly practiced mindfulness vs. subjects who did not. Their results indicate both reduced activation of two main nodes of the DMN (posterior cingulate cortex and medial prefrontal cortex) and that experienced meditators show 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 [9] 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 [10] 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 the functional connectivity of mediators, consistent with reduced mind wandering, a greater awareness of the present moment and self-referential processing than nonmediators [8, 11].

Neuroimaging studies have analyzed the brains of people with and without meditation experience. During the first 20 min inside the MRI machine, they had spontaneous thoughts, and for the next 20 min they developed a simple exercise task focusing only on breathing. As they began to practice this exercise, meditation with the usual respiratory concentration, the activity of the medial prefrontal cortex decreased in all patients. This part of the default neural network is considered relevant to the self-centred mental processes. Furthermore, although for those who had no experience in meditation, the blood flow in the medial prefrontal region decreased a few minutes later than it did for experienced meditators, the blood supply of the area was reduced for the duration of the exercise, suggesting that meditation causes calming effects [12].

Meditation may be able to reinforce positive feelings, particulary compassion and benevolence. To test this hypothesis [13], 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 particularly stimulated those areas that had been shown in other studies to process emotional stimuli (the insula, the anterior cingulate cortex or 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.

Functional Neuroimaging Techiques Based on MRI

A recent review article [4] separated the different areas of study of meditation with four characteristics: attention regulation, body awareness, the regulation of emotions, and changing perception of the self. This chapter focuses on each of the areas involved in these processes and the studies that have been done on neuroimaging. Thus, the area underlying the regulation of attention is the anterior cingulate cortex, the insula is the principal area involved in the consciousness of the body itself, and the prefrontal cortex is studied in the context of emotional regulation.

Attention plays a central role in mindfulness. If attention wanders to another stimulus, the indication is gently returned to the original focus. Some exercises seek to develop a focal attention, whereas others extend the focus of observation to larger objects (open monitoring). Although attention includes many aspects, four aspects are associated with meditation: sustained attention (ability to remain vigilant and alert), selective attention (ability to select certain information for further cognitive processing), change of focus (ability to shift attention from one focus to another), and monitoring capability (ability to detect whether the mind wanders) [14, 15]. Improvements in care would be linked to the type of practice used. Selective attention, for example, can be developed in earlier stages where attentional targeting is practiced, whereas other areas such as sustained attention, would relate more with later practices such as open monitoring, where care is comprehensive and without an object preset observation [16]. However, the main neural mechanism responding to these attentional demands is still unknown. In the next

section we will discuss studies that have been conducted with regard to meditators.

One of the first studies [17] that addressed the neural changes during the practice of sustained attention in long-term meditators (more than 4 years doing yoga) focused their attention on building animal names. This study resulted in changes in activation in several areas, including the anterior cingulate cortex, which began to appear repeatedly in functional studies dealing with various types of meditation. In more recent studies [12], long-term meditators (in this case, more than 2 years of experience with meditation) were compared with control subjects. Subjects performed two tasks, one in which subjects were asked to focus on breathing and another in which subjects performed arithmetic calculations. The attentional task focused on breathing reflected increased activation in the rostral anterior cingulate cortex and the dorsomedial prefrontal cortex.

The studies suggested that the cingulate cortex would also be activated for a higher level of attentional training, but [18] showed that with increasing experience in meditation, when care becomes superfluous, the activation of this area may decrease. The study was conducted with medium- and long-term meditators and showed that long-term meditators had a decrease of brain activation relative to medium-term meditators, and the inverted U-shape plot is explained by the authors as a decreased requirement of attentional resources for a task at which they are expert. It adds complexity to the study of the cerebral effects of the practice of sustained attention because when the level of experience changes, the level of brain resources devoted to the practice is altered. It is also interesting to note that the study detected different levels of activation in two brain areas depending on the number of hours of practice by the subject. These are increased activation in the right anterior cingulate cortex, a fact that the authors correlate to activation profile of the default neural network, and reduced activation of the amygdala to aversive acoustic signals, which seems to be related to lower emotional reactivity to aversive stimuli. The fact that both findings are sensitive to the degree of experience suggests that neuroplasticity may be induced by meditation.

A study could be considered controversial if results are obtained in a short period of time [19]. In this study, such a short period of training showed that 5 days of integrative mind-body training produced changes in the activation of the anterior cingulate cortex (specifically in the subgenual and adjacent ventral regions). The parts of the cortex shown are usually related to autonomic control over the rostral part, as shown in other studies, and are most commonly associated with sleep states interrupted by attentional demands. Other authors [20] conducted a study using painful electrical stimulation of meditators and control subjects, showing that non-meditators and controls could reduce pain and anticipatory anxiety, exhibiting greater activations in the insula right and dorsal part of the anterior cingulate cortex.

The ability to notice bodily sensations or pay attention to the body itself has been described as one of the main features of the practice of mindfulness. In fact, for most exercises in mindfulness practices, the object of attention, either focal or extensive, is a physical sensation. For example, this happens in the case of observing the physical sensation of the breath at the nostrils or the observation of sensations that emerge throughout the body in the body scanner. This feature has strong implications for the adaptive capacity to increase or decrease sensitivity to, i.e., thermal sensations that occur in our bodies, which will make us better and faster at adapting to our environment. Various pathologies present deficits in terms of various types of sensitivity (temperature, touch), and an improvement in this capacity may be of vital importance for these patients [21]. Improvements to this task have been described in meditating [4, 12], and it has been reported that 7 out of 10 meditators perceived a differentiated experience of bodily sensations and four perceived greater emotional awareness. However, the brain areas relevant for studying changes occurring during this process are unknown. Multiple studies have identified the insula as the main structure involved [22–24], and cited the insula as one of the regions most responsible for interoception [25].

A study on the self-consciousness [23] used fMRI to compare subjects with no previous

mindfulness experience with individuals who have completed an 8-week mindfulness course. In that study, the authors distinguished between two different forms of self, the "narrative", characterized by a flow of consciousness not anchored temporarily given to mental development, and the "experiential", which, unlike the former, is focused on the present and is aware, moment by moment, of thoughts and feelings without mediating the thoughts. In both cases, the subjects who had completed the course in mindfulness showed reductions in activity in the medial prefrontal cortex (related to the "narrative") and increased lateral processing in the insula (related more to the secondary somatosensory cortex "experiential self"). The authors noted that connectivity patterns between past experiences ("narrative self") and the present ("experiential self") can be differentiated and act independently after training in mindfulness, which would provide a neural basis to this fundamental aspect of the practice of mindfulness. An example of this importance is a study [22] in which pictures with emotional content were shown to subjects without meditation experience and subjects after the 8-week course mentioned above. Subjects who had completed the course showed lower activations to images with sad emotional content, and in turn lower rates of depression, when compared with subjects who had not taken the course.

Because experienced meditators show increased activation and density of the brain regions related to attention and awareness of the body, they would be expected to also have increased sensitivity to pain. However, meditators seem to have a higher pain tolerance. A recent review [26] described up to 17 studies in which the therapeutic potential of mindfulness in pain is analyzed. Another study [27] shows that Zen meditators have pain sensitivity thresholds higher than nonmeditator subjects. This is where regulation comes into play as a basic feature of meditation [4]. It appears that an effect of the reduced activation of certain areas is a reduction in the connectivity between them. In the last decade, fMRI studies have progressed from only observing changes in the activation of certain areas to investigating functional relationships between them [28, 29]. Thus, functional connectivity between two structures is correlated to the existence of signal fluctuations in blood oxygenation level; therefore, when activation is observed in one area, the other should also be observed. Connectivity has been associated with complex functions that are performed in combination by multiple brain structures. The study described above [24] showed increased activation of areas typically associated with pain such as the insula, thalamus, anterior cingulate cortex, and prefrontal cortex [30, 31]. 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 reduced the connectivity between these areas related to pain regulation.

Similarly, several authors have studied the role of the prefrontal cortex using emotion regulation tasks. In the first study to address this [32], meditators were asked to perform a task of emotion recognition, showing lower connectivity between the prefrontal cortex and the right amygdala than 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, which contrasts with the usual way of thinking and feeling emotions and thoughts, in which they are considered "facts" or "reality".

Results on this topic have been obtained by other authors [12, 23], but some of them [33] pointed to the amygdala as a major participant in the regulation of emotions. The authors studied the regulation of anxiety through meditation techniques, showing reduced amygdala activity after performing a workout. However, the studies were not limited to regulating emotions, and some [12] had separated two basic components of this regulation: reevaluation and fear extinction. The reassessment is based on the reconstruction of stressful events as beneficial for the person. Thus [34], they conducted a study in which college students had to reassess images with negative emotional content, and this process resulted in activation of the dorsolateral prefrontal cortex

and a negative connectivity with amygdala activity. Extinction is accepting bodily processes and emotional responses that come from fear. There have been few neuroscientific studies of this phenomenon, although studies in other fields have shown links between the ventromedial prefrontal cortex and other structures such as the hippocampus [35, 36] or the amygdala [37].

