Psychiatry and Neuroscience Update

From Epistemology to Clinical Psychiatry – Vol. IV

Pascual Ángel Gargiulo
Humberto Luis Mesones Arroyo
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In Memoriam
† Prof. Dr. José Ricardo Báez
† Prof. Dr. Armando Bermejo Moroni
† Prof. Dr. Abelardo Pithod
† Prof. Dr. Dorian Gorena Urízar

Commemorative volume for the 25th Anniversary of the Laboratory of Neurosciences and Experimental Psychology

Foreword

Sisyphus

The king of Corinth was the most cunning man of his time. But his tricks and cheating defied the gods until he was punished with eternal hard labor, pushing uphill a great rock only to see it fall just before reaching the top.

Myths remind men of their fate. Most human endeavors are an endless search for truth or happiness. If the goal is an ideal, by definition it isn't possible to succeed; death will find the hero still in the path. It seems that human science can't reach complete knowledge and has to keep endlessly toiling. But classical Greeks considered euthanasia the heroic death in battle. And the best life is that spent seeking beauty and truth for love.

When Springer asked us to update studies bridging the gap between Psychiatry and Neuroscience Update, an enthusiastic group of experts volunteered. Hard work and many hours of reading and selecting valuable papers laid the foundations for state-of-the-art conclusions.

The volume had a good acceptance and the next year Volume II covered a *Translational Approach.* When Springer asked for another *update* we thought that maybe every five years could be reasonable. But the concern was that five years are too full of changes.

Learning and leading with technology is the new tool needed for teaching and working in investigation. But more demanding and an ethical obligation is continuous medical education. *E-portfolio* is the name we have to include with our familiar books and instruments. Technology has made simulators a new teaching facility unavoidable in many medical specialties. Videos and Gesell cameras help psychology and psychiatry.

Biotechnology is defying ethical boundaries and post humanism is proposing superhumans in the near future. Microchips implanted in the brain enhance abilities present or lost. The temptation to be like gods forgets the spiritual dimension of human personality.

In spite of the evident difficulties and complexity of the task, this foreword is repeating our commitment to humbly keep up our efforts in aid of excellence in knowledge and practice of medicine. Let me underline the unending and untiring work of Pascual Gargiulo who is the soul and engine of these updates. My prognosis is that he will have to accept Sisyphus's fate.

Buenos Aires, Argentina — Humberto Luis Mesones Arroyo

Preface

Preserving the tradition assumed in the previous volumes, the included chapters are written by researchers fully dedicated to their subjects. The Part I is about epistemological considerations regarding the study of normal and abnormal human behaviors. It includes fourteen chapters.

In Chap. 1, Prof. Juan Ernesto Calderón considers that psychiatric clinical results should be considered behind the Popperian demarcation criterion. The author prefers the other method. He proposes that it is the case of the Inference to the Best Explanation (IBE), suggesting that this method has enough epistemic strength. By this way, he proposes an overcoming of Popperian falsacionism. Furthermore, this method is proposed as a tool which helps explain research and clinical practice, and enquire into Evidence-Based Medicine (EBM). This chapter constitutes a rigorous and interesting approach in the field of epistemology of science.

Chapter 2, written by Prof. Miriam Dolly Arancibia de Calmels, starts with some critical considerations about the notions extracted, most of the times decontextualized, from Kant. Recently, multiple disciplines, such as pedagogy and neuroscience, give new life to the old philosophical question regarding freedom or determinism. In this context, this chapter tries to find new anthropological approaches to resolve problems of today's teachers in the classroom. The studies of empathy according to Edith Stein is considered and analyzed in a bright form.

In Chap. 3 Prof. Ricardo Aranovich speculates in a valid way the links of happiness, agreeing with oneself, the life project, and the possibility and meaning of falling in love. The imperative of fitting with the personal self, with myself, and the condition of happiness is highlighted. The notion of happiness as the unrenounceable goal of life searching for unity with itself, and overcoming difficulties of its circumstance as means of realization of the life project is discussed in an interesting and profound way.

The Chap. 4, written by Gargiulo and Crespo, is dedicated to a differentiation between causal explanations and freedom. In this chapter, the role of nature is compared with psychological or psychoreactive influences. Ontological naturalism is described as a prevalent belief today in the world, inducing a naturalistic worldview in the narrow sense. It implies the reduction of the objects of knowledge to nature, to physical and biological things and processes. Some objections of philosophical origin are proposed, delimiting a level of "understanding" independent of naturalistic explanations.

In Chap. 5, Dr. Osvaldo Agamenonni describes in a very interesting writing the evidences in favor of self-organized mechanisms in the brain. This schedule may be applied to conditioning. He starts from Perceptual Control Theory (PCT), presented in the 1970s, aiming to try a causal explanatory approach to some behaviors. Special attention is given to feedback control and to self-organizing theories, starting from the cognitive cycle, also designed as an action-perception loop. This author highlights the importance of the brain's adaptive mechanisms.

In Chap. 6, Dr. María Teresa Gargiulo analyzes the concept of *dynamis* in the Treaty on Ancient Medicine, a part of the Corpus Hippocraticum. The complementation between philosophy and medicine is considered as an interesting paradigm. The author considers this concept of *dynamis* a core concept on which a particular epistemological view is built. Additionally, she considers that a very relevant hermeneutical tradition is presented in this writing as the birth of a scientific medicine. The author considers this concept as the origin of an integration of a new model of scientific explanation.

Prof. Dr. Rocco Genaro proposes, in Chap. 7, a rigorous analysis of consciousness, higher-order thought (HOT) theory, somatoparaphrenia, and inserted thoughts in schizophrenia. Various psychopathologies of self-awareness, such as somatoparaphrenia and thought insertion, might seem to threaten the viability of the higher-order thought (HOT) theory of consciousness since it requires a HOT about *one's own* mental state to accompany every conscious state. Somatoparaphrenia is a "depersonalization disorder" characterized by the sense of alienation from parts of one's body. Thought insertion is the delusion that some thoughts are not "one's own" in some sense or are somehow being inserted into one's mind by someone else. Gennaro argues that HOT theory has nothing to fear from thought insertion and can consistently explain what happens in this admittedly unusual case.

In Chap. 8, Prof. Francisco Rego establishes neatly a relationship between body and soul, following traditional concepts, and giving to this chapter a bright condition. This author sustains that the soul does not have to be considered a nonexistent or mythological reality. It is considered that it must not be understood as a sensitive reality. In the opposite way, it may be considered as a real order that links to the body as substantial formal essential principle. It determines the body in the order of being and its way of being. In other words, the soul makes man to be and to be what he is. At the same time, while enlivening him, the soul founds all his spiritual and organic activities.

Prof. Consuelo Martínez Priego develops, in Chap. 9, an elegant historical exposition of thinking about medical pathology in Spain, starting it in somatological medical criteria and arriving, with the thought of Juan Rof Carballo, at its fullest psychosomatic expression. In this proposal, the key concept is the affective warp. The author analyzes the various sources that make up the psychosomatic perspective. She gives an important emphasis to the role played by neurology, but also to "the constitutive process" of man. This study is mainly centered in an historical perspective. The intention is an attempt to shed light on the present, fundamentally in the neuroscientific advances but also in the humanization of medicine, which does not always share the same fate.

Chapter 10 is dedicated by Prof. Dr. Guilherme Messas and Prof. Melissa Tamelini to the basement that phenomenological psychopathology gives to psychiatry. Important attention is paid to the case of schizophrenia and its relation to substance abuse. A detailed phenomenological study is applied to both realities. The notion of dialectic is developed, highlighting the consequences of its use for clinical practice. It is a founding writing in many ways, thought with particular rigor by experienced phenomenologists with wide clinical practice.

In Chap. 11, Prof. Dr. Javier Bernacer and Francisco Güell develops the notion of person in neuroscience. They start from the notion of cognitivism leading later to a comprehensive phenomenology. They stand out clearly that the attribution of the category of person applied to a living being cannot be considered as irrelevant at all. They stand out that a person is entitled to rights, dignity, and duties by law. In this chapter, a very fruitful dialogue is established between classical philosophers and the recent conceptions of theory of mind.

Prof. Dr. Luis Echarte Alonso raises, in Chap. 12, the theme of the relationship between neuroethics and beauty. He analyzes the ideas of the theory of knowledge of Aldous Huxley and its relationship with this topic. Prof. Echarte presents Huxley's ideas about Science and Art relationship in a fluid and interesting manner. This analysis consists in the comparison of two of Huxley's distinctions. He presents, on one hand, knowledge and understanding. On the other hand are emotions and beauty. The author concludes defending Huxley's notions that *understanding* and *beauty* are not fully accessible to Neuroscience, but they orientate Neuroscience in the study of the human decision-making process.

In Chap. 13, Prof. Dr. Javier García Castro proposes solutions to the ontological and epistemological problems of consciousness. He considers that the problem of consciousness has been considered very late in science. This topic implies necessary to face very diverse and complex issues. It may be considered as the main reason of this delay. Some solution attempts are described, analyzed, and discussed. Authors conclude that there is an intrinsic difficulty in objectifying the phenomenon of consciousness. Future maturation of scientific and philosophical knowledge may be necessary to clarify the ontological and epistemological foundations of consciousness.

In Chap. 14, Prof. Dr. Michel Bourin and Prof. Dr. Monique Bourin refer to historical differences in the anxiety perception. A diagnostic consideration about the present condition is drawn remembering that we now have more risk factors in our environment due to stress, urban violence, and the vagaries of employment. In other words, it is attributed to modifications in the environmental conditions. For most of us, they argue, factors such as housing, sanitary conditions, and food have been improved over time. However, anxiety, panic attacks, and fear of old age, illness, and death remain. In this chapter, an historical approach is developed in an interesting and documented way.

In the Part II of the book, bridges between basic neurosciences and the human brain are attempted. The first example of this is Chap. 15. In it, Dr. Luis Felipe Sarmiento Rivera and Prof. Dr. Amauri Gouveia Jr. review the

effects of neurotransmitters and hormones in human decision-making. They take into account the difficulties in approaching this human behavior, considering the perspectives from which these decisions have been studied. It is the case of variables related to the cost and benefits of a decision (economic decisions), involving social bonding (social decisions), social behavior and mating, and moral decisions that relates moral principles. In this chapter, they focus on the specifically induced actions of neurotransmitters (dopamine, Arginine-Vasopressin) and neuro-hormones (oxytocin, testosterone, stress hormones). The psychobiological interaction between such hormones and neurotransmitters and their relations in the decision-making process is detailed and commented in a lucid and interesting manner.

In Chap. 16, Occhiepo and coworkers, directed by Prof. Dr. Claudia Bregonzio, describe in depth the role of glial cells in schizophrenia. The role of Angiotensin II is focused. They begin with the fact that it is well known that schizophrenia illness has been related to a wide pluri-factorial etiopathogenesis (gene factors, neuroinflammation, and the brain microenvironment's alterations, etc.). Glial cells are considered important elements in the brain microenvironment. They have a relevant role in synaptic establishment and function, neuroinflammation, metabolic and ion homeostasis, among others. They are today an important topic of study in numerous researches on schizophrenia etiopathology. The role of Angiotensin II in this context is carefully described. Angiotensin II has been involved in several pathologies, and now its role in schizophrenia is proposed. A special reference to AT_1-R involvement is developed regarding this illness. The chapter is written in a lucid manner.

The workgroup directed by Dr. María Graciela López Ordieres describes, in Chap. 17, the possible role of Somatostatin and Neurotensin systems in schizophrenia. Due to its complexity, it is thought that the pathophysiology of this illness goes beyond dopamine dysfunction, including also glutamate, GABA, serotonin, and acetylcholine systems. These neurotransmitters may be interacting with neuropeptide systems. In schizophrenic patients it has been reported that GABA interneurons, containing parvalbumin and somatostatin, are reduced, suggesting that it may be related to this illness. Neurotensin is closely related to dopamine transmission. After central administration it produces biochemical and behavioral effects that resemble those observed in a systemic administration of antipsychotic agents. Since peptides do not cross the blood-brain barrier properly, new peptide analogues are synthesized, aiming to resist enzymatic degradation, and entering to the brain crossing barriers. The above-mentioned peptides interact with neurotransmitter systems that has been related to pathophysiology of schizophrenia. These facts improve our knowledge of this psychosis and may constitute new alternatives for its treatment.

Prof. Dr. Michel Bourin actualizes, in Chap. 18, our conceptions about the mechanism of action of benzodiacepines. The role of these drugs on GABA and serotonin receptors, the action of serotonin reuptake inhibitors, and glutamine and neuropeptides are described and analyzed. The action of Neuropeptides in anxiety is a matter of extensive review (cholecystokin, corticotropin-releasing hormone, substance P and neurokinins, galanin, oxy-

tocin, atrial natriuretic peptide). Some interactions are studied (glutamate regulating GABA activity, Adenosine interactions with serotonin and GABA). The effect of a high number of neurotransmitters and hormones on anxiety is reviewed, giving an interesting preclinical and clinical relevance to this chapter.

In Chap. 19, the group led by Prof. Dr. Mariela Pérez focuses on the learning processes involved in benzodiacepine dependence. From its prescription to dependence is studied in a rigorous manner. The context-dependent associative learning process underlying BZD tolerance and dependence is analyzed. Authors postulate that long-lasting neuroadaptations are present in the limbic system. Repeated drug exposure is necessary aiming to induce alterations in synaptic plasticity, such as glutamate synaptic modifications in brain structures related to memory formation. These structures are mainly the hippocampus, the reward system, the ventral tegmental area, the nucleus accumbens, and the frontal cortex. The chapter is clear and comprehensive, with scientific and didactic merits.

The group of Prof. Dr. Mariela Pérez contributes again in Chap. 20, describing the molecular mechanisms implied in acute traumatic brain injury (TBI). A description of the effects of neuro-inflammation in higher-order cognitive processes is described. The fact that an important number of patients report chronic disabilities long after the initial injury event is analyzed and explained mentioning mechanisms acting after the early mechanical damage. A secondary brain injury is described as caused by metabolic, synaptic and vascular alterations, neuro-inflammatory processes, and oxidative stress. Behavioral, affective, cognitive, and general psychological changes are clearly detailed by authors. This chapter constitutes an interesting link between neuropathology and altered human behavior.

Prof. Dr. Michel Bourin contributes again with a very interesting chapter starting from the physiology and following with the pharmacology of melatonin. Chapter 21, didactic and detailed, starts with historical considerations about the role of melatonin. Prof. Bourin also describes an important number of pharmacological properties of melatonin, such as antioxidant effects, oncostatic, anti-aging, an agent in the immune system, and, recently, a hypnotic power of melatonin as a "sleep hormone." Its role as a hypnotic in women and the elderly is referred. The role of melatonin in the resynchronization of our biological clock is detailed. Its efficacy has been observed in the treatment of the circadian rhythm desynchronization syndromes. In these cases it must be emphasized that a melatonin intake may be useful in the therapeutic arsenal of the management of insomnia. It may also be interesting promoting a better sleep.

In Chap. 22, Daniel Cardinali and Daniel Vigo postulate a cytoprotective effect exerted by melatonin. They consider the metabolic syndrome (MS) as an example. In this clinical entity, an important number of risk factors for cardiovascular disease are recognized. It is the case of elevated blood pressure, hyperinsulinemia, glucose intolerance, and dyslipidemia. The authors discuss the role chronobiotic/cytoprotective properties of melatonin may have in the prevention and treatment of MS. As a relevant fact, Melatonin levels are consistently reduced in MS. Improvement of sleep efficiency and

antioxidant and anti-inflammatory properties that derive partly from its role as a metabolic regulator and mitochondrial protector have been highlighted. The chapter analyzes the actions of melatonin that are relevant for the attenuation of inflammatory responses in MS. It is found that melatonin is effective in curtailing MS in animal models of hyperadiposity and ischemic and non-ischemic heart failure.

José Burgos and Juan Galeazzi review, in Chap. 23, a neural-network simulation model of a possible role of the hippocampus in Pavlovian conditioning. In this model it is suggested by authors that the hippocampus sends a diffuse discrepancy signal. It would lead to modifications in modulation efficacies, starting from synapses of primary-sensory areas and to exerting effects on polysensory areas. The authors hypothesize that such modulations significantly modify signal Pavlovian conditioning. Additionally, it may be think that Hippocampal lesions have detrimental effects. The study was conducted using two computer simulations. Finally, the authors discuss the limitations and future directions of the model.

In the next four chapters, natural compounds are studied aiming to find psychopharmacological effects. A group of three chapters is dedicated to the search for compounds with biogenic effects. Two chapters were written by members of the Faculty of Agrarian Sciences of the National University of Cuyo, one comes from the Hong Kong Polytechnic University, and a final clinical chapter evaluates the possible application of natural compounds.

The first of these chapters, Chap. 24, is directed by Prof. Alejandra Beatriz Camargo. The team search for neuroprotective compounds in Brassica vegetables. This Brassicaceae family includes several vegetables such as broccoli, cabbage, cauliflower, radish, kale, and rocket, among others. This group has been associated with antioxidant, anti-inflammatory and anticarcinogenic effects. Brassica plants are rich in several neuroprotective compounds like isothiocyanates, phenolic compounds, vitamins, minerals, and carotenoids. The authors present a review of the main compounds and demonstrate the neuroprotective effects of Brassica vegetables. Their mechanism of action is also analyzed. Some considerations on their positive effects in neurological disorders are also given.

The second study in this line is Chap. 25, written by the team directed by Leonor Deis. In this chapter, the biological action of colored compounds present in fruits and vegetables is reviewed. The action of carotenes, lycopene, anthocyanins, flavanols, and lutein is evaluated. The authors mention previous studies in which the intake of plant foods like blueberries decreases the risk of obesity, diabetes, heart disease, and, mainly, overall mortality. The authors recommend the consumption of five portions of fruit and vegetables per day. They conclude that an important number of active biological compounds are present in vegetables, highlighting polyphenols. They mention epidemiological studies and associated meta-analyses strongly suggesting that long-term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, endothelial dysfunction, osteoporosis, and neurodegenerative diseases. Furthermore, it has been postulated that polyphenols may have relevant effects as antihypertensive, anti-inflammatory, bactericide, antimutagenic, and antitumoral

agents. The study constitutes an interesting set of arguments in favor of the consumption of natural products of plant origin as inducers of psychotropic effects.

Chapter 26, directed by Professors Benjamin Yee and William Chi-Sing Tai, gives additional arguments in the same line. They formulated an input from herbal medicines in the gut-brain communication perspective. This chapter is centered on herbal medicine, depression, anxiety, microbiome, and gut-brain axis. Herbal remedies are here proposed in neuropsychiatric diseases. The microbiota transplantation and the possibility of a herbal modifications of the gut microbiota are studied in an interesting and detailed manner, suggesting new treatment ways. Potential benefits of herbal medicines and related supplements are postulated as possible novel therapeutic perspectives.

In Chap. 27, Prof. Dr. Eric Wainwright proposes new treatment possibilities, including natural compounds. He analyzes the role of the Mediterranean diet, omega-3, and nutrigenomics in primary prevention. He recommends physical exercise as a cornerstone in the management of many disorders. It is the case of Alzheimer's disease, Parkinson's disease and stroke. He clearly points out that neurodegenerative disorders are frequently comorbid with depression. He proposes new strategies like modifiable lifestyle factors and psychoeducation. It is postulated here that these variables should improve current treatment results. This chapter constitutes a relevant place for reflection on new therapeutic possibilities.

Prof. Dr. Gustavo Tafet and Dr. Diego Feder review, in Chap. 28, the relationships between environmental and psychoneurobiological factors. This study emphasizes the role of environmental and psychoneurobiological factors in the interface between stress and depression. The relevance of resilience in this situation is highlighted. They remind us that chronic stress is involved in the origin and development of depression. The authors also discuss long-lasting effects of stressful experiences throughout life, and mainly in the early stages of life. Increased activation of the HPA axis, leading to hypercortisolism, plays a role in chronic stress and depressive disorders. This chapter is interesting, and links in a valid manner neurobiological findings and clinical situations.

In Chap. 29 the workgroup of Prof. Dr. Francisco Barrantes gives interesting evidences about the impact of Apolipoprotein E (ApoE) allelic variants on Alzheimer's disease (AD). ApoE is a lipid-transport protein. The main function of ApoE is to deliver lipids to cell-surface receptors. The authors propose that it is involved in the pathogenesis of AD. They propose that carriers of the *APOEε4* isoform of the gene are at higher risk than *APOEε3* carriers developing sporadic AD. ApoE is a lipid-transport protein. Its main function is to deliver lipids to cell-surface receptors. This chapter proposes new possibilities of late-onset variant of AD (LOAD) diagnostic and potential new therapeutics.

The group directed by Prof. Dr. Claudia Hereñú develops, in Chap. 30 an analysis of cognitive and emotional status in Parkinson's disease (PD). They claim that regardless of the well-characterized, well-known motor symptoms, the presence of non-motor symptoms (cognitive dysfunctions and emotional

disturbances) are underestimated. The pathophysiology of PD (degeneration of the nigrostriatal dopaminergic pathway) implies affectation of other critical areas, such as basolateral amygdala and hippocampus. Recent studies are addressed. Neuro-circuitries, regulation networks, and possible therapeutic approaches in different Parkinsonism experimental models are discussed. The chapter constitutes an interesting example of correlations between neural pathology and behavior.

In Part III the relationship between neurosciences, teaching, and the role of social environment are considered. In Chap. 31, written by Lucas Rodríguez, José Eduardo Moreno, and Belén Mesurado, relationships in children and adolescents are studied. Authors considere that positive development contributes to mental health problems prevention. Actually, social abilities improve the contact and relation with other persons. They propose, citing concepts of Harry Stack Sullivan, that friendship is an opportunity to learn about conflict and negotiation. In this context, authors sustain that children and adolescents develop perspective taking skills and empathic concern. Using specific tests, the chapter highlights the role of honesty, perspective taking, and empathic concern as predictors of friendship quality. This study contains a relevant set of findings applicable by parents and educators.

Santiago Resett and Belén Mesurado analyzes bullying and cyberbullying in adolescents in Chap. 32. A meta-analysis is done about the effectiveness of interventions. The intention is to critically evaluate the effectiveness of interventions directed to reduce behaviors like bullying, victimization, cyberbullying, and cybervictimization in adolescents. They found that bullying programs were a bit more effective than cyberbullying programs. Additionally, they found that the effect size of the intervention programs on victimization and cybervictimization was similar. The authors finally give some suggestions for future studies. This chapter, like the previous one, is a study of high interest for educators.

In Chap. 33 Celina Korzeniowski, Mirta Ison, and Hilda Difabio propose an interesting summary of the developmental trajectory of executive functions from birth to adulthood. They characterize firstly the concept of executive functions (EF), and its role in cognitive and socio-emotional advance. These authors point out that longitudinal design studies have shown the relevance of adequate EF development during childhood. They consider, according to the mentioned studies, that EF are reliable indexes as predictors of improved health, higher academic achievements, and better employment status. Conversely, these EF may be considered as acceptable indexes of a lower incidence of disruptive social conduct, addictions, behavioral problems and psychopathology in adulthood. The value of a measure of these functions seems a relevant predictive value, and prevention should be influenced by them.

Our group presents, integrated with Prof. Dr. Jesús Escanero Marcén, María Soledd Soria, Manuel Guerra, and Pascual Ángel Gargiulo, in Chap. 34, a review regarding approaches to learning at university, with special reference to learning to learn. According to the Bologna reform, which points out that teaching must be student-centered learning, we study here the competence of "learning to learn." Metacognition is the central topic in this chapter.

It begins remembering the contributions of our group in "learning to learn," working with learning styles, and the creation of a new questionnaire (CESEA, acronym in Spanish of the Questionnaire of Escanero and Soria) for exploration. The final purpose is to facilitate efficient learning for students. We mention also here contributions of our group. We worked with questionnaires exploring metacognitive and cognitive strategies. The intention was to provide students with the best means for meaningful learning. Additionally, we recommend some methodologies to teachers. The self-perception approach in students is also considered. Another goal here is improvement in teaching.

In Chap. 35, an interesting study about the labor conditions of state school teachers is realized by Víctor Quiroga Calegari and Carlos A. Bonantini. They present a comparative cohort study of Burnout Syndrome (BS). They assay an approach from psychosociology. In this interesting chapter, they display part of their study based on considering 9 cohorts (2008/2016). These cohorts were tested by a methodological triangulation, including application of Maslach Burnout Inventory (MBI) as a psycho-technical tool, and, simultaneously, the analysis of observations and interviews of the Areas of Work Life Survey (AWS). They conclude with some suggestions for prevention at the institutional level, considering the dimensions of the syndrome.

In Chap. 36, Prof. Dr. Raúl Gagliardi formulates a detailed review about traumatic situations and mental disorders in migrants, refugees, and asylum seekers. He meets here an important number of social problems. He mentions and relates migrants' mental health, mental health problems, traumatic situations, adaptation, cultural consonance, ethnic density, asylum seekers, refugees, children, adolescents, and the socio-cognitive niche. He mentions a number of important mental health problems that may be observed in migrants. The mental issues discussed here are psychosis, depression, stress, anxiety, post-traumatic stress disorder (PTSD), and paranoid-like states. In his reflection, Gagliardi uses different approaches. "Cultural consonance," "ethnic density," and "socio-cognitive niche" are discussed The experience and sharp observations of a scholar in this subject are presented in a very acute way.

In Part IV, an effort to explain human pathological behaviors, tendency bridges from brain disorders to psychopathology is made. Here clinical projection lines are cultured. The first chapter of the part, Chap. 37, is headed by Guillermo Alfonso and directed by Pascual Ángel Gargiulo. It is about the transition to a dimensional system for Personality Disorders. In it, the main advances and limitations are considered. Starting from the debate between categorical and dimensional systems, the authors consider the position of DSM-V. Here we retain the categorical classification of previous editions for Personality Disorders (PD). However, an alternative evaluation system with dimensional bases is proposed. It is considered that this "compromise solution" between the need to preserve the clinical tradition and the imperative of overcoming important diagnostic restrictions opened the doors for a better approach. The authors conclude that dimensional alternatives do not solve all the clinical problems but provide valuable resources tendency to a constant refinement in this discipline.

In Chap. 38, headed by Ángel José Martín Gargiulo and directed by Luciana D'Alessio, a range of the psychiatric comorbidities of epilepsy are revealed. New approaches and perspectives are provided. The coexistence of epilepsy, depression, stress, anxiety, and psychosis is exposed in a detailed and interesting manner. The relevant role of depression is highlighted because it affects patient's quality of life in a significant way. In the second place are anxiety disorders. They often generate severe problems that are sometimes underestimated at the clinical level, despite the abundant evidences supporting their relevance. The problem of psychotic disorders in epilepsy is even today often controversial. A low number of studies on this subject have been realized. Furthermore, the psychopathological mechanisms behind them remain largely unknown. The problem of diagnoses and management of psychogenic nonepileptic seizures is also reviewed. The chapter constitutes a very relevant review of psychiatric comorbidity of epilepsy. Finally, the authors argue that the management of stress and emotions in epileptic patients has a direct impact on their quality of life.

The group directed by Prof. Dr. Svetlozar Haralanov, in Chap. 39, proposes a very novel method in psychiatry. They present an integrative objective quantification of individual locomotor behavior (LMB) during the execution of the stepping test of Unterberger. It is here used in depressive patients. Empirical data from 200 depressive patients compared to 200 matched healthy controls was collected. The results demonstrated two contrasting poles of affective psychoses, overinhibited (hypolocomotion) and overactivated (hyperlocomotion). They propose a new dopamine hypothesis that links hypolocomotion with underlying hypodopaminergia, and hyperlocomotion with underlying hyperdopaminergia. Their findings and conceptual analyses are discussed in the light of their clinical implications. The authors postulate that the present method allows a more personalized treatment selection. The implications for their stratification and personalized treatment monitoring are here discussed.

In Chap. 40, the group headed by Prof. Dr. Antonio Lobo describes the Psychosomatic and Liaison Psychiatry as the contribution from Psychiatry to the general field of Medicine. In this analytical chapter, they postulate that this discipline is based on the traditional humanistic medical view but also on the empirical or evidence-based approach. Using data coming from epidemiological research, they present examples of this approach. It has contributed to document the prevalence and incidence of psychiatric morbidity in medical settings. The authors also mention other benefits of this methodology. They sustain that it contributes to the establishment of outcome, to the actuarial assessment of morbid risk, and to the efficacy of the treatment of psychiatric problems in medical patients. They also postulate that it may contribute to the conceptual construction of diagnosis and classification. The transcendence in this thought should be noted.

Prof. Dr. Michel Bourin contributes, in Chap. 41, to the study of emotions and cognitions in bipolar disorder (BD). The author remarks that organic or functional disturbances of processes of cognitive activities and the control of the emotional experience are likely to induce pathologies clinically characterized. Cognitive functioning and affective driving are commonly disturbed in

BD. They imply behavioral disorders, social interactions being one of the main alterations. These alterations may be observed during acute episodes but may also persist during the euthymic period. Emotional disturbances may influence cognitive functioning. He concludes that functional abnormalities of cortico-subcortical neural networks could be at the origin. As a consequence, improving cognitive and emotional processes is an objective of therapeutic management. The chapter opens new and interesting dimensions of therapeutic objectives.

The link between cannabis and psychoses is treated by Prof. Dr. Eduardo Leiderman in Chap. 42. He sustains that, despite initial controversies, a causal relationship may be established between tetrahydrocannabinol (THC) and psychoses. This author alerts about the fact that the concentration of this substance is increasing in confiscated cannabis products. The cause-effect association between cannabis and psychosis is possible due to the strength of evidences. It may be also be outstanding the consistency along the studies, biological gradient, temporality, plausibility, coherence, experimentation, and analogies. It has been observed that cannabis increases symptoms' severity, relapse, and length of hospitalization. Its use also deteriorates the functionality of those individuals already affected by psychotic disorders. Present findings contraindicate the use of cannabis in relatives of patients suffering schizophrenia, and mainly in teenagers, since they are the most vulnerable age group. Effort must be made to discourage the use of cannabis and treat addiction. The important risks related to its use are clearly exposed.

In Chap. 43, the group integrated by Lic. María Andrea Delgado, Lic. Adriana Fochesato, Prof. Dr. Luis Isaías Juncos, and Prof. Dr. Pascual Ángel Gargiulo develops the theme of gut microbiota biomarkers in Autism Spectrum Disorders (ASDs). ASDs are considered here as a complex neurodevelopmental group of disorders characterized by impairments in social and cognitive functions. The etiology is not clear, but it is thought that they are caused by a combination of genetic predisposition and environmental factors. ASD patients present frequently comorbid medical conditions like gastrointestinal (GI) symptoms. Relevant evidences allow to sustain the importance of the "gut-brain axis" in the pathogenesis of ASDs. Interestingly GI disorders are correlated with severity of the brain disorder. Gut microbiota produces metabolic products that may influence brain function and the correlative behavior using diverse humoral mechanisms. The importance of considering these mechanisms and their consequences in the clinical picture is highlighted in this chapter, opening possibilities for the diagnosis and control of these clinical entities.

Following with this thematic of child psychiatry, the group headed by Dr. Nicolás Fayed describes in Chap. 44 the findings of brain magnetic resonance imaging in attention-deficit/hyperactivity disorder (ADHD). It is characterized by attention deficiency, hyperactivity, and impulsivity. These symptoms are not present in all cases. New genetic findings are suggesting that ADHD may be a hereditary disease in most cases. However, the etiology appears to be multifactorial, but with a neurobiological base. Diverse elements like genes interaction, environmental, perinatal, and psychosocial factors are usually highlighted when considering its origin. In this chapter the authors review

a series of MR techniques, including functional MRI and MR spectroscopy. A relevant number of functional MRI studies in ADHD have shown abnormal blood flow or abnormal metabolism within the brain. Findings are not specific. However, the authors postulate that larger series and standardized methodology could delimitate groups and allow comparisons and generalization. An interesting group of complementary examinations are proposed here aiming to reaffirm the clinical diagnostic.

Continuing with the topic of complementary diagnostic methods in psychopathology, Dr. Fayed points in Chap. 45 the interest recently awakened by neuroimaging techniques applied to psychopathology. Among them, Magnetic Resonance Spectroscopy (MRS) stands out today. It obtains information by applying magnetic waves to living tissues and, particularly, to the central nervous system. The fact that it allows characterizing metabolic or chemical abnormalities in some nosological entities enables an outstanding presence to this complementary method of diagnosis in psychopathology. The authors detail their findings using hydrogen Magnetic Resonance Spectroscopy. This method has been used in a number of important disorders, like autism spectrum disorders, delayed psychomotor development, mild cognitive impairment, and attention-deficit/hyperactivity disorder in childhood. In the elderly it has been applied to Alzheimer's disease and Parkinson's disease studies. In the adult it has been used in somatoform disorders and fibromyalgia. The chapter gives additional relevance to the complementary methods in psychiatry.

In Chap. 46, Prof. Dr. Michel Bourin addresses the issue of prescriptions in the elderly. Important modifications in the pharmacokinetic and pharmacodynamic parameters must be considered in the elderly subject. Some of the main modifications are kidney functions. Furthermore, aging can alter the sensitivity of receptors in the central nervous system. It mainly compromises dopaminergic receptors. To address these changes, the author suggests some psychopharmacological strategies in the elderly. Anxiety should be treated with selective serotonin reuptake inhibitors (SSRIs), paying special attention to the doses. Hypnotics should be used with special care, due to the risk of falls during night. In the case of depressive disorders, all the tricyclics and tetracyclics can be used. However, it is important to note that they have atropinic effects. The author mentions that SSRIs have not yet demonstrated efficacy in depression in the elderly. Mood stabilizers may be used, but with the same care and limitations as in adults. Antipsychotics may be used in confused or delirious states. Haloperidol must be applied in low doses due to the risk of drug-induced Parkinsonism. The chapter represents the synthesis of an expert in psychopharmacology and clinical psychiatry, on a relevant topic.

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Part I

Epistemological Considerations About the Study of Normal and Abnormal Human Behaviors

1 Psychiatry and the Inference to the Best Explanation

Juan Ernesto Calderón

Introduction

One of the most influential currents in the field of the contemporary Philosophy of Science is Karl Popper's critical rationalism. This theory sustains that the best way of evaluating scientific theories is through the hypothetico-deductive method (H-D). According to the H-D, scientific theories are hypotheses which are tested deducing observational consequences from them. The hypotheses cannot be confirmed, but refuted through the H-D. When a hypothesis is falsified, it obliges us to formulate other surpassing hypotheses concerning their explanatory content and precision. A hypothesis is scientific if it can be falsified, in the sense that there exists a group of statements logically possible which can be deduced from the theory and which serve to refute it.

The key of this method lies in the possibility of achieving a refutation. However, the problem is that a hypothesis can never be absolutely refuted. According to the Duhem-Quine thesis, a theory can be made compatible with any evidence through an adequate adjustment of the auxiliary hypothesis. This implies that the H-D does not have the necessary epistemic force to refute the hypotheses, which indicates that the H-D is completely permissive but minimally probative. This problem, which affects any theory, also has an impact on the field of Psychiatry. In this area, the confounding factors are present more than in other types of object of study. This fact has made various authors deny the possibility of approaching the discipline from the categories of the Philosophy of Science. According to this position, concentrating exclusively on the clinical practice is more productive than enquiring into the implicit strategies of the Philosophy of Science which can appear inside these practices. In this same line, serious doubts about the possibility of applying the so-called Evidence-Based Medicine (EBM) are posed.