Morphometric Neuroimaging

Brain 'morphology' refers to the structure, shape, and composition of the brain; the measurement and analysis of brain morphology via various neuroimaging techniques is generally known as 'morphometry' or 'morphometric neuroimaging ([38, 39]; see Fig. 27.1a, b). Few studies have been directly replicated, and few have correlated behavioral changes with differences in brain structure.

One of the pioneering works in this field [25] found that meditators with an average of 7–9 years of experience meditating 40 min a day showed increased cortical thickness in the insula, in somatosensory cortex, frontal areas, and visual and auditory cortex in comparison with subjects without meditation experience. In addition, the cortex in the frontal areas (areas 9 and 10) of the meditators who were between 40 and 50 years old had the same thickness as that of control subjects of 20–30 years of age, which would indicate that meditation may promote the preservation of cortical areas associated with meditative activity.

Another research group [24] conducted a study in which the thickness of the cerebral cortex was assessed as high or low sensitivity to pain and by whether the subject practiced meditation or not. Those results showed greater thickness of the anterior cingulate cortex and secondary somatosensory areas in meditators. That study demonstrated that the practice of meditation is linked to increases in the thickness of those areas. Because the face is also linked to pain sensitivity and emotions, the practice of meditation may also be useful in the treatment of diseases such as chronic pain. In this same line, it was observed that a relatively brief intervention, 8 weeks of meditation practice for an average of 27 min a day, was sufficient to induce changes in left parietal structures such as the hippocampus, posterior cingulate, temporoparietal junction, and cerebellum [40].

These types of studies provide empirical evidence that the insula is implicated not only functionally but also structurally in the management of emotions [41]. In subjects performing a task related to interoceptive perception, the density of grey matter in this area correlated positively with the effectiveness at the task. Studies on meditators found similar results. If the subjects are trained in these tasks, they show greater efficiency at the tasks and higher density of the insula. In this field, one study that was conducted on long-term meditators [40] measured the density of grey matter in areas previously involved in meditation showed increases in grey matter in areas of the right anterior insula, confirming previous results [25] in which the same structure showed a greater thickness in meditators.

Despite the diversity of findings, it appears that the anterior cingulate cortex and other areas such as the medial prefrontal cortex or secondary somatosensory areas are changed by long-term meditation practice and even, as shown in some current studies [42], in beginners. These studies may shed light on future treatments for diseases involving attentional deficits [43].

The notion that a largely mental practice, such as meditation, can produce such changes is further supported by studies showing structural differences after short-term mental training for working memory [44] and reasoning abilities [45]. Nevertheless, we reiterate that evidence for meditation as the causative factor in structural changes in the brain remains limited. Ultimately, brain morphology differences are important only inasmuch as they relate to altered behavior and subjective well-being. Establishing such relationships should therefore be a paramount concern in future research.

DTI

Microstructural changes in white matter can be revealed by specialized MRI brain imaging techniques such as DTI. This method analyzes

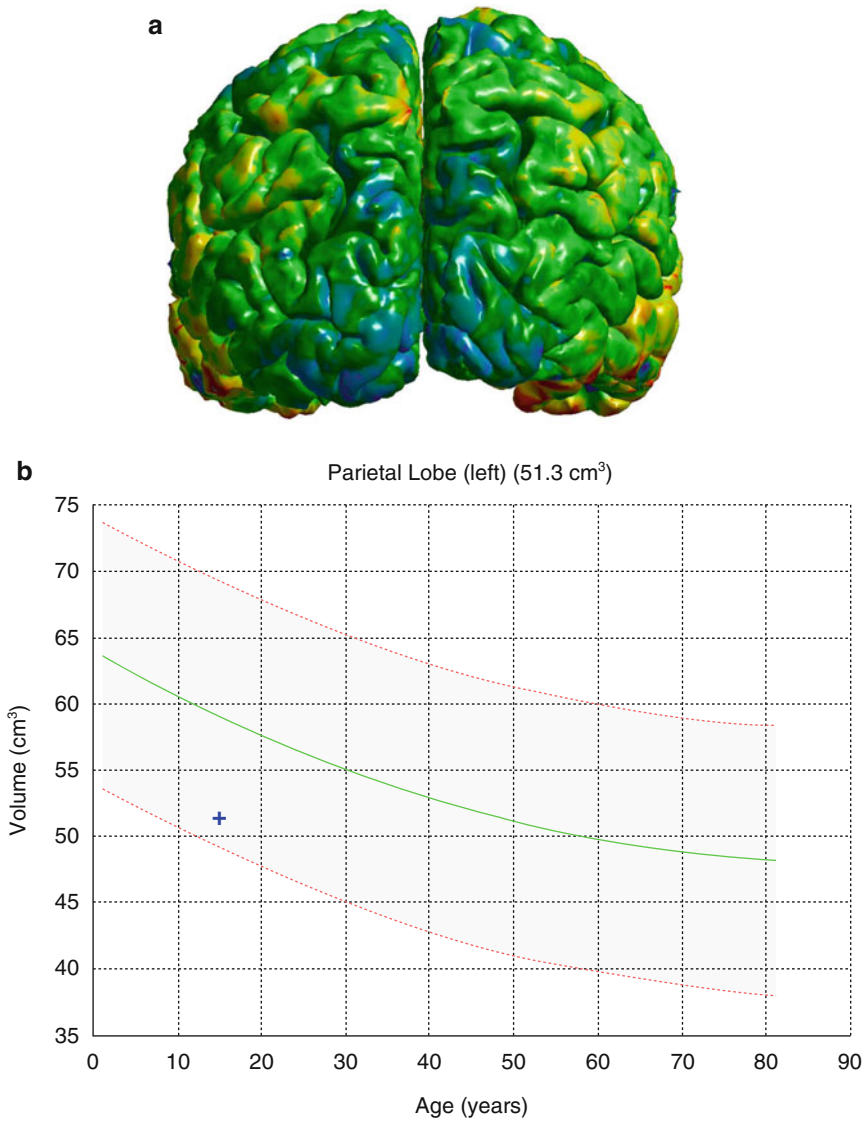

Fig. 27.1 (**a**) Total volume of the brain by morphometry comparing patients and normal controls of the same age and sex. (**b**) Volume measurements in parietal lobe comparing patients and normal controls of the same age and sex

proton diffusion in tissue, which is more restricted in white matter than in grey matter. Fractional anisotropy supplements increased myelination, diameter, and axon compaction (See Fig. 27.2). Although the adult brain was once seen as a rather static organ, it is now clear that the organization of brain circuitry is constantly changing as a function of experience or learning [46].

Several groups have recently[47] shown pronounced structural connectivity throughout the entire brain within major projection pathways, commissural pathways, and association pathways in meditators compared with controls. The largest group differences were observed within the corticospinal tract, the temporal component of the superior longitudinal fasciculus, and the uncinate fasciculus.

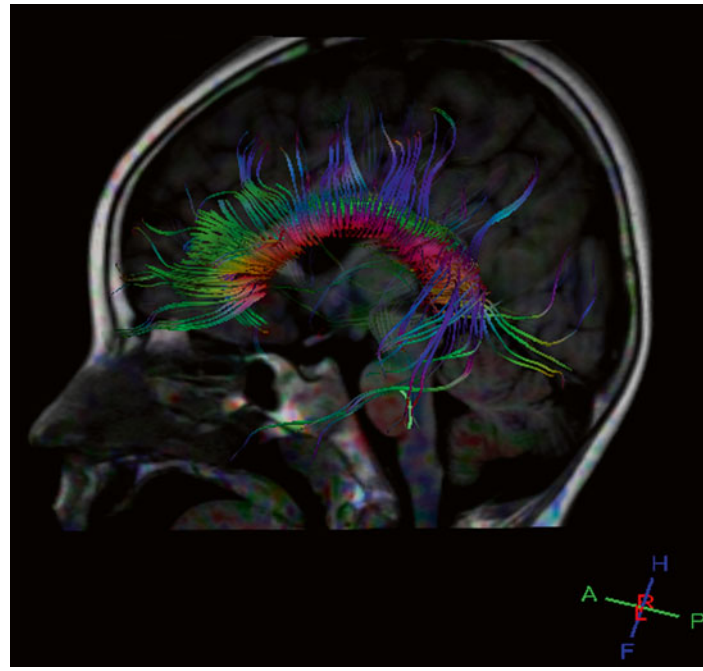

Fig. 27.2 Difusion tensor imaging (DTI) and fiber tractography of the corpus callosum

A previous study showed that 4 weeks of integrative body–mind training (IBMT) (11 h in total) enhanced fractional anisotropy in several brain areas involved in communication to and from the anterior cingulate cortex, including the corpus callosum and the anterior and superior corona radiata [48]. As with previous studies using fMRI, we must consider these data with caution because the finding that so little training can result in such profound structural changes has generated substantial controversy.