The objectives of the present contribution are the following: (1) showing that the difficulties presented by Falsacionism can be overcome by the Inference to the Best Explanation (IME); (2) demonstrating that the IBE can serve as a key element for the EBM; and (3) showing that, in the specific case of Psychiatry, the IBE is a tool which allows to complete the EBM and to explain medical practice and research. Upon this base, this paper will deal, in the first place, with the problems of Popper's Falsacionism; and in the second place, with the notion of the IBE as a methodological proposal able to overcome the difficulties presented in the critical rationalism in general and in the epistemological analysis of the Psychiatry in particular will be dealt with. Finally, the solutions given to the EBM by the IBE will be discussed.

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The Method in Psychiatry

In the case of Neurosciences, an important distinction between Philosophy of Neuroscience and Neurophilosophy can be found. Neurophilosophy aims at using the tools of Neuroscience to understand traditional philosophical questions as "What is an emotion?" [1], "What is the nature of desire?" [2], "How may social cognition be made possible?" [3], "What is the neural basis of moral cognition?" [1], and "What is the neural basis of happiness?" [4]. Philosophy of Neuroscience, however, deals with the traditional questions of Philosophy of Science: Which kind of investigation and which type of explanation is made in Neurosciences? These enquiries can be answered from a descriptive or from a normative perspective, whether we attempt to explain how Neurosciences proceed in fact or how they should proceed [5].

The Philosophy of Neurosciences has problems when framing its activity in relation to the general framework of sciences and its classification. Taking into account the nature of the object of study of Neurosciences, starting from a normative framework or applying the categories of science and pseudoscience according to falsifiability has not been productive [6]. As Rachel Cooper [7] states, the distinction between falsifiable and unfalsifiable becomes even more confusing since the Duhem-Quine thesis, which demonstrates the impossibility of forcefully falsifying the statements. For this reason, Cooper states that in Psychiatry before debating about the character of science and pseudoscience, we must answer concrete questions about Pathology and possible treatments instead of answering to the traditional rules of science. As John Pickstone [8] and Cooper [7] point out, the methods used in Psychiatry are not always the same in a strict sense but vary historically. For this reason, instead of asking if Psychiatry is a scientific research, we must ask about the method which has been used to study the case in hand.

The impossibility of strictly refuting a theory is seen in any theory analyzed with the hypothetico-deductive method (H-D). This fact can be shown through the so-called "Duhem-Quine thesis." According to Elliot Sober [9], the problem posed by the Duhem-Quine thesis can be summarized as follows: when a negative prediction is derived from the conjunction of a hypothesis (H) and auxiliary hypothesis (AH), which one should be rejected: H or AH? This problem affects the capacity of the H-D of evaluating any theory because any possible negative prediction does not bring along the epistemic force to reject H. For this reason, Stathis Psillos [10] (p. 181) affirms that "….H-D is minimally epistemically probative, since it does not have the resources to show how the undercutting defeaters can be removed."

If we depart from the base that the problem is not the theories but the method, the key is looking for another method which offers enough epistemic force and which can be broad-based at the same time. "Any attempt to characterize the abstract structure of scientific method should make the method satisfy two general an intuitively epistemic compelling desiderata: it should be *ampliative* and epistemically probative" [10] (p. 174). Evidently this ampliative character is essential if we consider science an activity which makes our knowledge expand. However, this growing is merely illusory if we do not have any way of proving this knowledge. To refute a hypothesis, we need to know concretely where the problem resides: if in the H or in the AH. Nevertheless, "All these considerations go a lot beyond the deductive link between hypotheses and data that forms the backbone of H-D and are not incorporated by the logical structure of H-D" [10] (p. 181). Which are the difficulties of making Psychiatry fit into Popper's model? Evidently, a basic problem to place Psychiatry into the Popperian framework departs from the object of study itself. That is to say, how scientists achieve a conclusive refutation when the factors influencing the results overlap with the experiment making it more difficult to accomplish conclusive results. Specifically, the verification of hypotheses is complex due to the great amount of deviation factors (*confounding factors*), which, according to Jeremy Howick [11] (pp. 35–37), have three characteristics:

1. It affects the results. For example, age can be mentioned as a factor that effectively influences the results. If the age of the members of the control group is not considered, the result will certainly be seriously altered.
2. If a key element, such as the age, is distributed in an unequal way in the experimental group and the control group, the inequity of the distribution is a deviation factor, that is to say, if the factor is distributed in inequitable way between the experimental and the control group.
3. The third characteristic is when a factor unrelated to experimental intervention appears.

As it is seen, "Each confounding factor provides a potential alternative explanation for the results of a clinical trial" [11] (p. 35). This means that each of the factors is perfectly compatible with the idea of the auxiliary hypothesis, which makes a complete and conclusive falsation impossible, as the Duhem-Quine thesis points out against Popper's Falsacionism. Each deviation factor gives an alternative potential explanation for the results of a clinical trial.

Irrefutability of the Scientific Theories and the Inference to the Best Explanation

According to Psillos, the difficulties presented by the H-D can be overcome by the IME. This can be summarized as follows:

> D is a collection of data (facts, observations, givens)
> H explains D (H would, if true, explain D)
> No other hypothesis can explain D as well as H does.
> Therefore, H is probably true. [10] (p. 183)

The evaluation made by the IBE implies, in the first place, we never work "in the vacuum." This implication means that the causal-nomological connection between the *explanans* and the *explanandum* is relevant because not only data is considered but also the available substantiated knowledge [10] (pp. 183–184). Based on this, why the IBE allows to compare hypotheses is clearly seen. H1 and H2 can be compared according to their verisimilitude degree. Scientists with their activity indicate which hypothesis gives more possibilities of development. Psillos [10] (pp. 184–185) mentions six key points which serve to determine which is the best hypothesis.

1. *Consilience*: if there are two hypotheses H_1 and H_2 and the "relevant background knowledge" favors H_1 over H_2, unless any relevant change appears, H_1 must be considered the best explanation.
2. *Completeness*: if there exists an only one explanatory hypothesis H which explains all the data, in spite of other hypotheses which explain in a partial way, H must be considered the best.
3. *Importance*: if there are two hypotheses, H_1 and H_2, which do not explain the entire relevant phenomena, but H_1 explain the most salient, H_1 is the best.
4. *Parsimony*: if H_1 and H_2 explain all the facts, but H_1 uses less assumptions than H_2, then H_1 is the best.
5. *Unification*: if H_1 and H_2 are compound hypotheses, but H_1 has less auxiliary hypotheses than H_2, H_1 is the best.
6. *Precision*: if H_1 offers a more precise explanation of the phenomenon, "… in particular an explanation that articulates some causal-nomological mechanism by means of which the phenomena are explained," H_1 is better than H_2.

Together with the six points mentioned above, there is another key element of the IME which we should consider: coherence. "In the end, IBE enhances the explanatory coherence of a background corpus of belief by choosing a hypothesis which brings certain pieces of evidence into line with this corpus" [10] (p. 188). When H is affirmed to be the best hypothesis, coherence is implied not only between the corpus of knowledge but also with the data tried to be explained. For this reason, through the IBE, the extending character which the method evaluating theories must have is rescued.

The problem of two competing theories could be posed, H_1 and H_2, which share up the criteria. Then, H_1 could be superior to H_2 in the three criteria and H_2 in the remaining ones. To solve this problem, the IBE states that the criterion never works "in the vacuum," which points out that the election is related to the state of the situation, where the scientific community decides.

Besides, the existence of several hypotheses is not only healthy from the point of view of scientific knowledge, but it is also a fact. We should also bear in mind that the IBE does consider the theories to be completely true. This interpretation is based on the way the IBE interprets the notion of verisimilitude (*truth-likeness*). "In our interactions with the world, the exact truth cannot generally be had, especially concerning the unobservable and spatio-temporally remote aspects of the world. A perfect match between theories and the world is almost impossible" [12] (p. 276). This situation is due to different reasons. One of these is that the complexity of the natural phenomena prevents it from being represented by scientific theories unless idealizations and simplifications are introduced. For this reason, "Demanding the exact truth in science would amount to demanding the exclusion of all approximations, simplifications, idealizations, approximate derivations, sources of error in measurements and calculations. Even were this sort of science possible, it would not be the science which we are familiar" [12] (p. 276). We should also state that the idea of verisimilitude implied by the IBE is an intuitive notion which is not realizable and it does not require it because "The conceptual schemes that science uses to study the world are revisa*ble* and revise*d*" [10] (p. 32).

Evidence-Based Medicine

The EBM emerged as a new "paradigm." This new paradigm tried to bring more strict canons for medical practice. Specifically, scientists tried to separate medical practice from less rigorous methodologies.

Evidence-Based Medicine de-emphasizes intuition, unsystematic clinical experience, and pathophysiological rationale as sufficient grounds for clinical decision-making and stresses the examination of evidence from clinical research [13].

As Jeremy Howick [11] (p. 15) points out, there are three terms which need clarification: for "clinical experience," the EBM representatives stem from the fact that clinical experience is not based on the relevant clinical experience available, but it depends on non-systematized personal experiences. Many times, these experiences are transformed into common practices through congresses and publications which are means of dissemination, but which are supposed to have a solid foundation although they have not been subjected to criticism.

For pathophysiology rationale (mechanistic reasoning), the EBM representatives understand the existence of treatments based on explanations which suppose the existence of a causal-explanatory relation between a cause and a determined object. For example, the belief that antiarrhythmic drugs would reduce mortality was based on (supposed) facts about the causes of mortality (arrhythmias) and the mechanism of action of antiarrhythmic drugs [11] (p. 15). This belief proved to be dangerously false. For this reason, the representatives of the EBM contrast mechanistic reasoning to a clinical research or comparative clinical study. The second aims at observing the results through empirical tests where the concrete effects of intervention can be evaluated. The classical example of Cardiac Arrhythmia Suppression Control is an attest designed to measure if the drugs controlling arrhythmia reduce the mortality in patients who have suffered a heart attack. As it is widely known, the result was negative, showing that the use of mechanistic reasoning can generate serious problems. These problems can be detected and overcome using comparative clinical studies. However, the representatives of the EBM do not see all comparative studies in the same way: the randomized trials are better because they give the best evidence to evaluate therapeutic effects (see Figure 1 [11], p. 13).

Some authors consider that it is impossible to apply the EBM categories of analysis to

Psychiatry, and for this reason, they question the attempt to propose an Evidence-Based Psychiatry (EBP). The questioning stem from part of two methodological problems: "…prognostic homogeneity of clinical trial groups and quantification of trial outcomes" [14]. Based on these methodological problems, two basic arguments against the application of the EBM in Psychiatry appear. The first states that the theory of EBM can be accepted, but not its actual practice. In other words, this criticism affirms that the research does not reflect neither the conditions of Psychiatry nor the patients' state sufficiently, so as to be useful in clinical practice. An example of this problem appears in the case of a diagnosis of depression. In this case, as the treatment is presented under the form of a randomized controlled trial (RCT), the fact that the patient has multiple conduct disorders is left aside. On the basis of this, data obtained in patients with a pathology are applied to patients with multiple pathologies harming clinical practice instead of benefiting it.

The second argument aims at the fact that the narrative structure and the meaning of personal experiences are essential components of living with a mental disorder and the psychotherapy treatment of those patients. This situation is hard to study with the tools provided by the EBM with a RCT because these aspects complicate when they do not make it possible a study described in terms of neurological processes. Life experiences cannot be completely explained in biological or quantitative terms. According to Holmes [15], these problems do not imply abandoning research on non-pharmacological treatments, but refining it or the methods.

These criticisms can be overcome with the help of the IBE. In the first case, when we mention that the results obtained cannot be put into practice, the IBE departing from the intrinsic relation between *explanans* and *explanandum* does not allow this to happen. The relation between theory and concrete practice is constantly reformulating and reinventing itself because it is not a static but a dynamic relation. In the specific case of Psychiatry, the patient cannot be left aside by any means. That is to say that the Inference to the Best Explanation acts upon the patient trying to give an answer to the pathology that presents. In this same line, the answer to the second objection must be understood. The IBE always departs from the fact that is tried to be explained, not the agreement between the fact and the theory. Apart from that, the fact that experience is individual and in this sense is narrated and has meaning only for the one who is suffering it does not invalidate the possibility of searching common and natural general elements inside this particular structure. In this sense, the IBE is the one which assures with a greater degree of truth the relation between a particular case and the theory which can help to understand the specific case without forcing it.

Conclusion

The problems posed by the H-D have led various scientists to disbelieve in the possibility of using the categories in the Philosophy of Science. This position tries to give answers to the problems that sprang from a discipline without inquiring the implicit methodology of these answers. These problems appear because the H-D does not take into account the relation between the data and the theory, between the *explanans* and the *explanandum*. The IBE does take this relation into account. According to the IBE, theories are never absolutely true, but they are the best possible explanation due to the actual state of knowledge. When it is stated that H is the best hypothesis, coherence is assumed not only between the corpus of knowledge but also between the data that it is intended to explain. For this reason, in the specific case of Psychiatry, the IBE is a tool which allows us to explain investigation and clinical practice, and it also gives to an action a framework of rationality which would be otherwise only a sophisticated variant of common sense. The EBP is an attempt to apply the MBE to the field of Psychiatry. The IBE can serve to solve the two basic queries made to that attempt. On the one hand, rescuing the essential relation between the *explanans* and the *explanandum* prevents the possibility of thinking that the theories are incompatible with the con-

crete clinical practice or the lack of awareness of the nature of the object. On the other hand, acknowledging that the theories are approximations to the truth clarifies the fact that it is not a question of rigid structures but of permanently revisable ones. The narrative structures of both the pathology experimented by the patient and the treatment applied are analyzable through the IBE, taking them as explanations feasible of being analyzed through the criteria previously mentioned.

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2 The Notion of Empathy According to Edith Stein

Miriam Dolly Arancibia

Introduction

In her early writing, *On the Empathy* [1], Edith Stein follows the example of her teacher Edmund Husserl. Then, she analyzes the issue of empathy by applying the phenomenological method convinced that phenomenology was the most appropriate approach to the investigation of the structure of the human person.

In 1905, Edmund Husserl introduced the notion of empathy, understanding as such the experience of other consciousness and their experiences, unlike the experience of consciousness of itself. However, the first extensive phenomenological study on this subject is the doctoral thesis of Edith Stein [2].

Although this work was her Ph.D. dissertation, Stein differs from Husserl in some respects. Many years after the completion of this work, Husserl presented his *Cartesian Meditations* emphasizing the possibility of other description of the empathy rather than the phenomenological. Thus, *Cartesian Meditations* [3] is more in contrast with his earlier conceptions than similar to them. Therefore, Stein's work on empathy is in contrast with *Cartesian Meditations* [1].

The main question that Stein investigates is about the experience of the subjects and their experiences. How it is possible that we get to feel the sadness that another person feels? To investigate this phenomenon, Stein begins with a historical review of the different positions on the empathy and the differences in empathy with other similar acts [4, p. 310].

One of the main objectives of the work is criticizing the different theories on the understanding of the minds of others. It focuses on the criticism of the theories of imitation, the theories of association, and the theories of analogy. According to the theories of imitation, we could understand the experiences of others through an imitation of what others have experienced. According to the theories of association, the understanding of other psyches is possible due to connections between different experiences until arriving to a similar experience of the other person. The theories of analogy propose that we are able to understand each other through analogies or parallels with what we have experienced [4, p. 310].

Stein criticizes all these theories for the fact that they deny the immediate experience of other experiences and understanding of others, because all these theories postulate that one gets to the understanding of the minds of others through reasoning, analogies, associations, or imitations. Against these positions, Stein offers a phenomenological version according to which empathy is an act that, as experience, is original and that makes us possible to capture the content of the experience of other person [4].

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On the other hand, descriptions of the psychophysical individual and of the spiritual person are necessary in order to show the full implications and applications of the doctrine of empathy. Stein affirms that all controversy over empathy is based on the assumption that foreign subjects and their experience are given to us [1].

Edith Stein notices that the description of empathy within consciousness after the suspension of the existence of empathy must be the basis for any other dealings with the problem by psychologists, sociologists, or biologists. The description must be a description of the pure transcendental phenomenon. This does not mean that Stein tries to develop a set of abstract theorizations without practical derivations. On the contrary, the contributions of phenomenology still enrich psychology.

The phenomenological attitude of Edith Stein reveals an independent position that resembles the fundamental conceptions of the phenomenology of Munich and Göttingen but takes very clear loans of Husserl and suggests certain affinities with procedures of Heidegger [5, p. 726].

According to Stein, the goal of phenomenology is to clarify and thereby to find the ultimate basis of all knowledge. To reach this goal, it considers nothing that could be in any way doubtful, nothing that could be eliminated. In the first place, it does not use any result of science [5, p. 3].

The entire surrounding world, the physical as well as the psychophysical, the bodies, as well as the souls of human being and animals are subject to the exclusion of reduction [1, §1]. We must exclude the positing of existence. However, we cannot exclude our experience of the thing: the perception memory or other kind of comprehension. The full phenomenon of the thing, it means, the object given, as the same in series of diverse perceptions or memories, is not subject of doubt.

On the other hand, Stein affirms that the world in which we live is not only a world of physical bodies but also of experiencing subjects external to us, of whose experiences we know. This knowledge is dubitable. The phenomenon of a psychophysical individual differs from a physical thing. This individual is not given as a physical body, but as a sensitive, living body belonging to an "I" that senses, thinks, feels and wills. The living body of this "I" is the center of orientation of such a phenomenal world [5, p. 5].