Another recent study of meditators compared with controls showed significantly greater cortical thickness in the anterior regions of the brain in both frontal and temporal areas, including the medial prefrontal cortex, superior frontal cortex, the temporal pole, and the middle and interior temporal cortices. Significantly thinner cortices were found in the posterior regions of the brain located in the parietal and occipital areas, including the postcentral cortex, inferior parietal cortex, middle occipital cortex, and posterior cingulate cortex. Furthermore, in the region adjacent to the medial prefrontal cortex, both higher fractional anisotropy values and greater cortical thickness were observed. These signs suggest that long-term meditators have structural differences in both grey and white matter [49].

In a recent study conducted by our research group [50], meditators showed a significantly lower mean diffusivity (i.e., apparent diffusion coefficient [ADC]) in the left parietal white matter than did the controls, and the mean diffusivity was correlated with time spent meditating (See Fig. 27.3). Our results show the time-course of white matter neuroplasticity in long-term meditation. The increased myelination would enhance communication among cortical areas resulting in enhanced performance. Our study also showed a negative correlation between the lower ADC in the left posterior parietal white matter and years of meditation. Thus, the improved self-regulation following IBMT might be mediated via the increased communication efficiency between the left posterior parietal lobe and other brain areas. These results imply that the enhanced integrity of white matter fibers through long-term meditation might reflect increased numbers of brain fibers or increased axonal caliber. Increased myelination may occur as a consequence of increased neural firing in active brain areas during training [51].

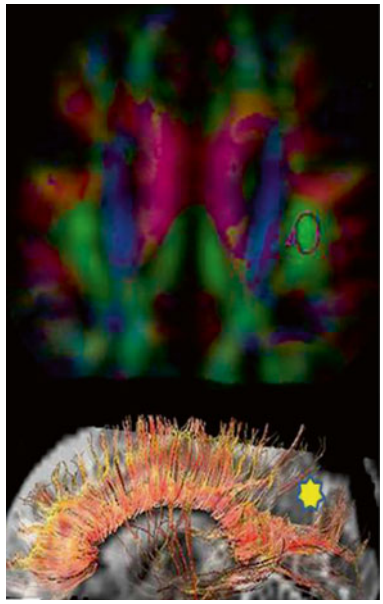

Fig. 27.3 Difusion tensor imaging (DTI) and fiber tractography. Area where this increased axonal connectivity in meditators compared with nonmeditators controls (*circle* and *star*)

In our study [50], the primary somatosensory cortex, part of the postcentral gyrus which receives the bulk of thalamocortical projections from the sensory input fields, showed no significant decrease in fractional anisotropy in meditators compared with age-matched nonmeditators. The results only confirmed a nonsignificant trend of reduced anisotropy in the postcentral gyrus. Asymmetry of anisotropy has been reported in the superior longitudinal fasciculus [52], showing asymmetry with the left greater than the right. Another study found that mindfulness meditators more robustly activated the left anterior, posterior, and mid-insula and the thalamus [53].

Findings converge on several brain regions hypothesized to be involved in meditation based on results from functional neuroimaging, behavioral and clinical research, and phenomenological reports of meditative experience. These include regions key to meta-awareness and introspection (rostrolateral prefrontal cortex/area 10), exteroceptive and interoceptive body awareness (sensory cortices and insular cortex, respectively), memory consolidation and reconsolidation (hippocampus), regulation of the self and emotions (anterior and mid-cingulate, and orbitofrontal cortex, respectively), and finally intra- and interhemispheric communication (superior longitudinal fasciculus and corpus callosum, respectively).

MRS

To our knowledge, there is only one spectroscopic study on meditators which was performed by our research group [50], in which mI was increased in the posterior cingulate gyrus and glutamate, NAA and ratio of NAA/Cr were reduced in the left thalamus in meditators. We found a significant positive correlation between years of meditation and mI levels in the posterior cingulate. We also found significant negative correlations between years of meditation and levels of glutamate, NAA, and NAA/Cr in the left thalamus.

The current finding of increased mI in the posterior cingulate gyrus in long-term meditators seems counterintuitive. Changes in mI concentrations might reflect disturbances in fluid homeostasis and cellular signalling.

The precise mechanism of action on the central nervous system is not yet known, but the evidence presented here implicates the activation of microglia following the peripheral injection of interleukin-2. In addition, there is evidence that interleukin-2-induced neurochemical changes might have a delayed functional relevance for affective conditions such as anxiety-like behavior. Consistent with this assumption, cytokines modulate serotonergic neurotransmission and enhance the catabolism of tryptophan (serotonin precursor), leading to a reduction in the levels of serotonin and an increase in tryptophan catabolites.

Glutamate activates several receptors, including N-methyl-D-aspartate receptors (NMDAr). A glutamate excess can kill neurons through excitotoxic processes. If glutamate levels approach excitotoxic concentrations during intense states of meditation, the brain may limit its production of N-acetylated-a-linked-acidic dipeptidase, the enzyme responsible for converting the endogenous NMDAr antagonist N-acetylaspartylglutamate into glutamate [54].

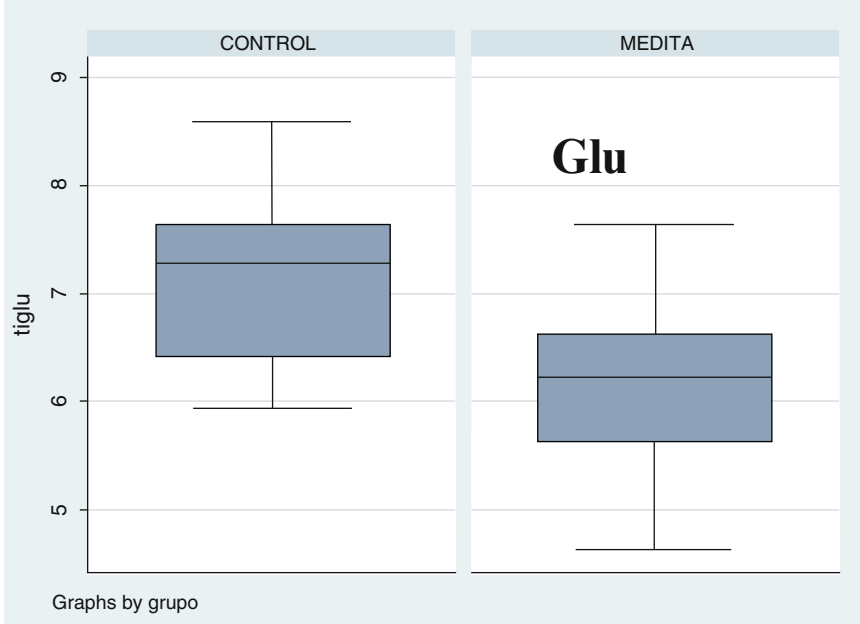

Fig. 27.4 Differences on glutamate (Glu) levels in left thalamus between meditators and healthy nonmeditators

MRS may detect decreases in glutamate content (See Fig. 27.4). There may be a consequence of a change in metabolic activity reflecting decreased function or viability of neurons because Glutamate, similarly to NAA, is located primarily in neurons [55]. The function of NAA within axons in the white matter is unknown, but it may contribute to the synthesis of neurotransmitters [56]. The question remains as to whether the depletion of NAA levels could signify a decreased activation of inhibitory neuronal pathways in meditators. The depletion of the NAA concentration may reflect decreased mitochondrial metabolism, which might correlate with years of meditation.

Pathology and Mindfulness

Beyond the finding that brain areas are involved in the practice of meditation, studies are beginning to focus on determining whether it has practical utility in the treatment of various pathologies, with the purpose of generating new or better interventions. Although there are a number of studies in which mindfulness and meditation are used to treat conditions such as psychiatric relapse into depression, other forms of anxiety or addiction relapse prevention [26], there are few studies to date that also involve scanning of brain substrate, but we have tried to introduce the most relevant examples. One such study [57] sought to evaluate how practicing mindfulness can prevent depression. Thus, with the use of fMRI, 19 participants performed a breathing task, and others performed a stress-inducing task. Non-reactivity was inversely correlated with vulnerability to depression and also with activity in the insula. These results show that in stressful situations, the practice of mindfulness can be protective and allow better responses to negative emotional stimuli.

Reducing anxiety has been associated with the emotional evaluation of external stimuli, leading to expect that people who practice mindfulness, as mentioned earlier in this chapter, have the ability to reduce anxiety. There is a recently published study [58] in which participants were trained in mindfulness for 4 days, achieving a reduction of anxiety in each session in which participants meditated. These studies should be considered with caution because, as the authors

comment, this reduction of anxiety can be a result of the relaxation produced by the distraction of the training from the causes of anxiety, and anxiety returned to initial levels after the training.