All these data of foreign experience point back of the basic nature of acts in which foreign experience is comprehended. Stein designates these acts as empathy. The acts given primordially are outer perception, which have something in common with the empathy: in both cases, the object itself is present here and now. However, empathy does not have the character of outer perception. Moreover, empathy is not ideation. Thus, empathy is a kind of act of perceiving sui generis [5, p. 11].

Let us take the burnout syndrome as an example to illustrate those differences. Burnout is a syndrome of emotional exhaustion, depersonalization and reduced personal accomplishment that can occur among individuals who work with people in some capacity [6]. It is the result of a gradual process of disillusionment. According to Maslach et al. [6], emotional exhaustion is the central quality of burnout, representing feelings of being emotionally overextended and exhausted. Of the three aspects of burnout, exhaustion is the most widely reported and a necessary criterion for burnout [6].

We will apply thoughts of Edith Stein at this example. Perhaps we notice external symptoms: anxiety, depression, anger, pessimism, apathy, isolation and irritability. According to Edith Stein, the outer perception is a term for acts in which spatial-temporal concrete being and occurring come to us in embodied givenness. Nevertheless, those symptoms are not a thing, and they do not appear to us as a thing.

The example shows that there are close relationships between phenomenology and psychology. Stein notices that there is a phenomenon of "foreign experience" and correlatively the "perception of foreign experience." The phenomenon is indubitable; it is the genuine object of protophilosophy. Thus, the first task in this domain is to comprehend the phenomenon in its pure essence, free from all the accidents of appearance. According to Stein, we must know this before we can ask how this perception occurs [1, §22]. The genetic psychological investigation of cause cannot answer this first question.

On the other hand, phenomenology and psychology are not complete independents from one another. Phenomenology investigates the essence of empathy, genetic psychology, presupposing the phenomenon of empathy investigates the process of this realization and, when its task is completed, must be back to the phenomenon [1, § 23].

According to Stein, the problem is that the traditional philosophical speech was dedicated to pure "I" and to subject of experience but not necessarily to another. Nevertheless, that is one of the central interests of Stein in dealing with the problem of empathy [7, p. 85].

Consequently, this article focuses on the empathy as comprehension of spiritual persons. Specifically, it pursues to elucidate the conditions of possibility of empathy between people in order to highlight the analyses of human experiences as facilitators to understand the nature of the human person.

Empathy as a Problem of Constitution of Psychophysical Individual

According to Edith Stein, in general, empathy is an act, which is primordial as present experience though non-primordial in content. This content is an experience, which can be carried out in different ways as in memory, in waiting or in fantasy. When it suddenly emerges before me, it faces me as an object, i.e., I realize the expression of sadness in another's face. Then, I try to explain the mood of another; this experience is not an object anymore; I turn not to the content but to the object of it. Thus, I am at the subject of the content in the original subject's place. Only after the clarification, the content faces me again as an object [1, §8].

Therefore, Stein distinguishes three levels of accomplishment:

1. The emergence of the experience
2. The fulfilling explanation
3. The comprehensive objectification of the explained experience

On the first and third levels, the representation corresponds the non-primordial way to the non-primordial perception, and on the second level, it corresponds the non-primordial way to the accomplishment of experience.

For Stein, empathy announces in the most direct manner possible the actual presence of the other's experience although it does not provide us with first-personal access to it [8, p. 294]. The subject of the empathized experience is not the subject who accomplishes the action of empathy, but another. These two subjects are separated and not joined together. This is different regarding memory, waiting and fantasy. The other subject is primordial although I do not experience it as primordial. In my experience non-primordial, I feel accompanied by a primordial experience, which was not experienced by me and manifests itself in my non-primordial experience. In this way, empathy is a kind of act of perceiving sui generis [1, §10].

Consequently, empathy is not a simple feel with or identification with someone. Empathy is not to accompany someone feeling sympathy or antipathy. Empathy is not to live in the same way that another lives.

First, empathy is recognizing another individual not only as object in the physic world as body between bodies, but as subject living and sensitive, as individual psychophysical. Then, the specific of the empathy emerges in comparison between memory and fantasy. In fact, empathy is not a representation of a mood but from a mood experienced by another [9, p. 7]. Stein defines empathy as a form of intentionality directed at foreign experiences [8, p. 290].

On the other hand, the individual "I" becomes individual in contrast with "you" and "he." This individuality means that it is "itself" and no other. This "selfness" is experienced and is the basis of all that is "mine." The otherness is apparent in the type of givenness [1, §10].

In a second sense, "I" is the unity of flow of consciousness. We begin with the "I" as the subject of an actual experience. However, when we reflect on this experience, we find that it is not isolated [1, §41]. Among our experiences, there is one basic experience given to us, which,

together with its persistent attributes, becomes apparent in our experiences: the substantial soul [1, §42].

It means that our experiences show us something that is at the base of them and manifests itself and its constant properties; this is a substantial soul. Some of these psychical properties are, for example, the energy that manifests itself in our acts. Tension or the weakness of our volitional actions manifests the vivacity, strength, or weakness of our will. If they persist, they manifest tenacity and are also shown, for example, in the intensity of our feelings, which can discover either the passion or emotion of our mood.

To better understand empathy, Stein proposes to explain what constitution is.

The constitution is linked to act of synthesis whereby consciousness represents an object from different set of data. "During a recent walk with him (Husserl) by Haslach, in which we have discussed about philosophy, I had a new confidence. Moreover, after that, I had a sudden intuition for which I guess to know, more or less what is constitution, but breaking with Idealism" [10, p. 34]. Stein refers to Ingarden that the presupposition for a constitution of evident nature is a physical nature existing and on the other hand a subjectivity with own structure. Stein is afraid of committing heresy regarding Husserl.

This heresy is not possible regarding the possibility of constitution of objectivity in natural world because is the topic in *Ideas II* [11], neither regarding the distinction of two poles of the constitutive act: consciousness and reality. According to Husserl, it is impossible to understand a reality without consciousness. Stein coincides with Husserl regarding the constitution of ideal realities, but she is questioning about the constitution of natural world [12, p. 47].

Therefore, Stein asks if it is possible to think nature without consciousness, if it is possible a reality not constituted by consciousness. Those questions do not pretend to undermine the absolute nature of consciousness neither questioning the formal cognitive aspect of experience, but it is an original aspect inside phenomenology [12, p. 48].

According to Stein, sensation is the limit between the constitution of natural world and the constitution of our experience, between transcendence and immanence. Sensation does not emerge from pure "I"; it is independent of the activity of conscience. Through sensation, there is a first level of immanence, which the main characteristic is passivity. Consequently, the main aspect of the sensation is the absence of intentionality [12, p. 49].

Meditations about empathy and intentionality reappeared as topics of the later work of Edith Stein, *Philosophy of Psychology and the Humanities*, published in the year 1922, after her doctoral thesis and when she was still assistant of Husserl [13, p. 176].

It is important to understand the notion of constitution proposed by Edith Stein in the analysis of the experience of natural world through sensations. Even in this level, we can appreciate the role of intersubjectivity in the objective constitution of the world and the nexus in the constitution of "I" through empathy [12, p. 43].

The problem of intersubjectivity and empathy interests Husserl from the beginnings of his work. Between 1914 and 1916, Stein was dedicated to the work of the empathy, as Husserl was concentrated to the problem of the constitution, one of whose key points is empathy [12, p. 44]. Stein narrates that in the seminar about *Nature and Spirit*, Husserl commented that we could know an external objective world only in the manner of intersubjectivity. It means from a majority of individuals who know their mutual cognitive exchange. Thus, the experience from other individuals is presupposed. Husserl called this experience empathy (Einfühlung), but he did not explain what it is. On the contrary, Edith Stein expressed "I just wanted to know what empathy is" [14, p. 246].

The various parts of the living body constituted for me in terms of sensation are various distance from me. In addition, the distance of the parts of our living body from us is incomparable with the distance of foreign physical bodies from us [1, §46]. In fact, the dependence of experiences on somatic influences characterizes to physical bodies.

On the other hand, the soul is always in a body. Our own physical body emerges to us in successive appearances only variable within very narrow limits. It is the same tangible nearness and more than other objects. It is always here while other objects are always there [1, §45].

The soul is not the flow of consciousness. We know the soul as a substantial unit consisting of categorical elements as causality and variability. It similarly occurs in the physical thing. The peculiar structure of physic unity depends on the peculiar content of the flow of experience; and, conversely, the content of the flow of experience depends on the structure of the soul [1, §44].

Stein maintains that sensations are spatial-localized while the "I" is non-spatial. "I" is at the "zero point of the orientation" of the living body. This zero point is at no particular place [1, §46]. The possibility of sensory empathy is guaranteed by the apprehension of my own body as a body and my body as my own body, thanks to the fusion of the external perception and own-body perception [1, §66].

The human body does not delimit the field of my objects of empathy, but rather defines a field within which it is possible to a defined degree of emphatic fulfillment. What I feel like non-originating can coincide with the other's original sensation. At the end of the process, empathetic presents a new objectivity, thanks to which we are facing of other as such. The comprehension of foreign experiences – sensations and feelings – involves a modification of consciousness and requires a uniform name. Stein selected the term empathy for those phenomena [1, §68].

This perspective about body and souls appears again in the later work *The Structure of Human Person* (1932–1933). Although the Thomistic influences are obvious, the basis remains. Stein asserts once more time that the living body is inserted in the unity of person. The body is something even without soul, but thanks to the soul, this body is in unity with her. The spiritual soul is the unity of human nature occupying a central place and dominating [15, pp. 146–147].

Empathy as the Understanding of Spiritual Persons

Summarizing, Stein distinguishes the individual "I" as a part of nature, the living body as a physical body among others, and the soul as founded on it. The soul is in causal correlation, suffers, and practices the influences. Moreover, Stein considers all that is psychic as natural occurrence and consciousness as reality.

However, this interpretation has some difficulties because in the constitution of the psychophysical individual, something appeared beyond this field. Consciousness appeared not only as a causally conditioned occurrence but also as an object constituting at the same time. Then, consciousness stepped out of the order of nature and faced it. Consequently, consciousness as a correlate of the objective world is not nature, but spirit [1, §102].

From these difficulties arise new problems, which motivate new meditations from Edith Stein in order to assume position about questions concerning the history of studies about empathy.

First, it is necessary to determine how far the spirit has already entered into the constitution of the psychophysical individual. Since the foreign body is a center of orientation of the spatial world, we assume the "I" as a spiritual subject for we have thus ascribed to the foreign living body an object-constituting consciousness and considered the external world as its correlate. All external perception carries out in spiritual acts. Similarly, in every literal act of empathy (i.e., in every comprehension of an act of feeling), we have already penetrated into the realm of the spirit [1, §102].

Therefore, as in the perceptual acts is constituted the physical nature, so a new object constitutes the feeling, the world of values. In joy, the subject has something joyous facing him; in fright, something frightening; and in fear, something threatening. Even moods have their objective correlate. For who is cheerful, the world bathes in a rosy glow and for who is depressed, bathes in black. All this is given with acts of feeling as belonging to them. The appearances of

expression make possible the access to these experiences.

Stein infers that, as we consider all those expressions proceeding from experiences, we have the spirit becoming visible in the living body. This is possible by the psychic reality of acts as experiences of a psychophysical individual and involves an effect on physical nature [1, §102].

This appears still more strikingly in the realm of the will [1, §103]. The act of volition not only faces an objective correlate, but, since volition releases action out of itself, it gives reality to the volition, and it becomes creative. The whole cultural world, all that the hand of man has formed, all utilitarian objects, all works of handicraft, applied science, and art are the reality correlative to the spirit [1, §103].

Natural science (physics, chemistry, and biology as the science of living nature, which also includes empirical psychology) describes natural objects and seeks to explain causally their real genesis. This perspective is present in the later work of Edith Stein: *The Structure of the Human Person*, written in the years 1932–1933 [15]. She refers there the need to search an answer about what man is. It is the topic of anthropology, but it is not only a group of morphological observations. The basis of pedagogy does not come from natural science [15, pp. 54–57].

The ontology of nature seeks to reveal the essence and the categorical structure of these objects. In addition, natural philosophy or the phenomenology of nature indicates how objects of this kind are constituted in the consciousness.

On the other hand, the science of the spirit or the cultural science describes the works of the spirit, pursuing the origins of spiritual works or their birth in the spirit. They do not do by causal explanation, but by a comprehension post-experienced [1, §103]. However, there are some occasions where cultural scientists proceed by causal explanation. Stein considers only permissible this proceeding for elucidating the genetic process of cultural products when processes are a natural event as in physiology of language or in psychology of language.

This comment from Edith Stein is very important because she is anticipating one of the topics of the last work of Edmund Husserl, *The Crisis of European Sciences and Transcendental Phenomenology* (1934–1937) [16], where he denounced the positivist vision of the world.

The exclusivity with which, in the second half of the nineteenth century, the total vision of modern humans is left to determine and blind by the positive sciences and the "prosperity" that are debtors meant an indifferent distance from the questions that are decisive for a genuine humanity. Merely fact-minded science makes merely fact-minded people [16, pp. 49–50].

The consequences for epistemology of psychology are significant. Stein refers to Dilthey in order to clarify two aspects: one regarding a nature of psychology and the other regarding introspection. Concerning a nature of psychology, Dilthey pretended to put a descriptive and analytic psychology in the place of the explanatory psychology. Stein considers that "descriptive" is not a proper word because descriptive psychology is also the science of the soul as nature. Only phenomenology makes clear the method of cultural science as well as that of natural science. Therefore, Stein considers Dilthey not completely clear in this point.

Concerning the introspection, Stein agrees that it is a way to get an epistemological basis. In addition, she recognizes too that it allows to sciences of spirit understanding the life of the spirit, comprehending it empathically. However, according to Dilthey, the subject of this understanding is a man as nature or the total life of the psychophysical individual. On the one hand, the descriptive psychology would be a presupposition of the sciences of spirit, and, on the other hand, it would give unity of them. Nevertheless, Stein realizes that it would mean suppressing the distinction between nature and spirit.

On the other hand, exact natural science is also a unity. Each one of these sciences has as object an abstract part of the concrete natural object. The soul and the psychophysical individual are also natural objects. Empathy was necessary for the constitution of these objects. However, spiritual understanding distinguishes

from this empathy. If empathy is the perceptual consciousness in which foreign persons come to givenness for us, then it is also the exemplary basis for the eidetic knowledge of nature [1, §106].

In brief, regarding Dilthey, Stein considers ambiguous his expositions, and she learns that there must be an objective basis for the cultural science beside the clarification of method, an ontology of the spirit corresponding to the ontology of nature. As natural things have a structure fixed to eidetic laws (the empirical spatial forms are realizations of ideal geometric forms), there is too an eidetic structure of spirit (and there are ideal types; historical personalities are realizations of these types).

Consequently, Stein takes important inferences for understanding what empathy is. If empathy is the perceptual consciousness in which foreign persons come to givenness for us, it is also the exemplary basis for the eidetic knowledge of nature.

For answering to these questions, Stein continues thinking about what spiritual subject is. Stein finds that the spiritual subject is an "I" whose acts constitute an objective world and which itself creates objects because of its will [1, §107].

According to Stein, the spiritual acts do not stand beside one another without relationship, like a cone of rays with the pure "I" as the point of intersection. On the contrary, one act experientially proceeds from the other. Not every subject sees the world from the same side; everyone has his peculiar "*Weltanschauung*." The "I" passes over from one act to the other in the form of what Stein called "motivation" [1, §107].

Then, this significant nexus of experiences, which is the center of causal physical and psychophysical relationships and which does not have parallels with physical nature, is the spirit.

On the other hand, the motivation is lawfulness of spiritual life. Thus, the nexus of experiences of spiritual subjects is a significant totality experienced (originally or empathically) and understandable as such [1, §107]. This meaningful proceeding distinguishes motivation from the psychic causality as well as the empathic understanding of spiritual nexus from the empathic comprehension of psychic nexus. A feeling by its meaning motivates an expression, and the meaning defines the limits of a range of possible expressions just as the meaning of a part of a sentence prescribes its possible and material complements. It means that spiritual acts are subject to a general rational lawfulness. In consequence, there are also rational laws for feeling and for willing, as well as laws for thinking which find their expression in the science a priori: logic, axiology, ethic and practice [1, §108].

This rational lawfulness is distinguishable from essential lawfulness. A feeling essentially motivates to the will. Therefore, an unmotivated willing is an impossibility, and it is not conceivable that a subject wants something, which does not appear as valuable. Willing by its meaning directed toward what is possible.

At this point, Stein arrives to significant contributions for psychology's researches. Rationally, one can only will the possible. Nevertheless, there are irrational people who do not care whether what they have recognized as valuable is realizable or not. They will it for its value alone, attempting to make the impossible possible. Pathological psychic life indicates that what is contradictory to rational laws is possible for many people. We call this mental derangement. Moreover, psychic lawfulness can here be completely intact [1, §108].

In mental illness, the comprehension is interrupted, but there is a series of pathological cases in which neither the psychic mechanism nor rational lawfulness seems to be interrupted. They are experiential modifications in the field of rational laws, for example, depression following catastrophic events.

Anyway, the conclusion of these considerations is that the spiritual subject is essentially subject to rational laws and their experiences are intelligibly related [1, §109].

For explaining what the "I" is, Stein refers to traditional distinction made by psychologists between senses and sentiments. She thinks that two designations indicate different kinds of experience, but only different directions of the same experience. Feeling is an experience when it

gives us an object or else something about an object. The feeling is the same act when it appears to be originating out of the "I" or unveiling a level of the "I." In feelings, we experience ourselves not only as present but also as constituted in such and such a way.

The subject not only perceives, thinks, but also feels. For as it feels it not only experiences objects, but itself. It experiences emotions as coming from the depth of us. The "I" experienced in emotion has levels of various depths. These reveal as emotions arise out of them [1, §110].