Another disorder addressed through meditation is bipolar disorder, in which patients have increased levels of anxiety and poor regulation of emotions. In the first fMRI study in patients with bipolar disorder [59], patients and healthy subjects were trained in the practice of mindfulness. Their results showed that patients had a reduction in the activity of the medial prefrontal cortex and improved outcomes for anxiety and emotion regulation.

Conclusions

We have discussed in this chapter how certain areas of the brain work differently in meditators compared with people who do not practice meditation. The most important areas addressed are the anterior cingulate cortex, related to care; the insula, associated with consciousness of body and various sections of the prefrontal cortex, which have been linked to the regulation of emotions. The field of neuroimaging is making great strides in understanding the utility of the practice of mindfulness, and the proof of this is the study of connectivity and resting. It is true that neuroscientific knowledge of this topic is still sparse, particularly relating to higher stages of meditation practice. However, the dialogue between research and contemplation is beginning to bear fruit. A major challenge for the future is to better understand how, and to what extent, meditation is associated with differences in brain morphology, and whether the magnitude of these differences indicates any practical significance. Meditation can change the brain and thereby make us different people.

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28 Clinical Magnetic Resonance Neuroimaging in Mild Cognitive Impairment and Alzheimer Disease

Nicolás Fayed, Javier Garcia-Campayo, and Laura Viguera

Introduction

Amnestic mild cognitive impairment (MCI) is a common condition in the elderly individuals mainly characterized by memory loss. Although there may be other subtle decline in other functions, the general cognitive function and daily living activities are preserved [1].

The annual rate of conversion to dementia is around 12 %, in general to Alzheimer type dementia. In this review, we are focusing on the application of biochemical markers [2] and imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). MRI can cover structural MRI that uses parametric quantitative methods such as volumetry, but also other techniques such as functional MRI (fMRI), diffusion/diffusion tensor imaging (DTI), arterial spin labeling (ASL) perfusion, and magnetic resonance spectroscopy (MRS) techniques. In this review we focus on the applications of MRI techniques and their role in cases of cognitive decline.

The relationship between dementia and parameters evaluated by imaging probably vary with age: amyloid load may not be as specific for cognitive impairment in very old patients as compared with younger patients, whereas indices of neuronal loss (regional volumes, metabolic activity, or absolute blood flow) might show a more stable relationship to dementia across ages [3]. Brain reserve will also influence the results from those studies. In Alzheimer disease (AD), large areas of medial temporal cortex are activated during cognitive tasks that do not occur in controls. This may represent a compensation for the reduction of function or a "cognitive reserve". Recommendations on the use of imaging techniques must be interpreted in light of such factors, whatever the technique used.

For the initial assessment of patients presenting with cognitive difficulties/symptoms of dementia, guidelines from several countries indicate that structural neuroimaging with CT or MRI is appropriate.

Space-occupying lesions, usually neoplasms or subdural hematomas, can be detected and may present progressive cognitive impairment. These lesions are uncommon, with estimations at approximately 3 % [4].

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Magnetic Resonance Spectroscopy

MRS enables us to study the chemical composition of living tissues. It is based on the chemical shift of atoms. The concentration of some metabolites is determined from spectra that may be acquired in several ways.

Physical Basis of MRS

Currently the spectra may be acquired with single-voxel (SV) or multi-voxel (MV) techniques. The SV technique is readily available on most scanners. Voxels must be positioned away from sources of susceptibility artifacts and lipids. For diffuse processes, a 2 × 2 × 2-cm (8 cm^3) voxel is routinely used (See Fig. 28.1). A voxel (*volumetric pixel* or *Volumetric Picture Element*) is a volume element, representing a value on a regular grid in a three-dimensional (3D) space. In contrast to pixels and voxels, points and polygons are often explicitly represented by the coordinates of their vertices. A direct consequence of this difference is that polygons are able to efficiently represent simple 3D structures with a lot of empty or homogeneously filled space, while voxels are good at representing regularly sampled spaces that are non-homogeneously filled. Voxels are frequently used in the visualization and analysis of medical and scientific data. Some volumetric displays use voxels to describe their resolution. For example, a display might be able to show 512 × 512 × 512 voxels. For local lesions, the SV can be reduced in volume. The SV technique offers the advantages of better spatial location, more homogeneity, better water suppression, and speed. However, only one spectrum can be obtained per acquisition and the MV technique makes it possible to obtain multiple spectra simultaneously per acquisition and to assess a greater area of the brain but with smaller spectral resolution.

To date, the SV is still superior to MV on the grounds of reproducibility [5, 6]. For both SV and MV, the magnetic resonance scanner uses a process known as shimming to narrow peak line widths within the spectra. For SV studies, improving field homogeneity is performed with basic, zero-ordered shimming on clinical magnetic resonance scanners. For MV, the simultaneous production of uniform field homogeneity in multiple regions requires higher order shimming. To obtain high-quality spectra, blood products, air, fat, necrotic areas, cerebrospinal fluid, metal, calcification, and bone should be avoided. In such areas differing magnetic susceptibility results in a non-homogenous field that hinders the production of diagnostic quality spectra.

Two different approaches are generally used for proton spectroscopy of the brain: 1) SV methods based on the stimulated echo acquisition mode (STEAM) and 2) point resolved spectroscopy (PRESS) pulse sequences and spectroscopy imaging, also known as chemical shift imaging. These latter studies are usually done in two dimensions, using a variety of different pulse sequences (spin-echo, usually PRESS). The basic principle underlying SV localization techniques is to use three mutually orthogonal slice selective pulses and design the pulse sequence to collect only the echo signal from the point (voxel) in space where all three slices intersect. The PRESS mode is used more often than STEAM because it increases the signal/noise ratio and is less sensitive to movement artifacts [7].

Echo time (TE) have not yet standardized so far in MRS. In degenerative, demyelinating, and vascular disease a short TE is advocated. A short TE (20–40 ms) allows us to increase the signal/noise ratio and to visualize most metabolite peaks, with the inconvenience of some degree of overlapping of peaks. Intermediate TE (135–144 ms) inverts the lactate peak to better distinguish it from lipids peak. Long TE (270–288 ms) gives worse signal/noise ratio but allows better visualization of some peaks (N-cetylaspartate [NAA], choline [Chow], and creatine [Cr]). Time matters in clinical practice, so short TEs are preferable. In our experience with a 1.5 T General Electric Signa Horizon-clinical scanner a TE of 30 ms and a repetition time of 2500 ms has proven valuable [8].

A TE averaged PRESS technique has been yielding highly simplified spectra with better suppression of signals not pertaining to assessed