According to Stein, the constitution of personality is a unity entirely based in experience and distinguished by its subordination to rational laws. Person and world, more exactly value world, are in correlation. Thus, Stein considers impossible to formulate a doctrine of the person without a value doctrine. The ideal person with all his values in a suitable hierarchy and having adequate feelings would correspond to the entire realm of value levels [1, §121].

Regarding soul, it is important to add that Stein affirms persistent attributes in the soul and the person. The qualities of the soul are constituted for internal perception and for empathy; it is the reason why they have the experiences as object, while the personal qualities are revealed in primordial experience or in empathic projection [1, §122].

Finally, the significance of knowledge of foreign personality is that it carries a "knowledge of self." We not only learn to make us ourselves into objects, but through empathy with related natures (persons of our type), we develop what is sleeping in us. By empathy with differently composed personal structures, we become clear on what we are not, what we are more or less than other. Thus, together with self-knowledge, we also have an important aid to self-evaluation. Since the experience of value is basic to our own value, at the same time as new values are acquired by empathy, our own unfamiliar values become visible. When we empathically run into ranges of value closed to us, we become conscious of our own deficiency or disvalue [1, §130].

Conclusion

This phenomenological foundation is the basis of the structure of the human person according to Edith Stein. In fact, on her youth book *On the Empathy* [1], she is opening a way for searching the Truth, which was her lifelong passion.

This first work revealed a young woman serious and eager to a rigorous philosophical study. A young woman enthusiastic to learn from her contemporary great masters, as Husserl, Max Scheler and Adolf Reinach, among many others. It also discovers a young woman avid of knowledge who enjoyed the vicissitudes of the academic university life.

Her endearing memories of Gottinga and the philosophical encounters with friends as Roman Ingarden, Conrad and Hedwig Martius and Hans Lipps express how much she enjoyed that student period.

The years with Husserl – as his student, disciple and assistant – were not easy. However, neither difficulties nor the moments of solitude in which weighed his indifference diminished the intellectual respect that she professed for her master.

The printed traces in the beginning of the way served as gateways to a higher path. Her thirst for knowledge was not satisfied neither with the responses of the phenomenology nor the idealism. Therefore, his work shows this vital route. From phenomenology, but beyond it. The work *Philosophy of Psychology and the Humanities* [13] was written when she was still assistant of Husserl, but this work shows that she was in the beginning of a break with him, and that deepened after her immersion in the philosophy of Saint Thomas.

So, *Finite and Eternal Being: An Attempt at an Ascent to the Meaning of Being* [17] is more than a late work and is the fruit of a vital maturity. The book contains deep reflections on the human person, the body as such, and the first entity God. Anthropology, metaphysics and theology all that as a final reflection due to the abrupt end of her life a bleak August 9, 1942.

The elaboration of a so comprehensive and so deep work is not a result of chance. It is the result

of a long road, which began in the youth doctoral thesis.

Therefore, these words of Edith Stein show this maturity and depth: "The human person carries and includes his body and his soul, but it is at the same time taken and included by them. His spiritual life rises from a dark background, rises as a flame of a candle bright but nourished by a material that does not light up. And she shines without being absolutely light" [17, p. 380].

In this book, Stein says that a creature endowed with reason is one that can understand the standard of his own being and remain subject to her for his behavior. To that, we must add understanding – or gift of understanding – and freedom that is the gift of informing the behavior as such. If to have reason belongs to the personal being, then the person as such has also necessarily understanding and freedom [17, p. 378].

The human being is a being that it has a body, a soul and a spirit. As soon as man is spirit according to its essence, he comes out of himself with his spiritual life and enters a world that opens up to him, without losing anything of himself. He exhales not only its essence in a spiritual way expressing itself in an unconscious manner, but it also acts personal and spiritually [17, p. 380].

The human soul as spirit rises in her spiritual life above itself. However, the human spirit is conditioned by what is superior and inferior: it is immersed in a material product that he animates and forms in view of his body shape [17, p. 380].

Stein distinguishes two areas in which the human spirit penetrates his life awakened and aware: the external world and the internal world. We can understand the external world in two senses: First, it is everything that does not belong to "I," the monadic unit of my being. In this case, it would also involve interior worlds of other spirits. Second, it is only accessible to the outside perception, to the world of the body with everything that belongs to him.

The own internal world refers to not only the self-conscious life of the "I," i.e., the unit of the experience (present, past, and future), but also what is not immediately aware and from which arises the conscious life.

The original form of the experience is the starting point for judgments and conclusions and retained in memory, which leads us to gather a solid set of experiences that allow us to know ourselves. According Edith Stein – following Husserl – this form is the interior perception, which is distinct from the consciousness that accompanies inseparably the life of the "I"; it means the life of the pure "I" [17, p. 381].

The interior life is conscious, the "I" awaked, the eye of the spirit looks inward and outward, and he can assume with intelligence everything that is directed to him; equipped with a personal freedom, he can respond in one way or another. The spiritual life is the truest field of liberty; the "I" can really generate a thing from itself [17, pp. 386–387].

The knowledge of itself is also possible by the knowledge of others. For this reason, empathy plays a special role. In addition, it is an important link to the world of values.

Every comprehension of different persons can become the basis of an understanding of value. Since, in the act of preference or disregard, values often come to givenness that remain unnoticed in themselves, we learn to assess ourselves correctly now and then. We learn to see that we experience ourselves as having more or less value in comparison with others [1, §130].

As a final synthesis – the empathy, the knowledge of self and of others, and values – all this is included in the word: encounter. Therefore, as final words, we quote a thought from Józef Tischner: "The key of axiology is an encounter with another. To encounter is to obtain an immediate sense of tragedy permeating all the modes of being of another person. Not until we encounter another can the path to God open before us" [18, pp. 48–49].

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Coincidence with One's Self

3

Ricardo Aranovich

Introduction

Ortega y Gasset's work's proposal as foundation for psychotherapy reaffirms itself in the fact that the thinker has set the phenomena of "human life" at the center of its philosophy. He has declared his way of looking at men as rational vitalism, to differentiate himself both from the irrational vitalism and from the rationalism that does not study life. Life stands above thought, because to think, we must first be alive. We think, therefore, because we were previously given life, and to this previous reality, Ortega y Gasset grants the name of Radical Reality:

> Living is the radical way of being: I find all other things and ways of being in my life, within it, as a detail of it and referring to it… The most abstruse equation of math, the most solemn and abstract concept of philosophy, the Universe itself, God himself are things I find within my life, things I experience throughout life, as I live. (3)

And the first fact which the living encounters is that its life develops in a circumstance of unavoidable conditions. There is no human life as we know it without taking place in the world or circumstance:

> Our life, human life, is to each the Radical Reality. It is the only thing we have and are. Now, life consists of man finding himself, not knowing exactly how, and having to exist in a determined circumstance. (4)

In the same instant in which we are born, we get thrown into a circumstance. It might be in more or less favorable conditions, from both the physical and care-related conditions, which we may receive from our families. From there onwards, our existence's viability shall depend on the circumstance's conditions, meaning human life is the result of a relationship between the individual who lives and the circumstances in which life develops. This has been expressed by Ortega y Gasset in his known saying "I am myself and my circumstance" where the "I" (as living being) is the product of the relationship between I (as individual) and the circumstance in which I may be immersed. I, as an individual, do not fulfill my life, my existence. My life is as much part "I" as it is part circumstance, in an indissoluble amalgam. We have witnessed that the newborn requires certain conditions from the circumstance to survive. This, in different ways, continues throughout life. That's the reason the human being permanently looks towards its future, because it knows that its survival depends on the alternatives it is granted. This condition, necessary to understand human life, Ortega y Gasset defines as the need of knowing what to expect:

> It therefore is, to man, impossible to exist being disoriented regarding the problem which is its life. Precisely because life is always at its root disorientation, perplexity, not knowing what to do, and at the same time effort to orient itself, to know what things are and what man is among things. Because

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> it has to manage those things, it needs to know what to expect regarding them. (5)

The human being finds itself exposed to circumstance, and to face that risk, it creates a life project in opposition to circumstance. In effect, what may mitigate the anxiety of the situation is developing a plan which will allow, as it develops, to ensure a favorable and stable circumstance. The life project is a characteristic of man. Animals do not need to create a life project; its instincts take care, in a better or worse manner, of leading it through its existence. The human being, instead, must make decisions all the time, regarding what to do with its life, and must think, and those thoughts may be adequate, or not. He may be right or wrong:

> Life is an operation made moving forward. We originally live thinking about the future, heading towards it. But the future is essentially problematic: we cannot stand in it, it is not fixed, it lacks a defined state. (6)

However, man possesses a resource regarding uncertainty, which is, precisely, the possibility to anticipate the future, to develop a life project. The project is the spontaneous answer to circumstance. We can say that living and projecting oneself are one and the same. But, even if the project has to consider the current and likely conditions of the circumstance, it can also respond to the individual's characteristics. In effect, the project which develops energy in an individual is not the same that excites another, and it is precisely that energy or excitement which is *essential* to deploy the action required for any and all life projects. As a consequence, this condition, which may seem random, of the life project being what it is to each person and not a different one, has the vital value of ensuring the necessary force of action. Ortega y Gasset defines this condition as the necessity of the project to be authentic:

> This project in which the I consists is not an idea or plan thought by man and freely chosen. It is previous to all ideas its intelligence may develop, to all decisions taken by its will. Furthermore, we only have but a vague knowledge of it. However, it is our authentic "being", it is our destiny. Our will is free to "fulfill or not" that vital project which we ultimately are, but it cannot change it. We are only that specific character which needs to fulfill itself. (7)

We understand then that the human being has the necessity and the possibility of projecting its life, taking into account the difficulties and possibilities that circumstance offers. But this project is not just a simple adaptation which aims towards simply surviving. It just so happens that the human being, along with wanting to survive from a biological point of view, which requires ensuring the necessary material conditions to that ends, wants to do something else with its life. That "something else" is previous, and it conditions and, sometimes, even puts in danger the material aspects necessary for survival. Let's consider a mountaineer who risks his life to… reach the top of a mountain, where nothing "useful" awaits him. We could say the same about all dangerous sports. The people who fly with artifacts resembling wings, or those who jump, without it actually being a necessity, with parachutes. Those who set sail to the ocean in small boats or race in different ones. This list can go on and on with many other examples regarding that unique activity we know as sports and to which Ortega y Gasset pays special attention since, precisely, it is the evidence that the human being projects its life towards goals and objectives that transcend biological survival:

> The athlete, instead of running away from danger, runs towards it, that's what makes him an athlete. (8)

But this strange and unnatural human condition is not limited to just sports. The different callings may come to put life at risk, the same life which they try to preserve. Besides the clearly dangerous callings, such as being in the military and the police, being a race driver, and other things of the sort, depending on the time and place, it may be dangerous to be a politician, a news reporter, a lawyer, a taxi driver, and in nearly any human activity which, at some point, is involved in a conflict.

In some cases, it could be said that the risk is inherent to any profession which has the objective of "making a living." But in the same way that sport seems to look for danger because of the

danger itself, in many cases, people who choose certain activities do it being aware of the risks it may involve. But what is this attitude related to? Biological survival is not enough for human beings. If that were the case, the ideal state would be of imprisonment, being ensured food and shelter. However, that situation is considered far from being idealistic; it is even considered the worst, since it acts as punishment for those who fight against life and coexistence. And the worst part of that condition is, precisely, that it acts as an obstacle to the fulfillment of the life project.

The explanation is that human beings do not just search for biological survival, like animals undoubtedly do, but instead run after a singular state, which we know as happiness. And the human being feels happy when it is completely immersed in an action. When he fits within himself, when he finds unity in himself. These are all one and the same. Let us see what Ortega y Gasset has to say about this.

Happiness

Now, we could evaluate more precisely just how important the life project is. Why does each person have a different one, and why must they respond to that internal impulse which takes the form of a "calling"? Because as the action that takes place throughout his life coincides with this calling, he will achieve the state of "coincidence with itself," and when his actions do not match his calling, he will drift away from his happiness:

> Man does not recognize his "I", his singular calling, by anything else other than his liking or disliking of it in any and all situations. Unhappiness tells him when his life accomplishes or not its vital objective, and when he drifts away from it… *Only his suffering and his joy teach him about himself…* Who is that "himself", that only becomes clear after the clash with what happens to him? Evidently, it is our life project who, in case of suffering, does not match our effective life: man breaks apart into two halves – who he was meant to be and who he ends up being. This breaking manifests itself as pain, as anguish, as anger, as hollowness; unity between these two halves, however, produces that prodigal phenomena we call happiness. (9)

A notable characteristic in Ortega y Gasset is the coherence he maintains throughout his extense and multifaceted works. Writings of different times and with different topics converge in a conceptual background through which they reaffirm one another, without this fact being the consequence of rigidity, but exactly the opposite. He reaches coinciding places through different paths, respecting the course of each thought regarding each topic.

We find a clear example of this in the chapter "Theory of Happiness" of an essay regarding literary criticism about Pio Baroja in 1916 (10). Regarding happiness, he there says:

> People often make the mistake of believing happiness lies in the fulfillment of our desires, as if our desires were the only thing making up our personality. (11)

Regarding this, Ortega y Gasset points out that there are people who may be in a situation where they can fulfill all their desires, yet feel profound sadness. Regarding this, Ortega y Gasset says possessions and material goods do not ensure happiness. Once more, Ortega y Gasset opposes common thought:

> In no way can that be the role material things play out in our joy. It's not that they contribute to our happiness since we own them, or obtained them, but because they are the byproduct of our actions, as matter where we apply our energy, turning from idea into action. (11)

Here, as seen above, it is reaffirmed that life, being project and "action done moving forward," requires a permanent tension that maintains the individual in a proactive state. A distinction is imposed: we live in a constant agitation from which all anxiety disorders, stress-related diseases, and depressions derive. How can we say, then, that this state of tension we call "living projected" is desirable? Here is where we must consider calling/vocation and authenticity. When the human being is developing his calling of *coinciding with himself*, his efforts and difficulties are not lived as conflicts. Pathologies appear when the individual feels pressured, exposed to a series of never-ending commitments which are imposed unto him by the medium, without choosing them himself, without being free to choose. Freedom! Freedom is a condition so thoroughly

linked to happiness that they are often mistaken for one another. But what do we mean by freedom? Human being is the most conditioned being on Earth we can imagine. He needs oxygen, food, warmth, and shelter, he cannot fight against the law of gravity, and he does not choose when to be born or when to die nor the time, place, loved ones, and family he is given. Where is freedom? Well, human beings feel free when, despite all limitations, they can organize themselves by actions born through their will. We might say that man feels free not when he does what he wants, but wants what he does:

> *All life is struggle, the effort of being itself.* The difficulties I run into to fulfill my life are precisely what awakens and mobilizes my activities, my capacities. If my body did not weigh down on me, I would not be able to walk. If the atmosphere did not oppress me, I would feel my body as a vague, flabby, ghostly thing. (12)

Where do freedom and happiness meet? In actions corresponding to the calling! In that case, action stops being an effort and becomes a necessity that fulfills itself. Someone, when in this favorable situation, said: "yes, I earn money with my work, but I like it so much I'd still do it without getting paid for it":

> When we ask existence for an answer to its meaning, we are just demanding to be presented something that may *absorb our activities*… Who could consider himself unhappy when completely absorbed in any activity?… Melancholy, sadness, unpleasantness, are all inconceivable when our being as a whole is at work. (11)

Here Ortega y Gasset repeats that, for the activity to be completely absorbent, it requires the being to be *completely* at work, and for that to happen, it must fit within itself and agree with itself. Of course there may be deviations and exaggerations. One such case would be "workaholics," those addicted to their jobs. But this addiction is far from being a calling. Addictions end up impoverishing the individual spiritually and also affect other aspects of its life. On the other hand, those living through their callings feel more and more happiness, which they share with those around them. Now, what if, for whatever motive, the action stops? Sadness and hollowness appear:

> There we find an imbalance between our potential being and our actual being. And that is what we call unhappiness. (13)

It is clear that that state of absorption regarding an activity, where the totality of the being is compromised, is an ideal. Reality is never so helpful towards our desires, and difficulties always come up to discourage even the most determined. In this case, Ortega y Gasset utilizes the war metaphor of when troops must reorganize and the trumpet calls them all to gather around the flag. That capacity of falling back to reorganize and stay as a whole will be further looked into when we discuss "absorption and alteration." Now let us see another pleasant state of absorption which we consider as a part of happiness: falling in love. Ortega y Gasset would not have been able to leave this topic aside, considering his interest regarding human life.

Falling in Love and Love

A situation that presents itself as paradigmatic in our culture of happiness is the state of being or falling in love, and it is precisely the man or woman in love who finds him-/herself unified with the figure of their partner. We must not ignore this state, because in it, happiness and unity converge. It is often confused and related to the idea of love itself, but these are actually two very different states. Ortega y Gasset handles the topic in a series of articles which would later make up a book titled *Studies Regarding Love* (14). To this work, the interest towards falling in love takes origin in the fact that it is an important example of a state of unity. The one in love experiments a state of focus centered in the one who awakens their passion:

> Being 'in love', at first is not more than just that: paying an abnormal amount of attention towards someone else… It is not an enrichment of our mental life. Quite the contrary, there is a progressive elimination of things which used to take up our time… However, the one in love feels their life as richer, better… and this gives rise to a false feeling of superlative intensity. (15)

Let us look at the term "passion": it has a passive connotation. The individual is "moved" by

passion and has lost control over him. This "happens" to him. In consequence, unity is facilitated, thanks to the presence of an external object which absorbs the subject's whole attention. More than "agreeing with himself," he agrees with someone else, his partner, coming to unity through different means. However, this situation is a clear example of what we call the relationship between unity and what we consider happiness, since the state of being in love has been considered as a paradigm of happiness and has shown up in novels, poetry, theater, and, currently, the cinema and television.

It means, on the one hand, we see how unity and happiness merge, but, on the other, unity is not part of an endogenous process, but the consequence of a very peculiar state brought about by another person. Such an extraordinary phenomenon deserves a certain amount of thought. This process is only considered "natural" because of how often it occurs, but if we look into it, it is actually quite amazing. We have two people who a while back knew nothing about each other, who were not even aware of each other's existence, and who may have run into each other without paying attention to one another. Suddenly, they fall in love and place more importance into their relationship with one another than in all their prior relationships, even the ones involving parents, siblings, and friends, to the extent that they might abandon all these relationships if they agreed in a life project that implied going abroad. Even in the Bible, it is stated that "you shall leave your mother and father and form one body with one another," which gives some back-up to this situation. This process of "forming one body" starts with falling in love, but is also influenced, to some extent, by the previous relationships. However, how does this event which we consider extraordinary affect human life? Well, through this process, these two people, who had been strangers, become primary figures to each other, displacing the previous ones. Without that process, it would be hard, if not impossible, to form a new family, which would be necessary to accompany the growth of new human beings who are born in a defenseless state, prolonged for a long time. But that internalization process of someone who had been a stranger cannot be done without a profound modification of the mind. One must pass through a state of alteration which restructures and reorganizes all relationships where the life of individuals had rested until then. What Ortega y Gasset calls "beliefs." Once more, let us say that were it not for the fact that this is such a common state, it would be considered pathological, to say the least:

> When considered subtly, we may say that whoever falls in love wants to fall in love. This distances the act of falling in love, which is a normal phenomenon, from obsession, which is a pathological disorder. Someone who suffers from an obsession does not consider whether he wants to be obsessed or not. (16)

In all these cases, what we find is a state of attention where the fixation on a certain object displaces all other objects. But without this alteration, or disorder, a great part of humanity would not exist.

The state of falling in love, sadly, lasts a fixed amount of time, be it short or long, and past this point, to stay together it is necessary for this "exogenous" unity to become an "endogenous" one, through a stage where falling in love brings about what we commonly call "love." From being an object of passion, meaning a passive attraction, it must become an object to the dedication of what now is the will which unifies each individual with the other. The other individual, which had until then "charmed" with just its mere presence (and even absence), stops producing this effect. This brings about many breakups in relationships which may have been fruitful, but stop because "their love ran out." What came to an end was not their love, but the state of alteration we called falling in love, leaving behind the result of two people who, no longer strangers to each other, are now very meaningful to one another and, as such, are the opportunity for a relationship to grow, a relationship where they shall nurture their individual unity in relationship to the other person's unity, going from symbiosis to the coincidence of two individualities, together in harmony, which in a reciprocal way favor each other's unification process. That is what we call

love, and that is a very important opportunity of fulfilling happiness in this world:

> Love… takes care of affirming his subject… Loving one thing is being focused in ensuring that thing's existence… This would be the same as constantly giving it life *intentionally*. Loving is giving life, creating and preserving something we cherish intentionally. (17)

Vitality, Soul, and Spirit

Reality, with its constant demands and attractions, makes this state of unity difficult to achieve. We must pay attention to what each moment requires and offer what we can without being able to ask ourselves if that's what we really want to do. How can we fight back against this tendency? Around which axis can that coherence be rebuilt, over and over? Ortega y Gasset distinguishes three instances within the human being, which he names vitality, soul, and spirit, not as separate entities but as functions we can perceive within the human actions:

> Those three names, then, do nothing but name the differences we find in our most intimate events, they are descriptive concepts, not metaphysical hypothesis. (18)

Regarding vitality, he says:

> Each of us is, before anything else, a vital energy: big or small, overflowing or deficient, healthy or sick. The rest of our nature will depend on what our vitality is like. (19)

Ortega y Gasset says that if we compare hunger or sexual pleasure with the thought through which a scientist develops an abstract theory or an act of heroism which endangers life, we can clearly see that the corresponding actions depend on the different parts of our intimacy. Some may seem bound to the body, and the need for survival and self-preservation, as well as being reactions of fight or flight, the physical component of sexual attraction, and the pleasure of dancing or sports. This would be the corporeal soul or vitality, upon which the rest of the person rests upon:

> This carnal soul, this root and foundation of our being, is what we call «vitality»,, because in it is where the psychic and the somatic merge, where the corporeal and spiritual combine, and not only that, but grow as well. (19)

Vitality is not just a part of human beings but, for example, we can find it in animals, to some extent. With human beings, not only can we perceive the degree of vitality but also the fact that it is "contagious." Some encounters grant us vitality and some take it away, and at different points in time, we may find ourselves with different degrees of vitality. We might say, metaphorically speaking, that life can reach different degrees of intensity depending on the individual and this degree may change from time to time and depending on the case.

Here, Ortega y Gasset analyzes what he calls spirit:

> I consider as spirit the union of all intimal acts which each person considers as their own work. They are their authors and protagonists. (20)

While vitality aims to preserve life, human beings bring about the extraordinary situation where they actually decide to endanger their vitality or even sacrifice it because of their feelings, such as a sense of duty or sacrificing oneself to save others, be they family or not. Even though this behavior can be seen occasionally in some animal species (such as the protection of the offspring), in that case, the actions are dictated by instinct, without there actually being a choice, like with men. The spirit also manifests itself in thoughts. Whenever we understand something, be it a chemical reaction, a math problem, or a philosophical reasoning, this understanding originates in the spirit, because at the time when we *understand*, we also come to understand that whoever finds himself facing the same question or problem will come to the same conclusion as us. Our spirit puts us in touch with universality, with the norm, and makes us see and appreciate values, allowing our lives to follow a path that matches our will:

> …The spirit does not rest on itself; it has its roots and foundations in that universal and transubjective orb. (21)

Now, between the vitality, which does not get involved consciously, and the spirit, where the human being is in touch with himself, we find the soul, where feelings, emotions, impulses, and appetites reside. The spirit's function is deciding, through will and its notions of what he considers valuable, but it is not the source of

impulses, desires, and emotions, and without them, life would not possess the richness and drama which make it what it is, something singular and different for everyone. The spirit, when regarding a transcendental order, fulfills the role of organizing the emotional world which would otherwise be in chaos, because there may be multiple and contradicting emotions, desires, impulses, and appetites. The will, when defined, is only one. None may have two wills simultaneously:

> Volition, despite its delay to develop, is a ray of intimate activity which fulminates its decision. On the other hand, everything belonging to the soul's fauna lasts and extends in time. (20)

Here is where Ortega y Gasset establishes a difference between the manifestations of spirit and those of the soul. Emotions last in time; one may be sad, happy, and excited. However, will and thought are instantaneous:

> Understanding that 2+2=4 happens in an instant. (20)

What relationship holds this resemblance regarding coincidence with oneself? Achieving and maintaining that coincidence is the undeniable function of the spirit. "Reality, with its constant demands and attractions, endangers this state of unification" be they dangers, needs, impulses, desires, fantasies or anything else in the varied menu of attractions social life has to offer. That's the reason why spirit must sustain its order enforcing function, along with the purpose of avoiding life's dispersion. Even more so nowadays, when common sense appears to be fun.

Does humanity possess any resource to favor the spirit's intervention in life itself and develop a certain extent of freedom regarding pressures and distractions coming from reality? Luckily yes, and that is what we will now see as "absorption and alterations."

Absorption and Alteration

Since time immemorial, the outside world has presented man with survival challenges, regarding defense or subsistence. And those challenges, or problems, which may be unforeseen yet requiring an urgent solution, forced man into a constant state of alert. As consequence, our species has grown used to responding to the environment and expects both good and bad to come from it. It is because of that that he sometimes forgets about an ability which puts him apart from his less evolved relatives: the ability of withdrawing into oneself and to reflect upon things internally and, through that reflection, modify his conduct to present the best possible answer towards reality's problems:

> The world is the complete exteriority, the absolute *outside*, which denies the existence of anything beyond it. The only thing outside that *outside* is, precisely, an *inside*, an interior, man's intimacy, his *self* which is composed mainly by ideas. (22)

And in that *inside* we can:

> …suspend actions for a moment, to withdraw into ourselves, and revise our thoughts regarding our circumstance, to forge a strategic plan. (23)

Ortega y Gasset distinguishes two states: the most common one, which is paying attention to the outside world, which he calls "alteration" (from *alter*, the other) and "absorption," meaning to pay attention to what's within us, to *ourselves*. Through absorption, we can withdraw and acquire a degree of freedom regarding our current situation, which will allow us to see alternatives and choose the most appropriate one. But society grants a growing amount of value towards actions, and that's the cause of a great number of disorders, because actions, on their own, do not solve vital situations:

> The ability of withdrawing, of falling back into ourselves to think objectively, is in danger of extinction. Only action is considered important. Demagogues harass men to stop them from pondering; they intend to keep them crammed as crowds, so as to stop them from rebuilding their persona in the only place they can do it, in solitude. (24)

Now, Ortega y Gasset does not propose that we get away from the harsh reality and find refuge in our comfortable internal world, alone with our thoughts:

> Man's fate is, primarily, action. We do not live so we may think, but the opposite: we think so that we can keep on living. (25)

But:

> *Action* is not just moving around, bumping into the things in our environment, or into other human beings: that's beneath our humanity. THAT is *alteration. Action* is acting being aware of the outline of the material things, and of other men, following a preconceived plan, which was formed during a previous contemplation or thought. (26)

As seen above, man's soul is home to all emotions, which have a certain duration and may even contradict one another. We've also seen that the spirit's function is to order and organize, so that man may achieve a coherence between emotions and actions. Without the spirit acting, human beings would be tumbling around, led blindly by the emotions that may arise as consequence of external stimuli. It would be hard to maintain a course of action which would allow us to fulfill our life project. So far we have seen how absorption acts as a possibility to adapt actions to reality. But that adaptation has a double aspect, because to reach it, man must first find an internal agreement, so that he may agree with the action he will perform. A double agreement is necessary: the action must be fitting with the expected outcome, but must also respond to the performer's preferences and interests. Without these conditions regarding action, how would it be possible for someone to fulfill a harsh duty, instead of choosing a more pleasant situation? This double function regarding absorption, adapting action to reality and to the individual's preferences, makes it all the more important in our current time. Indeed, our current society places action and its outcomes on a pedestal but, in doing so, forgets about the subjective or emotional elements. As a consequence of this mistake, the individual grows further and further apart from himself, meaning it becomes harder and harder to reach coincidence with himself, to enter the state of absorption. And if Ortega y Gasset is right, the individual drifts away from the possibility of happiness and instead moves closer to anxiety, depression, alcoholism, drug addictions and other distractions, in an attempt to fill his internal void, instead of resorting to absorption, through which he would find the serenity that coinciding with one's self grants.

Earlier we were wondering: "Does the human being possess any means to favor the Spirit's intervention in his life, so that he may achieve some degree of freedom regarding life's pressure and distractions?" Ortega y Gasset responds:

> There's no other way to be yourself other than through absorption; meaning, before acting, before giving your opinion about something, one needs to stop for a moment and, instead of doing anything or thinking the first thing that comes to mind, rigorously agree with himself, withdraw into himself, and in that state of solitude, decide what action or what opinion among all the possible ones is the one that's truly ours. Absorption is the opposite of living life letting the environment choose what we do, pushing us mechanically towards this or that. (27)

The Man-Mass

Ortega y Gasset considers that this lack of coincidence with one's self, of coming into contact with oneself, of absorption, has caused men to life "adrift" and has become more and more common in our current time because of a change in culture, which he analyzes in his most famous work *The Rebellion of the Masses* (28), where he analyzes what he calls "man-mass." Man-mass is the result of all the commodities that technology and medicine have brought to our daily lives. Ortega y Gasset published his book in 1930, and since then, the exponential growth of technology and the resources it brings in touch with man made his reasoning more and more current. Even though every person can live his life as a problem, which may be easier or harder to solve, the average individual is not aware of how many problems have disappeared throughout the years, thanks to technological progress. It is true that this development has not brought about the happiness it was expected to bring, but it has given men a feeling of safety bigger than it ever had:

> Before, even to the rich and powerful the world was full of poverty, difficulties and danger. (29)

Moreover, when met with a harsh situation, man may actually become indignant, because science, which "solves all trouble," has not yet found a solution to its difficulty:

> What had previously been considered a benefit of luck, which caused a humble feeling of gratitude towards fate, has become a right which is not thanked, but instead demanded. (30)

Of course there are places in the world where "poverty, harshness and danger" rule, but in those regions, the existence of the man-mass, if present, is not so common as to consider it a consequence of that specific time and place. An aspect of man-mass is its tendency to comfort and lack of commitment. He enjoys all the advantages the environment puts within his reach, without acknowledging the efforts those before him went through to put those advantages in everyone's life. Civilization's advantages are equivalent to the fruits of nature, which exist to be freely utilized by everyone. But nature is stable, it sustains itself, and those living in it are savages, with all due respect. But culture, civilization, does not sustain itself, and if we consider it as stable as nature and take advantage of it without caring about it, we endanger it. We may be standing at the edge of the abyss without even knowing it:

> If you want to take advantage of the advantages of civilization without worrying about sustaining civilization… you will lose that civilization. (31)

But man-mass, who has at his disposal, without any efforts whatsoever, resources that allow him to feel master of his life and destiny, does not believe he has to do anything to pay back such privilege:

> Since they do not see in the advantages of civilization an invention or construction, brought about and maintained through great efforts, they think that all they are meant to do with it is demand it, as if it were a birth right. (32)

This is the reason man finds it difficult to limit his desires, because he considers himself ruler of his life, not having to account to anyone. To him, there is no hierarchy he should rule his life upon:

> This man has learnt to use many of civilization's artifacts… he is characterized by his ability to ignore the very principles of civilization. (33)

A fact we must point out is that these quotes come from a book written in 1930. Nine years before the Second World War! Where "civilization's artifacts" played a huge role in fighting against everything civilization stood for.

The presence of man-mass has, as expected, a manifestation in the political plane. Since he makes up the great majority of the voters, he is capable of handling power and he does so with the same self-sufficiency and lack of auto critic that characterizes the rest of his actions. Politicians, being aware of this, dedicate themselves to flattering and pleasing the man-mass, simplifying topics and creating slogans. As a consequence, it may seem there are no proper rulers in Occident. The different governments in the different countries cannot be the right ones. What occurs is that the man-mass is the voter and the candidate must please him. The populist governments are criticized, but deep down, all governments are like this. Polls are used to know what the voter expects, so that the candidates may adapt to it. The ruler does not "rule," but instead changes his image to fit what is expected of him. But this work's goal is not to make political comments. We use that topic as an example of a spiritual state which expects things from the outside and not the "inside." Instead of trying to agree with oneself, the members of the current society appear to be trying to run away from themselves. They search for success by working constantly and use fun as a replacement or equivalent of happiness.

As a consequence, the man-mass does not question. He accepts as valid the repertoire of ideas that have been passively deposited within him and passively consumes the reasoning that the mass media offers him. Instead of accepting the reasoning of other, more qualified individuals, he imposes his own ideas or accepts those that match the ideas he already had:

> Man-mass…is in a vegetative state, suspended in space. This is the reason his life, with no weight and no root…. is dragged by the lightest of currents. This is the time of *currents* and *letting go*. (34)

The importance of this matter is that, like cosmic rays, it goes through all reality without catching anyone's eye. And one of reality's situations is psychotherapy. Psychotherapy requires the patient to dig into himself and perceive emotions, connect memories, and acquire a reflective attitude towards his life, so that he may find a lost state of

well-being, if he ever had it. On the other hand, the therapist finds himself with a patient who comes from a situation where living a shallow life and searching for easy immediate results are encouraged. Maybe the greatest difficulty is accomplishing that the patient understands the therapeutic work and becomes interested in following it. What occurs is that the therapeutic situation is immersed in society and cannot avoid its influence. Pondering has succumbed to acting. And instead of seeing this as a serious issue in culture, it is medicalized, meaning medicine is granted the task of "healing" those who cannot integrate with society. In those cases, the therapeutic tasks consist of the patient developing his inner world and acquiring the necessary independence to live following his own personal project:

> We live in a time where it feels terrible possible to fulfill goals, but we do not know what goals we should fulfill. Man dominates all things, but does not own himself. He feels lost in his own abundance. With more means, more knowledge, more techniques than ever, it seems the current world is the unhappiest there has ever been: completely adrift. (35)

Internal Coherence in Ortega y Gasset's Work

The texts quoted in this work correspond to volumes and text written in different times and regarding different topics. Despite this, and the fact that it is nearly impossible to find an aspect in human life left out of Ortega y Gasset's philosophical gaze, no contradictions can be found between them. They all converge in an exhortation declaring that the human being must withdraw unto himself and, through that encounter/meeting with himself, define his life project and calling as a way to find plenitude in his life. A shallow life only leads to hollowness, anguish and dissatisfaction:

> All life is the fight, the struggle to be itself. (12)

Conclusion

This lifestyle, which only becomes shallower as time goes by, leads to the lack of contact with oneself. The human being finds himself more adrift and living a life that does not match his own will, and does not even know his own will, because its attention is completely poured unto the outside and the immediate. The consequence of this is a growing state of emptiness, of lack of meaning, which derives into anxiety, depression, addictions, violence and the epidemic spread of these and other disorders. Interventions, both psychiatric and psychotherapeutic, must have as goal the recovery of touch with oneself, which would allow the coincidence with oneself to occur. This is the greatest challenge, because in many cases the troubles originated in not accomplishing goals or achieving success, and the patient must earn to accept that reality is not at his service. This does not mean he should give up, but quite the contrary. By recurring to his inside world and utilizing his capacity of introspection, a new space opens up, where he can find balance and peace, which will allow him to think about his life and realize where he lost his path, so that he may return to action with greater energy than before, because he now knows:

> By living I have been thrown into my circumstance, into the chaotic and painful mess that are things: I get lost in them… and in them I lose myself. I no longer know what it is that I truly want or not, feel or don't feel, believe or not believe. I get lost in things because I lose myself. The solution, the salvation, is to find oneself, coincide with oneself once again, and keep in mind what my sincere attitude towards everything is… The substantial problem, the original problem, and in that sense, unique problem, is fitting me within myself, to coincide with myself, and to find myself. (1)

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4 Naturalism, Psychology, and Culture: Nature vs. Nurture?