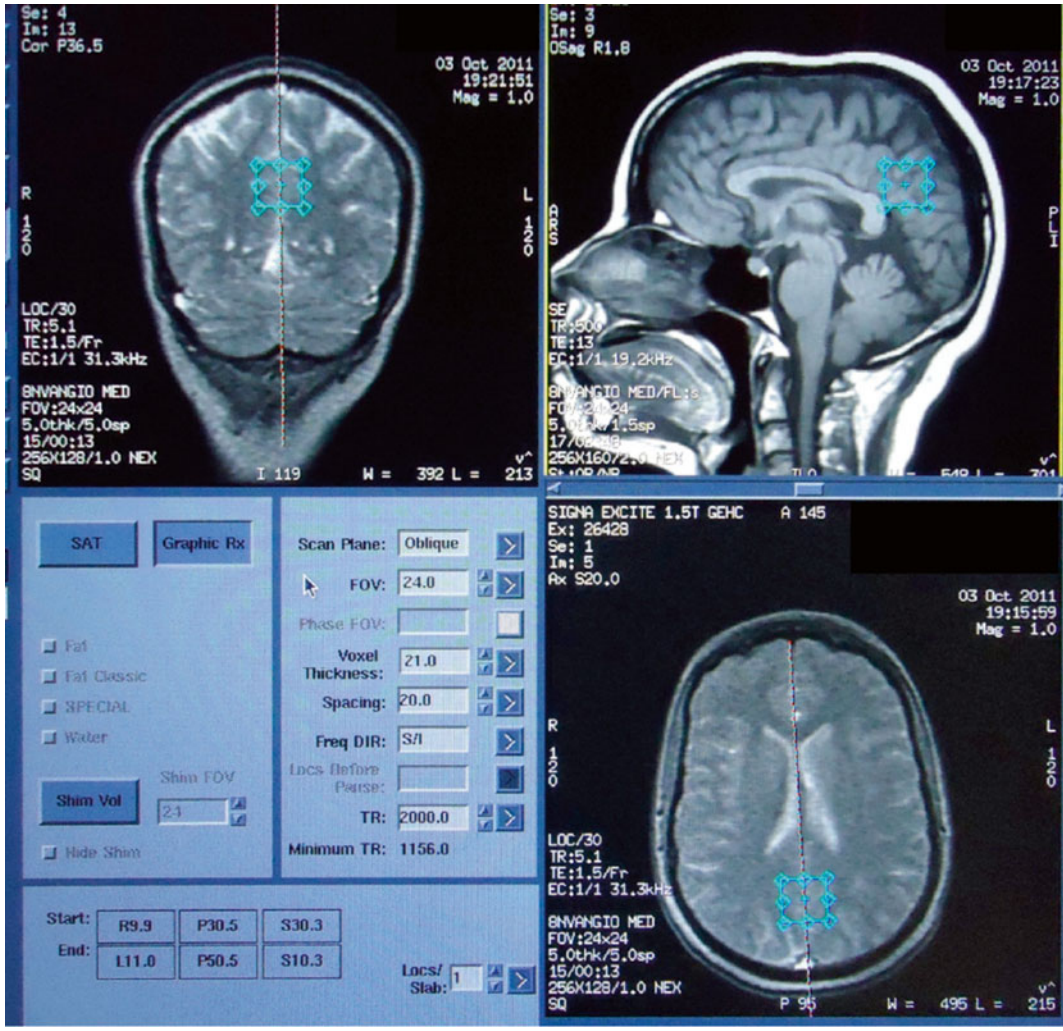

Fig. 28.1 Positioning of a single-voxel in the bilateral posteromedial parietal cortex for study with frontal, sagittal, and axial slices. The area explored includes the posterior cingulate gyrus

metabolites, such as that of macromolecules. TE is increased from 35 ms to 355 ms in steps of 2.5 ms with two acquisitions per step [9].

The most commonly used spectroscopy is that originating from a hydrogen nucleus (proton 1H-MRS). This technique is based on the differences in resonance obtained from hydrogen nuclei depending on the surrounding atoms (chemical shift). Each metabolite being assessed discloses a different hydrogen resonance frequency and appears in a different site in the spectrum. 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). With the appropriate factors considered, such as the number of protons, relaxation times and so forth, a signal can be converted into a metabolite concentration by measuring the area under the curve. Because water is the main component of living beings and its concentration is much higher than that of metabolites, it becomes necessary to suppress the resonance signal from the hydrogen of water. A plot showing peak amplitudes and frequencies is obtained.

Each spectrum shows peaks corresponding to the different metabolite values: myo-inositol

(mI), 3.56 and 4.06 ppm; Chow, 3.23 ppm; Cr, 3.03 and 3.94 ppm; NAA, 2.02; 2.5 and 2.6 ppm; glutamine and glutamate, 2.1–2.55 ppm and 3.8 ppm (See Fig. 28.2). Ratios between metabolites and Cr are also of great value as they counteract the systematic errors of measurements.

A program, called a linear combination (LC) model [10], fits in vivo spectra as a linear superposition of high-resolution "basis" spectra that are acquired from model solutions of the metabolites present in the region of interest. Advantages of an LC model are that all pre-processing steps, automatic phase correction, as well as modelling of a smooth baseline are included. Standardized basis sets are available for the most common clinical magnetic resonance machines (both 1.5 and 3 T).

Evolution of Brain Metabolites over the Lifetime

When analyzing metabolite levels in the whole sample while controlling for age and gender, we observe that all metabolites are correlated with age. NAA, glutamate and glutamate + glutamine and their ratios to Cr show a negative correlation (increase in age with a decrease in metabolite levels and vice versa), while the remaining metabolites, such as mI and Chow, show a direct correlation (See Fig. 28.3). A decrease in glutamate and glutamate + glutamine over one's lifetime, which is associated with a certain cognitive deterioration, could be expected as significant lower levels of these metabolites are found in AD. There is a certain degree of controversy in the literature regarding the changes in metabolite concentrations and ratios that occur with aging. Estimates of age effects based on such designs are interferred by secular changes in nutrition, medical care, and other factors.

MRS in Mild Cognitive Impairment and AD

Altered levels of NAA or NAA/Cr ratios are the most common finding reported in patients with AD and MCI [11–13], although alterations in other metabolites including mI [14] and glutamate

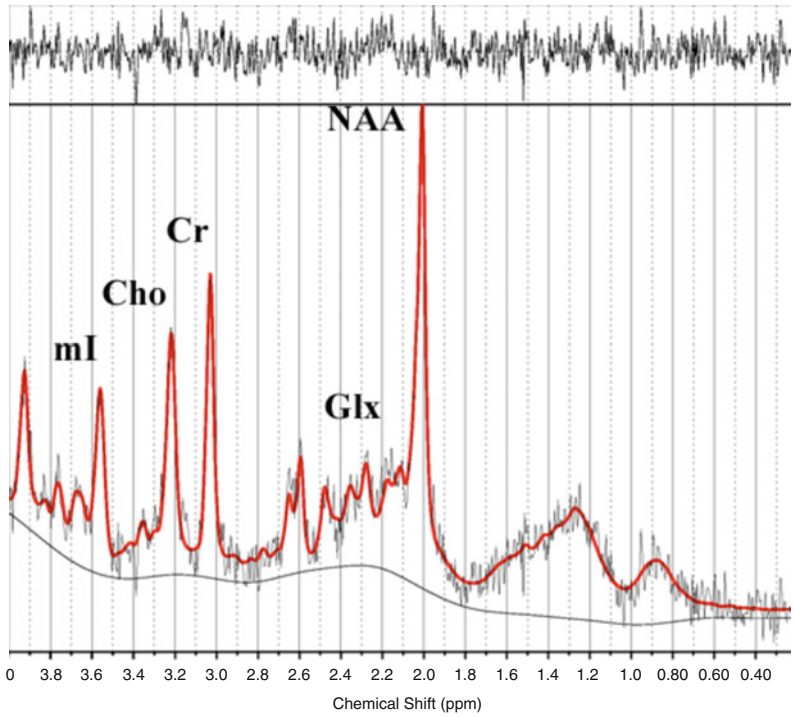

Fig. 28.2 A typical in vivo example of linear combination model spectrum in the same area with metabolite peaks. mI: myo-inositol; Chow: Choline compounds; Cr: creatine, Glx: glutamate + glutamine; NAA: N-acetyl-aspartate

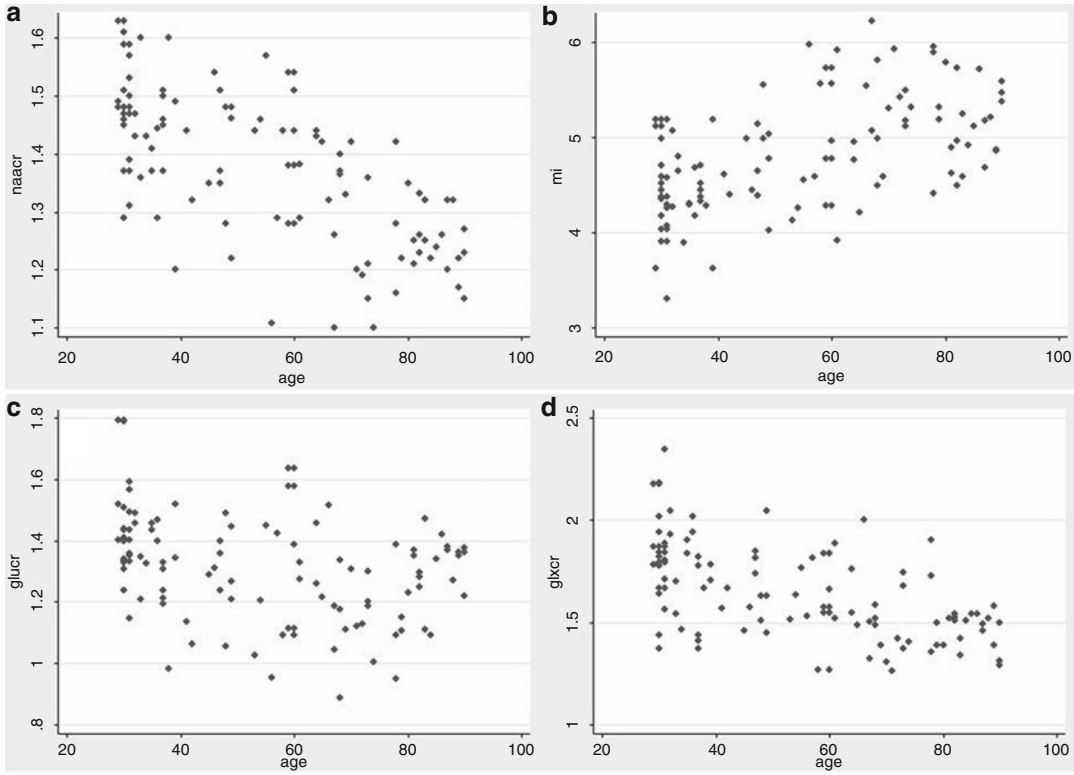

Fig. 28.3 Metabolite levels controlling for age and gender. NAA/Cr (**a**), Glu/Cr (**c**) and Glx/Cr (**d**) ratios show a negative correlation with age, while mI (**b**) show a direct correlation

[15, 16] are also found. Decreased NAA has been documented in patients with AD. This reduction may reflect a combination of the loss of neural cells, reduced neural metabolism, loss of dendritic structures, and reduced myelination. As NAA is almost entirely located within neurons in the central nervous system, the reduced neuronal density may reflect neuronal death or decreased tissue volume. As the reduced NAA signal could be interpreted as a sign of neuronal dysfunction, it does not necessarily indicate cell death. The depletion of NAA concentration could reflect decreased mitochondrial metabolism, which may correlate with the patient's age. There are cross-sectional studies dealing with MRS in AD. A study including 206 normal elderly subjects and 121 patients with AD demonstrated a decrease in the NAA/Cr ratios as well as increased mI/Cr and Chow/Cr ratios in the left posterior cingulate gyrus in patients with AD as compared with controls [17]. Some studies suggest a continuum between normal aging, MCI, and AD with regard to the values of NAA in the brain [18, 19].