Pascual Ángel Gargiulo and Ricardo Crespo

Introduction

The role of nature and the influences of nurture have been considered a matter of interest. The pertinence of naturalistic methods application is considered as an adequate way to explain human behavior. However, early observed limitations regarding these methods have been historically developed. Last years, some doubts have been drawn concerning the pertinence of this application that may be considered in some cases automatically and blindly used. The role of biological causes and intentional motivation constitutes an axis that cannot be ignored. Some lines, like evolutionary psychology and its possible role explaining the entire human condition, must be critically discussed.

It has been said that "Ideas rule the world." Indeed, throughout the ages, a set of philosophical ideas, a "metaphysical worldview," has greatly influenced our conceptions of life and science. The beliefs that prevail today in the world make up a naturalist worldview, in the restricted sense of the term that ultimately reduces nature to physical or biological stuff and processes. It has been denominated "ontological naturalism." This point of view equates science to natural science upholding that its methods are applicable to the explanation of any reality. It may be considered as a methodological naturalism.

Early perceptions of experimental psychologists found some limitations to naturalistic approaches to human realities. In this way, Wundt, founder of the modern experimental psychology, was owed to recognize that an important number of realities were not possible to be reduced to experimental "naturalistic" approaches. It was the case of language, history,

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and literatures, between others. He proposed, coming from experimental naturalistic approaches, a different category of disciplines destined to study human behavior [1–3]. He created by this manner the Peoples' psychology (*Völkerpsychologie*). This thought tradition was fruitful and decisively influenced by philosophers such as Dilthey and, through him, psychiatrists such as Jaspers and Schneider, relevant authors linked to Central European psychopathology. This implies an outstanding influence in the field of medical-psychiatric praxis and, more generally and comprehensively, in the conception of human behavior. The qualitative difference between naturalistic and the so-called "spiritual" phenomena was temporally differentiated. Natural phenomena were attributed to previous and past "causes" determining behavior. It is the case of a somatologic illness, like intoxication or brain compromise. The so-called "spiritual" phenomena were conditioned for the past, but actually projected to future proposals. Normal healthy human beings play trying this possibility. They build its own destination [1, 2].

Cognitive and evolutionary psychologies are naturalist developments of psychology. Evolutionary psychology is the intersection between the cognitive revolution and evolutionary biology. Evolutionary psychologists Tooby and Cosmides propose a program that involves developing a new social science [4]. They claim that mental phenomena are the expression of a complex functional organization in biological systems. Starting from there, they argue that complex organic functionality is consecutive to natural selection. Consequently, they consider that the sciences of the mind and the brain are "adaptationist" and psychological sciences and in this sense the mechanisms would be computational adaptations [4, p. 10].

Influenced by the naturalist wave, evolutionary psychology provides a fitting theoretical framework for the explanation of human thoughts and behaviors. However, this is not necessarily the true or last explanation of all human reality. The evolutionary psychologist Jerry Coyne [5] states, in a self-critical attitude, that the problem that has happened to evolutionary psychology is that it suffers something like a scientific equivalent of megalomania. Most of his followers would seem to have the belief that all human actions and conditions would be or would be generated by a single, evolutionary key, which would be introduced into our brains by natural selection [5, p. 27]. That is, evolutionary psychology tries to explain all conducts as determined by psychological evolutionary mechanisms, interactions of computational systems, or neurocognitive processes. However, are those the deeper or true drivers of human conduct?

They [4] end their article postulating that a new explanation of the differences between the laity or profane of the economy and the views of economists on a number of economic issues. These authors prefer to consider the views of the popular economy as results of principles-based cognitive systems and not merely as irrational deviations from normative understandings of economic processes. According to them, they appeared in human evolution as an adaptive response to specific challenges. They would be activated automatically when a situation meets your entry criteria. From this point of view, intuitions support deliberate, explicit, and thoughtful thoughts. These thoughts are transmitted culturally through the so-called popular economic beliefs. This implies that "economic rationality" should be considered as a narrow form of rationality that does not consider the entire human condition. This condition would involve the natural feeling of values such as justice and caring for others, derived from the social nature of the human being.

This psychological school is not an isolated case. A progressive modification of the views has been observed over the past two centuries. The crass naturalism underlying evolutionary psychology has long been overcome. In this chapter, we will relate the general lines of recent psychological thinking and its abandonment of naive naturalisms.

Neurosciences and Behavior

The study of neurosciences has experienced explosive growth in recent decades. Its scope has been growing exponentially, showing its influence on numerous physical and mental processes. Paradoxically, it has also found its limits, and this is one of the most valuable points. Neuroscience, reaching maturity, has found what it is and what it is not, thus abandoning an initial oceanic omnipotence, almost adolescent. In historical terms, neuroscience is discovering the algorithms of the Central European psychological schools of the late nineteenth and early twentieth centuries. Nothing more and nothing less.

The study of the brain is irremissibly related to the study of behavior. No other organ of the economy has such a close connection as the one observed here. The sensory organs provide the information that the brain registers and it is in this that it is processed and it is from this that a behavioral response is specified. These processes, typical of the brain, began to be studied from an experimental perspective.

Physiology and Causality: Physicochemistry and Mathematics Go Through the Soul

The physical sciences began to experience a prodigious expansion, from certain findings that occurred towards the end of the eighteenth century. A notable fact in this regard arises from an area outside of psychology. The fact in question occurs in the field of astronomy. In 1796, the young David Kinnebrook is expelled from the Greenwich Observatory. What was the reason? The real astronomer Nevil Maskelyne warned that the stellar transit observations made by his assistant differed from his own [3, 6]. Regarding his findings, there was a difference of … half a second!

The problem could have ended there if it had not been because a German astronomer, Bessel, decided to study what happened, returning to the theme around 1816. With insight and very good mind, he decided to find an explanation. He evaluated in a group of prestigious astronomers the differences in the recording of the moment in which a star crosses a certain line in the telescope. There were always small differences. What had astronomer Bessel discovered? An astronomical constant? No. He had discovered reaction times. A psychological variable he called "personal emotion." What did this finding imply? Nothing more and nothing less than the notion of the role that, faced with the physical fact and its correlative perception, the observer plays, supposedly, neutral [3, p. 63].

From this point on, attention is drawn to the processes that mediate the relationship between the physical object and the psychological perception. Special attention is given to the relationships between the eye and the seen thing, between the receiving organism and the perceived object. It describes phenomena such as post-image (Plateau), in which the sensation continues even when the stimulus has already been extinguished. Studies are performed on stereoscopic vision (Brewster and Wheatstone) and on twilight vision and its peculiar properties (Purkinje) [3, p. 63].

Johannes Müller establishes the theory of specificity of nerve energy. He maintains that, beyond any possible stimulus, a nerve reaction that has its own characteristic and shape is activated. The stimulus may differ, but the reaction is characteristic and characteristic of the nerve. This is the case of pressure on the eyeball. What appears is a light sensation. The sensation is not then a phenomenon uniquely determined by the stimulus, but rather a product of the encounter between the organization of the percipient and the inducing stimulus. The idea of univocal determination is still abandoned here, even at the level of the causally determined [3, p. 63].

Müller begins to investigate the psychology of the senses, printing a course to this science that will shape it in a particular way in the following years. Physiology appears studying the conditions of the reactive organism, thus giving rise to a psychology with an experimental expression. The idea arises of studying the observer organism as a previous and necessary step to the study of the universe [3, p. 64]. In order to see the stars, it is necessary to study the characteristics of the eye

that sees the stars and the reaction times of the brain of the person who sees the stars.

It is at this time that studies on the touch of Ernst Heinrich Weber appear. He performs studies on the perception of weight and its relationship with muscular perception. It states that this is thinner when the muscles actively intervene, lifting the object whose weight is studied. This gives advantage to the participation of the muscular sense. Secondly, the so-called Weber's law appears, which consists in establishing a "minimum appreciable difference." The perception of a difference, here of weight sensations, does not depend on the absolute magnitude, but on a relationship, expressed as a ratio or proportion between the difference and the standard used. When the muscular sense was used, the ratio was from 1 to 40. It was observed, expanding the experiments, that this "minimum appreciable difference" constituted a *constant relationship*. Later, Weber tried to generalize these concepts trying to apply them to other visual and auditory experiments. However, this generalization was considered by Weber an empirical rule and not a law [3, p. 65].

All of the above in the empirical plane. But Gustav Theodor Fechner enters the scene. He is a person who is divided between science and mysticism, a person who tries not to abandon either of the two ascriptions. After the statement of Weber's considerations, Fechner considers them located at the center of psychological investigations because of their importance. According to this researcher, when establishing a mathematical relationship between the physical and the psychic, which was revealed as accurate, a bridge was established, a formal relationship between the physical and the psychic. It would seem to have constituted, for him, something like a *revelation*. From his knowledge, Fechner decided to devote his entire life to the experimental verification of "Weber's law". He sought to establish a universal unity between the physical and the psychic through sensation, in accordance with a mathematical relationship defined between them. The methodology used allowed psychology to be established as a science. Beyond their motivations, the methodology used, quantitative experimentation marked the dawn of a new era. His book *Elements of Psychophysics* constitutes a milestone that cannot be ignored and as relevant as the Leipzig Laboratory. For this reason Fechner is considered, together with Wundt, Helmholtz, and Galton, as one of the Fathers of Modern Psychology. Obviously the nature of motivation could be studied here. It could be a passion and more related to a mystical quest for unity than with coldly empirical precepts [3, pp. 66–70].

The Discovery of the Limits and Their Alternatives

While there are other relevant milestones in this story (Hermann von Helmholtz), it is our intention to point out the role of Wundt and his Experimental Psychology Laboratory at the University of Leipzig. Wundt had begun, like Weber, Fechner and von Helmholtz, in physiology. His work *Elements of Physiological Psychology* is considered the one that initiates, in fullness of formulation, the independence of psychology as an autonomous science. His fundamental thesis is that psychology deals with mental contents and that these are accessible to the methods of introspection and experimentation. Wundt states that the psychological experiment must be of the same type as the physiological experiment. In this, the procedure for the study of the phenomenon remains very close to some essential elements. One is that the stimulus is controllable. The other is that the response is objective and correlated with an introspection, which must have been properly planned and its observation limited and exhaustive. The higher mental processes were not, in Wundt's conviction, liable to study using these procedures. These could only be studied through the Psychology of Peoples (*Völkerpsychologie*), an extensive work in ten volumes that culminated shortly before dying. There he states that a study of the so-called social products is only accessible to the *historical method*. This is the case of language, laws, art, institutions and customs [3, pp. 75–80].

This idea of two different and differential methods is developed by Dilthey. This author gives a philosophical expression to the idea that there are two different types of sciences. He talks about "nature sciences" and "spirit sciences" and lay the foundations of two differential methods of approaching to one and another: explanation (*Erklären*) and understanding (*Verstehen*). This difference between understanding and explaining is taken as a central paradigm of phenomenological psychopathology [1, 2, 7, 8].

From this perspective, there are two planes of differentiable phenomena, which are evident in their discrepancy in psychopathology. The first are the anomalies of the psychic way of being [1, 2, 7–11]. These do not constitute diseases in the strict sense. These are anomalies, when not mere findings. They do not have a cause or a physiopathogenesis. At most, we can talk about conditioning, never determination. Successive experiences, with all the elements of significance that the individual interposes with a margin of freedom against the given, are happening and giving rise to a development [8], understandable continuity of the self in time. This development conforms to the temperamental characteristic elements and, finally, the personality. We are in the field of the motivated, the understandable, the biographical. The prospective plays here, making motivation evident as a movement towards future goals. The motivational goes from the present to the future. The psychiatrist's approach in this field necessarily involves understanding, giving here a central place to empathy. We settle here on the level of the sciences of the spirit.

The second type of phenomena constitutes diseases in the strict sense [1, 2, 7–10]. Here the fields of causality and the consequent determination become evident, in a temporary game that goes from the past to the present. The present is explained by a cause that previously worked. Here we are in the field of causality, with all its natural load, imposing a determination and not a conditioning. A head injury causes a loss of consciousness. Several injuries over time give rise to a psycho-organic syndrome. Jaspers calls this "process." The process is a natural event that interrupts development, interrupts continuity of meaning. Intense brain trauma and schizophrenic psychosis are here "processes." Due to the action of a particular cause, the individual is never again the one he was. The approach is here explanatory, with the clinical diagnosis in the strict sense, with the objectification of the natural cause that produced the determination. We are here, strictly, in the "natural sciences" field.

Final Considerations

Thus, two areas are well defined, concordant with Dilthey's approach and updated by Popper when he speaks of "worlds" as tectonics [1, 12]. There is a causal plane, with determinations, explainable phenomena, and a dynamic generated in the past to give a reason for the present. This plane is approachable from the physical-chemical-mathematical, from the natural sciences. And there is another spiritual plane, in which the personal is defined. This plane can be influenced by conditioning, but it is not determined. There are no efficient causes here, physical or chemical elements that determine the personality. There is a continuity of sense of self in time [13], a development, an understandability, a motivational projection towards a future, an intentionality. In this plane, there are no causes, there are reasons, and there is not a teleology. This difference is not always taken into account in recent research and conceptualizations of the brain and behavior, as noted [14]. The notion of personal development allows to limit the speculations of the neurosciences to the corresponding level, without causal excesses and without ignoring a stratigraphy with diverse planes and diverse study methodologies [1, 12].

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The Self-Organized Feedback Brain

5

Osvaldo Agamennoni

Introduction

As we know, the brain is a system made up of a complex network of neurons, being responsible for a large number of functions, from homeostasis to our human mental activities encompassed in what we usually understand as cognition. The brain structure is not clearly defined in terms of functionalities. However, we know that the information flows in this network of neurons by means of electric pulses, making our brain the most amazing dynamic system known.

Time is an elusive phenomenon that is difficult to grasp with our senses [1]. This is the reason why it is hard for us to deeply understand the dynamic nature of our being. Our dynamic nature is constantly present in the processing of perceiving what happens and predicting our future actions. Rodolfo Llinas [2], one of the founding fathers of modern brain science, presents an original vision of the evolution and nature of the dynamic predictive functions of the brain. According to Llinas, the brain evolved to allow predictive interactions between mobile creatures and their environment. He illustrates the early evolution of the mind through a primitive animal called ascidia. The mobile larval form of this animal has a brain ganglion that receives sensory information about the surrounding environment. When it reaches adulthood, ascidian adheres to a stationary object and then digests most of its own brain. This suggests that the nervous system of animals evolved to allow their active movement.

Is clear that humans, as well as all biological beings, are continuously interacting with their environment and the *Homo sapiens* have distinguished, from the other species, by significantly increasing the cognitive resources applied to that interaction process. Visual attention is controlled by cognitive top-down factors (knowledge, expectation, belief, current goals, etc.) and bottom-up factors coming from sensory stimulation. Other factors that influence attention, such as novelty and unexpectedness, indicate an interaction between cognitive and sensory information [3]. The dynamic interaction of these factors controls where, how, and to what we pay attention in the visual environment.

This interactive process is clearly depicted with the cognitive cycle of Fig. 5.1. The information coming from the perception of the environment is processed with the stored in the memory, and, as a result, an action is produced. The action modifies the environment, and a new cycle begins. Naturally this is a feedback loop, and its dynamic is clearly affected by the perception, the information processing, and the actions performed as a

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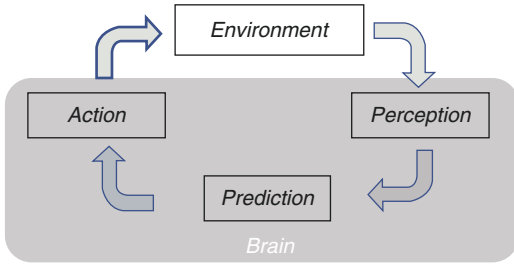

Fig. 5.1 Cognitive cycle: The environment is perceived through the senses. Its resulting information is integrated and processed with the information stored in the memory. A set of possible future action are considered through prediction. An action is carried out which modifies the environment. The modified environment is perceived, and so on

consequence. Most aspects of the cognitive cycle are unconscious; however, each cycle also has moments of conscious processing. An important feature of mental dynamic is the ability to consider alternatives to events in relation to the environment, and this is also possible to be analyzed in the context of the cognitive cycle as a simulated process.

Darwin clearly showed how the natural selection with its feedback loops improved our evolutionary process, shaping our body and brain. This means that our behavior is, to a great extent, a result of the whole historic dynamic process of interaction with the environment. In our environment, there are animate as well as inanimate objects and other people similar to us, but not equal. Then, other's brains are also included in the feedback loops in which our brain is involved. For this reason, many different researchers have found appropriate the use of dynamic system concepts and tools in the behavior analysis and research.

When a goal (reference value) is introduced in the cognitive cycle, it is natural to consider the implicit dynamic in the framework of feedback control loop. Feedback control theory is a well-known discipline in engineering and in the last century has been applied to diverse areas including to psychology in the 1948 book, *Cybernetics* [4], by Norbert Wiener. Later, PCT [5] has provided one of the most important contributions of the discipline to study the human behavior.