Longitudinal studies also confirm the utility of MRS as biomarker. The use of spectroscopy in the occipital cortex and posterior cingulated aiming to determine the rate of NAA/Cr, may be a valid tool for predicting the conversion of MCI to AD. It has been demonstrated that receiver operator curve analysis for NAA/Cr <1.61 predicted conversion with 100 % sensitivity and 75 % specificity [20]. The area under the curve was 0.91 with a positive predictive value of 83 % and a negative predictive value of 100 % with 88.7 % correct classification. Similarly [21], it has been shown that NAA/Cr <1.40 in the posterior cingulate predicted conversion of MCI to probable AD with sensitivity of 82 % and specificity of 72 % and an area under the curve of 0.82 and correlates closely with clinical severity scales [22].

Finally [23], showed that NAA/Cr <1.43 in the posteromedial parietal cortex predicted conversion to probable AD with 74 % sensitivity and 84 % specificity and an area under the curve of 0.84.

Additional longitudinal studies showed valuable results with magnetic resonance spectroscopy. In a large cohort of 151 MCI patients (most of them being of amnestic type) followed-up for 3 years, MRS was individually predictive of conversion to dementia but the accuracy of prediction improved when MRS was used in combination with hippocampal volumetry and the presence of cortical infarctions [24].

The value of proton MRS as a biomarker was assessed ante-mortem in a single study with 54 patients ranging from low to high likelihood of having AD and who underwent autopsy. Decreases in NAA/Cr and increases in myo-inositol/Cr ratios in the posterior bilateral cingulate gyrus correlated with higher postmortem Braak neurofibrillary tangle staging [25]. Godbolt and coworkers [26] noted that presymptomatic ApoE 4 subjects had decreased levels of NAA/myo-inositol and NAA/Cr by 10–25 % compared to controls, and that these differences appeared years before clinical symptoms [26]. Kantarci and his group found that the choline/Cr ratios decreased for 13 months in stable patients with MCI, whereas no changes were seen in patients with MCI progressing to AD. This may reflect a compensatory cholinergic mechanism failing in MCI patients who progress to AD [27].

There is also a growing appreciation of common risk factors for AD and vascular dementias (VaD), and that both pathologies may contribute to cognitive decline in an individual. Other primary degenerative dementias, such as fronto-temporal degeneration (FTD) may present atypically. Consequently there may be considerable overlap between clinical and imaging features in these conditions.

Metabolic changes in fronto-temporal dementia are similar to Alzheimer's disease, with low levels of NAA/Cr and higher than normal levels of myo-inositol/Cr [28]. MRS studies in common dementias are limited to comparing the signs of MRS in Alzheimer's disease with other dementias such as fronto-temporal dementia [29, 30], vascular dementia and Parkinson disease [31].

It is important to note that the reliability of these values requires a good reproducibility and depends more on the technical characteristics of the study of resonance (magnetic field homogeneity, good signal to noise ratio, peak width of the metabolites) than post-processing methods [32].

Monitoring of Treatment

With the recent availability of many pharmaceutical agents modestly effective for treating symptoms of AD, medicine has entered a new era in treating AD. Neuroimaging may provide a useful tool for monitoring the progression of AD. Several published trials measured the effect of drugs on AD progression with MRS and, we can see in general that drugs produced small changes in metabolite levels and ratios which correlated with the modest clinical or no effect of the drugs on AD progression. Decreases in choline/Cr and choline/phosphocreatine in parietal cortex in comparison with controls have been demonstrated using MRS in patients with AD when receiving xanomeline, a muscarinic agonist [33]. Decreased choline/Cr ratios in patients with AD when treated with cholinergic agonists [34] and increased NAA/Cr were detected under treatment with donepezil, a cholinesterase inhibitor [35].

A randomized trial included 67 patients who were treated with either donepezil or placebo for 1 year [36]. The NAA levels elevated transiently in the donepezil group at week 12 and 18 but the differences were not significant at endpoint, and cognitive improvement correlated with NAA elevations in the cortex. Conversely, in the placebo group the NAA concentration tended to remain near baseline values or to decrease modestly [36]. By comparison, other studies have found only a slight increase of NAA/Cr in patients with AD when treated with rivastigmine [37]. In a randomized trial, donepezil and memantine were compared by analysis of metabolite values in several areas of the brain. The general trend was towards a small elevation of NAA/Cr ratios. However, the results were not statistically significant. In the global sample there was a significant correlation between the clinical changes and changes in NAA/Cr values [38]. A recently published study, including 42 patients with AD and 22 controls all of whom underwent six MRS

studies over a 2-year timespan, showed that there is a progressive decline in the NAA/Cr ratios independent of treatment with cholinesterase inhibitors, which is consistent with the lack of efficacy of these drugs [39].

Glutamate is another neurotransmitter studied with MRS. Increased glutamatergic excitotoxicity has been reported in AD but several cross-sectional reports showed decreased levels of glutamate in AD in comparison with controls. In a small open trial, galantamine treatment for 4 months tended to elevate glutamate levels in the hippocampus [40].

Diffusion Tensor Imaging

White Matter Structure

Neuroimaging reveals changes in the white matter (WM) structure in the human brain. WM comprises half of the human brain and consists of bundles of myelinated axons connecting neurons in different brain regions [41]. Grey matter is composed of neuronal cell bodies and dendrites concentrated in the outer layers of the cortex.

Microstructural changes in WM 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 WM than in grey matter.

DTI Measurement

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 diffusion tensor imaging, rather than DWI, is that DTI reflects the underlying diffusion properties of the sample independent of the orientation of the tissue with respect to the direction of measurements (See Fig. 28.4). DTI is thus a robust quantitative technique that is independent of how the subject has been oriented inside the scanner magnet and gradient coils. In regions with few or no constraints imposed by physical boundaries, such as cerebrospinal fluid (CSF) in the ventricles, water movement is random in every direction and is isotropic. In contrast to CSF, the path of a water molecule in a

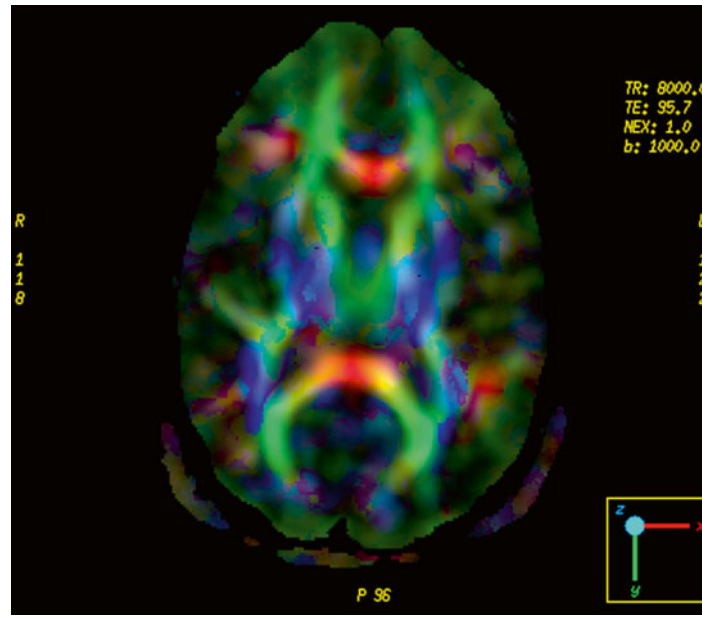

Fig. 28.4 Diffusion tensor imaging and example of color-encoded fiber orientation maps. Fibers that are predominantly oriented *left–right* are shown in *red*, anterior–posterior fibers are shown in *green*, and superior–inferior fibers are shown in *blue*

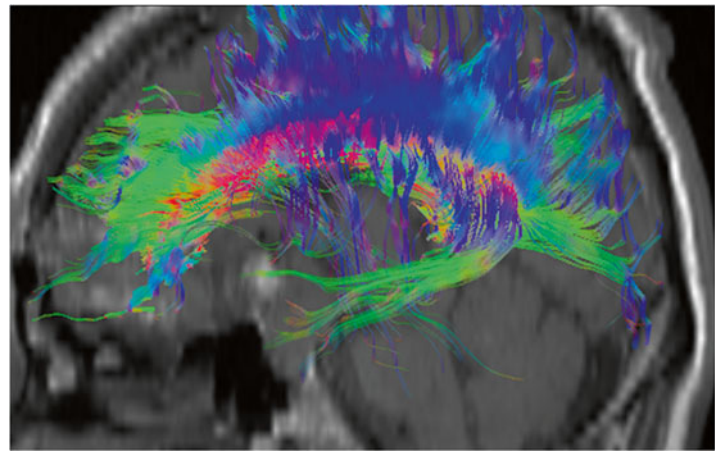

Fig. 28.5 3D DTI-based reconstruction results of association fibers in the limbic system (*green*) and corpus callosum (*blue*)

WM fiber is constrained by physical boundaries such as the axon sheath, causing the movement along the long axis of a fiber to be greater than across the radial diffusion. These data can be used to calculate the probable anatomy of WM bundles in living brain, a process called tractography (See Fig. 28.5). Orientation is calculated from the eigenvectors defining proton diffusion in three dimensions in each voxel. Using algorithms, the principal eigenvalue vector is connected to the next voxel to trace the fiber structure and orientation in WM tracts.

DTI yields quantitative measures for tissue water mobility as a function of the direction of water motion and is probed by application of diffusion sensitization gradients in multiple directions. Baser and coworkers [42] described the use of multivariate linear regression to calculate diffusivity, D, from a non-diffusion-weighted image plus six or more diffusion-weighted measurements in a non-collinear direction. The diffusion weighting is obtained by simultaneously applying diffusion gradients along combinations of the three physical axes.

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. The anisotropy increases with increased myelination, diameter, and axon compaction. Myelin is a major diffusion barrier for water, and gives WM its high anisotropy. Demyelinating diseases are characterized by partial or total loss of myelin, with consequent loss of neuronal function.

MCI and AD

Increase in the ADC has been described in multiple regions of WM, corpus callosum, and cingulum of patients with AD as compared with controls [43]. Huang and coworkers [44] found functionally relevant microstructural changes in patients with AD and MCI. These changes were present in brain regions with high cortical functions, but not in regions of primary functions, and are consistent with a hypothetical decrease in axonal process in the temporal lobe [44].

Neuroimaging in MCI and AD generally shows medial temporal lobe atrophy and diminished glucose metabolism in the posterior cingulate gyrus. However, it is unclear whether these abnormalities also impact the cingulum fibers, which connect the medial temporal lobe and the posterior cingulate regions. Assessment of the cingulum fibers using DTI may be of help for an early diagnosis of AD [45].

It was proposed in a recent review that using analysis of regional mean fractional anisotropy (FA) and mean diffusivity (MD) values, it was possible to show that MD values are different in all WM regions of the brain between controls and AD patients, and that FA showed similar results except for the parietal lobe and internal capsule [46]. Furthermore, a few studies in healthy older subjects at risk for AD showed abnormalities in

MD values in regions known to be affected in AD [47, 48]. Besides showing early alterations in MCI patients, DTI appears to correlate with cognitive performance independent of cortical atrophy, which suggests access to an upstream process in the neurodegenerative cascade [49]. The search for appropriate DTI and high angular resolution diffusion imaging parameters for the diagnosis of cognitive impairment is still a work in progress [50]. Various parameters behave in different ways according to localization [51]. In addition to the choice of diffusion parameters, recent tractography studies illustrate the superiority of analysis methods that can manage crossing fibers [52].

Perfusion MRI

PET and single-photon emission computed tomography (SPECT) have been used to identify focal changes in regional cerebral blood flow in patients with MCI and AD. However, the low spatial resolution of PET and SPECT, and the ionizing radiation emitted from the nuclear medicine tracers are major concerns. PET imaging offers a variety of techniques that have a significant role in investigating patients with cognitive impairment. Amyloid imaging with [11C]-labeled Pittsburgh compound-B (PIB) amyloid and [18 F]flurodeoxy glucose PET are covered elsewhere. A molecular probe with high affinity to tubulin associated unit fibrils and a low affinity for synthetic amyloid-β1–42 fibrils is in the early phase of development [53].

Magnetic resonance perfusion techniques have also been developed and offer higher spatial resolution without the use of ionizing radiation [54]. Magnetic resonance perfusion techniques are based on exogenous or endogenous tracers. In the method based on exogenous tracers, a paramagnetic agent such as gadolinium dimeglumine gadopentate is injected, and the resulting decrease and subsequent recovery of the magnetic resonance signal is used to estimate perfusion (See Fig. 28.6). In the method using endogenous tracers, the magnetization of the spins of arterial water are noninvasively labeled using radiofrequency pulses, and the regional accumulation of the label is measured in the tissues by comparison with an image acquired without labeling. In the case of ASL, there is no need to use exogenous contrast material; it uses endogenous water magnetization as diffusible tracer and works with modifications of the magnetization state of blood [55]. Arterial spin labeling-MRI studies of patients with AD and MCI have reported a similar pattern of regional hypoperfusion to that described in previous PET and SPECT studies. Moreover, arterial spin labeling-MRI offers several advantages over PET and SPECT: (1) it is free of exposure to ionizing radiation, intravenous contrast agents, and radioactive isotopes; and (2) it can be rapidly repeated because labeled water is cleared after a few seconds. An additional advantage is that perfusion and structural images can be acquired at the same imaging session.

Previous studies using this method have shown hypoperfusion in some brain areas in patients with MCI and AD compared with controls, including the right inferior parietal, bilateral posterior cingulate gyri, and bilateral middle frontal gyri, a pattern of hypoperfusion that is similar to the one seen with PET and SPECT scan studies in this population [56, 57]. Chao and coworkers [58] compared the predictive value of cerebral perfusion as measured by arterial spin labeling-MRI with magnetic MRI hippocampal volume for determining future cognitive and functional decline and subsequent conversion from MCI to dementia [58]. Results from linear mixed effects modeling suggest that baseline perfusion from the right precuneus predicted subsequent declines in the Clinical Dementia Rating, Functional Activities Questionnaire, and selective attention, whereas baseline hypoperfusion in the right middle frontal cortex predicted subsequent episodic memory decline in the California Verbal Learning Test. These results suggest that hypoperfusion as detected by arterial spin labeling-MRI can predict subsequent clinical, functional, and cognitive decline and may be useful in identifying candidates for future AD treatment trials.

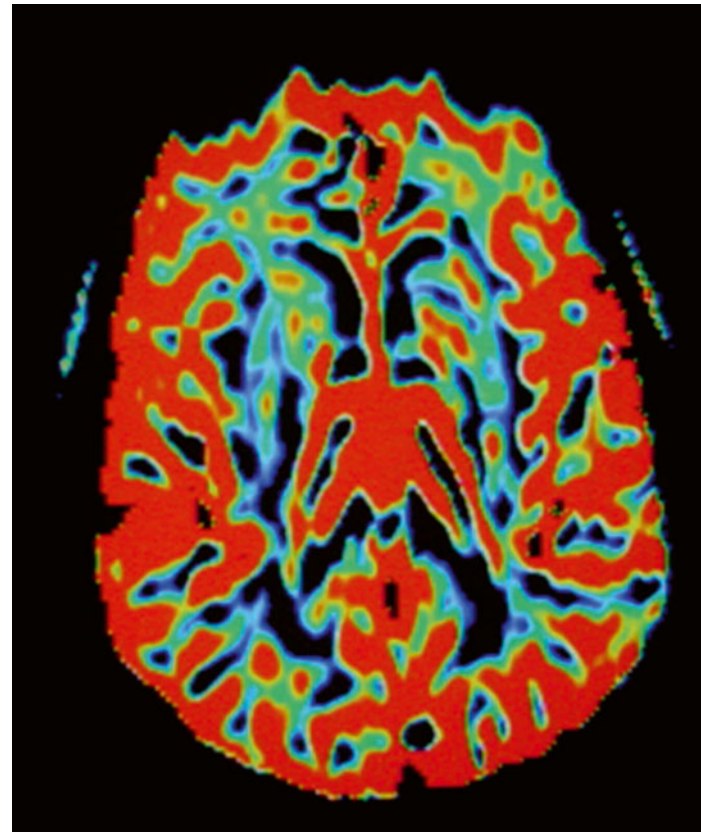

Fig. 28.6 Example of cerebral perfusion contrast-enhanced dynamic susceptibility. (DSC) with decreased left frontal cerebral blood

Structural Neuroimaging

Structural neuroimaging has also been validated as a tool in the detection and progression monitoring of preclinical AD. In AD there is cortical atrophy including thinning of gyri, widening of sulci, thinning of the cortical ribbon (coronal plane), reduced volume of the centrum semiovale, and lateral ventricular enlargement (one third of cases). The atrophy is evident in the medial temporal lobe, particularly the amygdala, hippocampus, and parahippocampal gyrus. Temporal lobe MRI may have an important role in assisting with the clinical diagnosis of AD, particularly its differentiation from other disorders that may cause diagnostic difficulties in the clinical practice. Tissue volumes in the central nervous system, and in particular changes in volume over time, are sensitive markers of a range of neurological disease states and disease progression. Measurement of brain volume requires segmentation of the brain from the rest of the tissues in the head and neck. While this can be performed manually or in a semiautomated manner, automated procedures are likely to be more reproducible and rapid. This is understandable because the size of the structures involved is usually relatively small, making the analysis less tedious than a manual segmentation of the whole brain. Manual segmentation to measure the hippocampal volume is recognized as the gold standard. However, an initial survey of the 12 most cited manual segmentation protocols revealed a 2.5-fold volume measurement difference [59]. The group that included Barnes performed a meta-analysis of hippocampal atrophy rates in patients with AD and matched controls from studies reported in the peer-reviewed literature [60]. Meta-analysis and meta-regression were then

performed with nine studies from seven centers, a total of 595 patients with AD and 212 matched controls. They found strong evidence of between-study heterogeneity, and finally concluded that the overall hippocampal atrophy rate was 1.4 % in normal controls with an age range of between 69 and 83 years. In patients with AD, the overall atrophy rate was 4.6 %. Automated validated measures of hippocampal volume will help to increase reproducibility of results. Additionally, many structures such as the hippocampus, are difficult to segment in an automated manner, but are relatively easily identified and manually or semiautomatically outlined, given the appropriate software. Some progress has been made in automating segmentation procedures, with methods including the use of deformable shape models.

Tensor-based morphometry (TBM) is a relatively new image analysis technique that identifies regional structural differences in the brain, across groups, or over time from the gradients of the deformation fields that warp images to a common anatomical template. The anatomical information is encoded in the spatial transformation (See Fig. 28.7). Therefore, accurate inter-subject non-rigid registration is an essential tool. With the advent of recent and powerful non-rigid registration algorithms based on the large deformation paradigm, TBM is being increasingly used [61] but at the moment is restricted mostly to research settings. Various automated methods to classify people with AD and MCI using structural MRI T1- weighted images have been proposed and have been reviewed [62]. The authors concluded that most of the techniques accurately classified normal controls and patients with AD. However, these methods had lower sensitivity in diagnosing prodromal AD. Again, the diagnostic value of specific hippocampal atrophy measurements has been well established in referral clinic populations, but its diagnostic value has not been demonstrated in unselected primary care patients, and this will remain a challenge for the foreseeable future.

fMRI

Another more recent imaging method for the mapping of activation patterns in the brain is fMRI. This is an important technique for better understanding brain function. When a brain region is activated, new energy must be transported to this region which leads to increased blood flow to that part of the brain. This can be

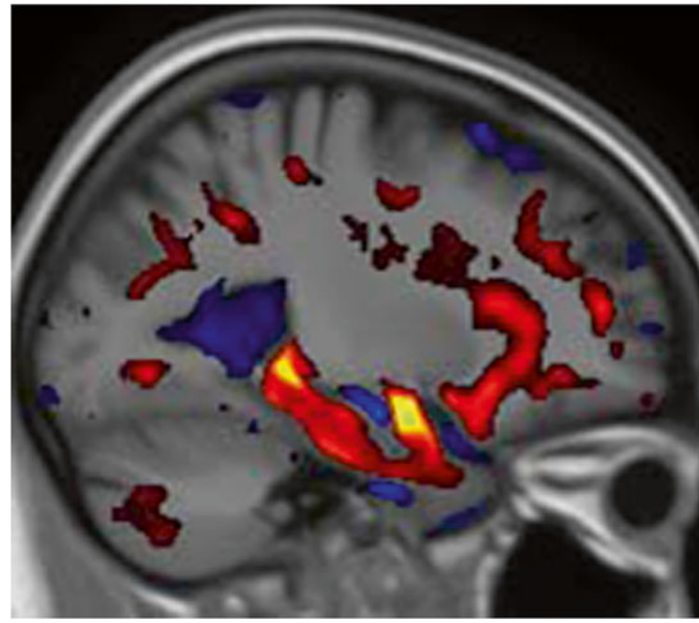

Fig. 28.7 Example tensor based morphometry of the AD patients vs. controls subjects. Statistical significance maps show dilatation (*blue*) and contraction brain volume (*yellow* and *red*) in AD

imaged by repetitive magnetic resonance scans and detected by appropriate signal processing methods.

Episodic memory encoding function is most commonly investigated because of its early and consistent involvement in AD. During episodic memory encoding, patients with AD onsistently show lower activation in medial temporal lobe structures, particularly the hippocampus [63], failure of the normal deactivation in posteromedial cortical areas such as the posterior cingulate and medial parietal cortex, [64] and increased activation in the prefrontal cortex, probably as a compensation mechanism [65]. fMRI studies have shown a decrease in intensity and/or extent of activation in the frontal and temporal region of patients with AD compared with normal subjects. In the genetic risk groups (ApoE4), activation with memory tasks has been shown greater extent and intensity of frontal and temporal brain activation, suggesting a compensatory brain function [35].

Dickerson and coworkers have extended a preliminary analysis of functional magnetic MRI as a predictor of dementia in MCI. Over a follow-up interval of more than 5 years after functional MRI scanning in 25 MCI subjects, some did not show change and others progressed to dementia [66]. The degree of cognitive decline was predicted by hippocampal activation at the time of baseline scanning, with greater hippocampal activation predicting greater decline. These data suggest that functional MRI may provide a physiologic imaging biomarker useful for identifying the subgroup of MCI individuals at highest risk of cognitive decline for potential inclusion in disease-modifying clinical trials.

The brain network referred to as the default mode network (DMN) includes several cortical areas that are particularly active at rest and deactivate during cognitive tasks. This network includes the medial prefrontal cortex, posterior cingulate cortex, precuneus, anterior cingulate cortex, and parietal cortex. The hippocampus is functionally connected to this network. A significant alteration in the intrinsic functional connectivity between the hippocampus and areas in the DMN at rest and during cognitive tasks in patients with MCI and AD has been reported [67].

Summary, Conclusions, and Future Directions

Several techniques used for the diagnosis of MCI and AD have been discussed in this chapter. Structural MRI alone has also proven insufficient to predict early AD and additional biomarkers are needed in combination to make reliable predictions in MCI. Additionally, an excess of significance bias has been suggested in volumetric studies according to data synthesis from 41 meta-analysis [68]. At present, there are no other non-invasive techniques that can provide equivalent information and, as a consequence, MRI, DTI tractography, and fMRI are expected to be a powerful combined technique for researching brain anatomy and disease in situ in human beings [69]. MRS in combination with DTI and fMRI may provide clinicians with information about ongoing pathological changes in AD. DTI and MRI are non-invasive and do not require the use of radioactive tracers, suggesting its potential safe application for longitudinal follow-up and repeated assessments. Mandal and coworkers have shown that brain oxidative stress can be determined non-invasively and quantified in various regions of the brain in both healthy young male and female subjects as well as in patients with MCI and AD [70]. It was also demonstrated using MRS technique that detection of glutathione in specific brain region may provide crucial information related to clinical status. In order to have diagnostic value in the individual patient with these neuroimaging modalities, they must have established validity, sensitivity, specificity, predictive value, and test–retest and interrater reliability.

The MRI scans show that the death of brain cells precedes symptoms of AD by 5 or 6 years. The goal of newer imaging methods is to detect these changes even earlier, and more precisely track disease progression. The accumulation of neurodegenerative biomarker abnormalities might reflect a more severe brain pathological stage that could potentially increase the risk of longitudinal cognitive decline [71] as well as development of clinical AD [72–74].

A recent study comparing neuroimaging modalities for the prediction of conversion from

mild cognitive impairment to Alzheimer dementia shows that among individual modalities, MRI had the highest predictive accuracy (67 %), which increased from 9 % to 76 % when combined with PIB-PET, producing the highest accuracy among any biomarker combination. Individually, PIB-PET generated the best sensitivity, and fluorodeoxyglucose PET had the lowest. Among individual brain regions, the temporal cortex was found to be most predictive for MRI and PIB-PET [75].

Larger longitudinal studies with improved homogeneity of participants and methods, combining neuroimaging and other diagnostic data, will probably give to the modalities discussed in this chapter clinical utility in the near future. The improvements in brain imaging techniques will help scientists working with AD to better understand this devastating and deadly cognitive decline.

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