The control theory was initially developed to deal with synthetic systems. Initially in the regulation of steam engines in the eighteenth century up to now with a wide spectrum of applications in all the different technology applications. People from the feedback control theory community claim that it is a hidden technology. The mathematical foundations of the control theory allow the study of the dynamical system, basically the feedback effects on its responses, how to analyze its stability properties, and how to design a proper controller to improve its performance. According to Cowan et al. [6], the control theory provides different tools for understanding systems with regulation via feedback, including biological ones such as regulatory gene networks, cellular metabolic systems, sensorimotor dynamics of moving animals, and even ecological or evolutionary dynamics of organisms and populations.

The feedback control theory approach applied to human behavior follows the simplified block diagrams of Fig. 5.2. The results of the action are perceived and compared with the desired reference value, and a consequent control action is performed over the manipulated variables. Let us assume, as an example, a violin student. In this case, the manipulated variables are its arms and hands that are the responsible to produce a given resulting sound in the instrument. The sound is perceived, compared with the corresponding reference, and the brain performs an action to improve it.

Even though PCT, and other control-based approaches, allow to understand many different aspects of human behavior, the emerging dynamics of a social group are more complex and difficult to analyze under these perspectives. Self-organized system concepts are needed as a base platform in the analysis of interactive dynamic between individuals. A number of researchers have proposed models of psychological systems based on the concepts of nonlinear dynamics, self-organization, and chaos. Some of these former approaches are described in the work of Scott Barton [7] of 1994. By self-organization, it is understood as the evolution of a system, to an organized form by changing their internal structure and their function, in response to changes in its environment without the inter-

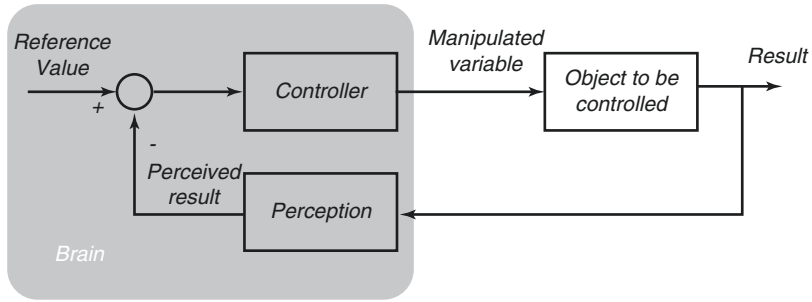

Fig. 5.2 A feedback control loop in the context of human behavior. In the case of a violin student example, the manipulated variables are its arms and hands, responsible to produce a given resulting sound in the instrument. The resulting sound is perceived, compared to the reference, and the brain performs an action in the manipulated variables to improve it

vention of any external action. Self-organization framework allows to evaluate the emergence of stable patterns through autonomous and self-reinforcing dynamics at different levels of complexity. The elements of a self-organized system are able to manipulate or organize other elements of the same system in a way that stabilizes either structure or function of the whole against external circumstances.

Figure 5.3 shows the extension of Fig. 5.1 to depict the interaction with other individual. Basically there exist two types of interactions between the elements of a system in relation to the resources of the environment: competition and cooperation. During competition, the elements of a system are individually striving to maximize the use of the resources, and during cooperation, they are engaged in mutual beneficial activities. Depending on context conditions, the emergent social behavior will be more probably oriented to cooperation or competition. It is possible to favor the appearance of certain behaviors, but not control them. In section "Social Behavior as the Emergent of a Self-Organized System Dynamic," some basic necessary conditions for cooperation will be addressed.

Social structures emerge from cooperative and competitive actions of individual actors, and individual actions are shaped by social structures. This system scheme, shown in Fig. 5.4, was proposed by Wolfgang Hofkirchner [8] in 1988 and has been used in a large number of later works. In the cyclic process that shows this scheme, there

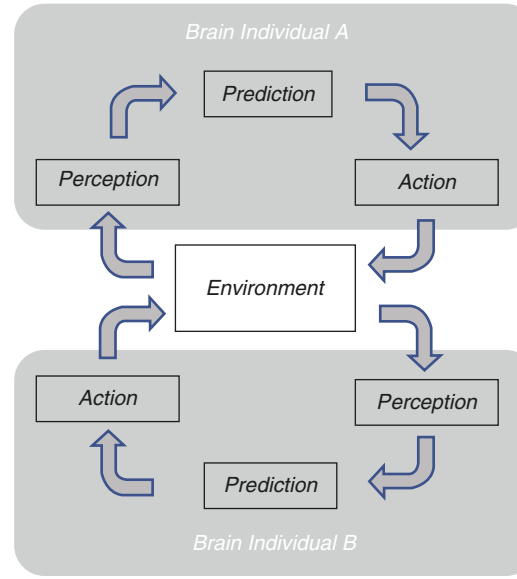

Fig. 5.3 Cognitive cycle with two individuals interacting in the environment. When two or more individuals share an environment, the dynamics are more complex due to the different kinds of iterations that could be generated in relation to the environment, competition and cooperation, and the differences between individual's goals

are two levels. At the micro, lower level, are the elements of the system, that is, the individual agents. The interaction of individual actions design relatively stable relationships between them that achieve a relative independence of the interactions. These structures that arise at a macro level exist in their own right and influence individual agents. On the one hand, they establish conditions (e.g., norms of behavior) that limit the

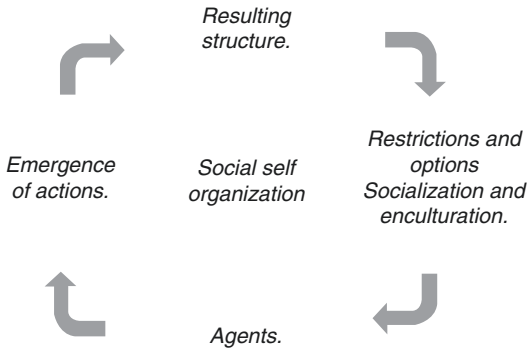

Fig. 5.4 Outline of the social self-organization proposed by Wolfgang Hofkirchner in 1988. Through their interactions, individual agents promote the emergence of social structures that in turn shape the individual actions of agents

scope of the possibilities of acting, and, on the other hand, by doing so, they offer them options that they would not otherwise have. That is, it provides socialization and inculturation. In this way, one can clearly appreciate the self-organized dynamic nature of a society.

Individual Behavior as a Dynamic Emergent of the Cognitive Cycle Feedback Loop

Behavior is the externally visible part of a process by which a person controls their environment. As disturbances affect the achieved results, behavior allows to control the changes introduced on the available manipulated variables (possibilities of action) to achieve the desired objectives (reference values). Behavior is the emergent of actions produced to cancel the effects of the disturbances on whatever a person is controlling or trying to do it.

In 1990, Carver and Scheier, in an article titled *Origins and Functions of Positive and Negative Affect: A Control-Process View* [9], addressed the nature of certain aspects of emotion, from a control theory perspective on behavior. They focused on the feedback-based processes through which people self-regulate their actions to minimize discrepancies between actual acts and desired or intended acts. The authors concluded that this view on affect is a useful supplement to other theories and that the concept of emotion is easily assimilated to feedback models of self-regulation. As the authors said: *to put it more simply, when people pay attention to what they are doing, they usually do what they intend to do, relatively accurately and thoroughly.*

Dag Forsell [10], editor of the book *Perceptual Control Theory (PCT): An Overview of the Third Grand Theory in Psychology Introductions, Readings, and Resources*, affirms that understanding the phenomenon of control provides an explanation for the way living organisms behave, what behavior is, how it works, and what it accomplishes. Control theory helps to explain how organisms control the different activities they are continuously conducting. On the other hand, the lack of control also helps to understand other phenomena. It explains why people deprived of any major part of their ability to control soon become dysfunctional. Fear of losing control is listed as a possible symptom of panic attacks in panic disorder [11].

In the framework of the PCT, a conflict is the state when two control systems attempt to control the same quantity with respect to two different reference values. Internal conflict is an inevitable consequence of learning to adapt to the world, but unresolved excessive conflict is disruptive, distressing, and damaging to the individual [12]. Different theories suggest that compulsive behaviors, characteristic of obsessive-compulsive disorder and addiction, are caused by shared deficits in goal-directed control. However, recent studies have shown that deficient goal-directed control accompanies several disorders, including those without an obvious compulsive element [13].

Feedback control framework explains what a goal is, how goals relate to behavior, how behavior affects perceptions, and how reality emerges from the perception. An example of how distortion in perception affects behavior is the anorexia nervosa. Slade and Russell [14] have shown that patients with anorexia nervosa have a perception of the size of their body (face, chest, waist, and hips) between 25 and 50 percent higher than in the case of healthy people. This problem in perception and its consequences on behavior can

only be understood within the context of feedback control. A person with anorexia thinks he/she is overweight because he/she perceives himself/herself as such, and, consequently, his/her goal in terms of the weight of his/her own body becomes unattainable.

Behavior is not triggered simply by features of the environment, but by the interaction of those features with the properties of the individual [15]. People choose between many possible courses of action within a particular situation. Since Power (1973), many different authors have followed the idea that behavior is organized hierarchically, and later, Carver and Scheier assume a cascading loop structure.

Using brain imaging in humans, Koechlin et al. [16] showed that the lateral prefrontal cortex is organized as a cascade of executive processes from premotor to anterior prefrontal cortex regions that control behavior according to stimuli, the present perceptual context, and the temporal episode in which stimuli occur, respectively. Specifically, they hypothesized that cognitive control involves at least three nested levels of processing, implemented in distinct frontal regions. First, sensory control involved in selecting motor actions in response to stimuli. Second, contextual control involved in selecting stimulus-response associations according to external contextual signals accompanying stimulus occurrences. Third, episodic control involved in selecting consistent sets of stimulus-response associations according to events that previously occurred or to ongoing internal goals.

Consider, as an example, the learning process of a musical instrument. Music, like all artistic activity, has technical aspects related to the dexterity in the execution of the instrument and others related to the artistic expressiveness itself. However, one of the most important factors in music learning is the virtuosity that the student has as desired objective to be achieved, i.e., the motivation. The behavior of a music student may be expressed following the hierarchical idea, which can be shown as depicted in Fig. 5.5.

The first (inner) control loop is related to the motor control of the body (hands and other parts depending on the instrument) to achieve a desired technical level in the instrument execution. The second loop controls the musical expression, i.e., the art of playing with a personal touch transmitting a feeling. On a practical level, this means making appropriate use of the dynamics, phrasing, timbre, and articulation to give life to music. Finally, the outer loop controls the overall musical learning process, being the over-riding reference value, i.e., the desired level of virtuosity to be achieved in the future. It is important to note that the controller of the outer loop states a reference value for the middle loop and that the controller of the middle loop states the reference value for the inner loop.

It is natural to understand that the over-riding reference value, i.e., the desired level of virtuosity to be achieved, will have an important effect on student behavior. The personal idea of virtuosity will also determine the levels of expressiveness and interpretative technique. The reference values are dynamically increasing with the learning process, and its relative valued with the achieved virtuosity will dominate the overall behavior. If after a given period of time the expected results are not achieved, the student may become frustrated. Depending on the relative values expected in terms of expressiveness or technique, a whole range of different behavior can be expected. It is clear that the over-riding reference value and the perception process of the student will have a determining role in the behavior of the student.

As we have seen, the theory of control allows us to explain an important amount of personal human behavior related to the pursuit of certain objectives. When we focus our attention to the social dynamics, the control theory does not allow us to analyze all the problematics. The different personal objectives may conflict, and the individual control actions may not lead to a stable solution. It is not possible to drive a car with double command if each driver has different objectives. For this reason, when the study is focused to social groups, a self-organization framework is needed. In any case, before analyzing social behavior, we must deepen in how we construct our reality, our particular vision of our environment.

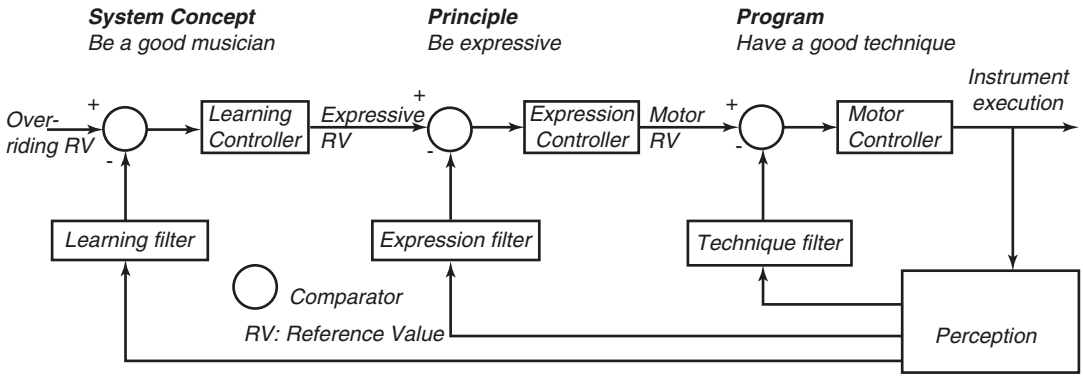

Fig. 5.5 Closed-loop hierarchical control process in the learning a musical instrument example: The first (inner) control loop is related to the motor control of the body (hands and other parts depending on the instrument) to achieve a desired technical level in the instrument execution. The second loop controls the musical expression, and the outer loop controls the overall musical learning process, being the over-riding reference value, i.e., the desired level of virtuosity to be achieved in the future

Sensitivity, Stability and Resilience

An appreciation that the whole is more than the sum of the parts can be intuitively visualized through the analysis of the dynamic properties of the closed-loop system like sensitivity, stability, and resilience. These dynamic properties, most of the time, are the direct result of the energy exchange in the interactions processes with the environment.

Sensitivity is a measure of the relationship between the cause (stimulus) and the resulting effect. In some cases, it is desirable that the performance of a system be as less sensitive as possible with respect to changes in its elements that compose them. For example, it is desirable that an electronic system does not vary its performance due to variations in the values of its components.

To deeply analyze the meaning of the sensitivity of a system and the effect of the feedback on it, it is necessary to introduce a little of mathematics related with control theory. Figure 5.6 is a simplified version of the feedback loop of Fig. 5.2 to facilitate the analysis. In the case of the violin student's example, A represents all the elements of the loop in the forward path, i.e., brain controller and motor control system, while B represents all the elements in the feedback path, i.e., perception systems.

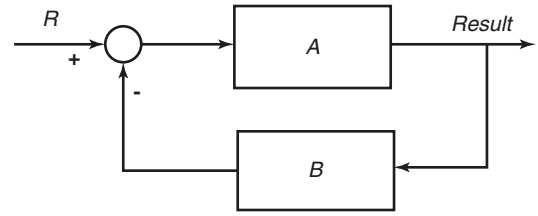

Fig. 5.6 A simplified feedback control loop: A represents all the elements of the loop in the forward path (brain controller and motor control system), and B all the elements of the feedback path (perception systems)

The relationship from the reference value R to the achieved results *Result* is given by the following transfer function:

$$T = \frac{Result}{R} = \frac{A}{1 + AB}$$

which is obtained by solving the equations derived from the block algebra of Fig. 5.6. Remember, in our example of the violin student, R is the desired sound to be obtained and *Result*, the achieved sound. The sensitivity of the *Result* with respect to the feed-forward path elements (A) is given by the relative variation of the transfer function T with respect to the relative variation of the forward elements (A) which is given by:

$$Sens_A = \lim_{\Delta A \to 0} \frac{\Delta T / T}{\Delta A / A} = \frac{\partial T}{\partial T} \frac{A}{T} = \frac{1}{1 + AB}$$

where ΔA represents any change in A as well as ΔT any change in T and $\frac{\partial T}{\partial A}$ is the derivative of T with respect to A. In the same way, the sensitivity of *Result* with respect to the perception system (B) is given by:

$$Sens_B = \lim_{\Delta B \to 0} \frac{\Delta T / T}{\Delta B / B} = \frac{\partial T}{\partial T} \frac{B}{T} = \frac{AB}{1 + AB}$$

Without having to understand in depth the mathematical process of calculating sensitivity, it should be considered that the forward path system (A) has higher values that the feedback path system (B), which results that the $Sens_B$ is higher than $Sens_A$. The conclusion is straightforward: variations in the forward motor control path are less relevant in the performance of the closed-loop system. The performance of the closed-loop system is much more sensitive to variation in the perception system. If the achieved sound is not accordingly perceived, the student will have no chance to improve the resulting sound. The value of A is directly related to the attitude of the student, in the sense of the predisposition to achieve the desired reference value R. The greater the predisposition, the greater the value of A.

There is a range of values of A within which an appropriate behavior can be expected. Smaller values make it difficult to achieve the objectives; it slows down the feedback system dynamic and is more sensitive to variations (uncertainties) of the motor control system. On the other hand, high values of A can cause oscillations or, if it increases further, make the system unstable. However, it is possible to get a whole range of values of A within which the dynamics of the system are appropriate (resilience).

Consider a simple example, the heating system of a house. It is a closed-loop system that has, in the forward path (A), the boiler and radiators. In the feedback path, the thermostat (B) senses the temperature of the room, feeds back the same to compare it with the desired temperature, and sends an ignition signal if the temperature of the room is lower than desired. Otherwise, a shutdown signal is sent. Different boilers and radiators of different sizes can achieve the desired temperature (resilience). Obviously there is a limit; if it is too small, it will not be able to generate the necessary heat to reach the desired temperature. If it is too large, it will be on and off too often (non-stable oscillatory state). But if the thermostat has a fault and does not feedback the true temperature value of the room, the desired value could never be reached.

Constructing Our Reality

Human beings are an intrinsically social and gregarious species, and practically all our thoughts, desires, feelings, and actions are directed or produced in response to others [17]. People and groups interacting in a social system create, over time, mental representations of their own actions and those of others processing the perceived information. In the dynamics of the individual or social feedback loops, the appreciation of the obtained results is highly relevant. There is a verifiable reality that exists; however, sometimes our perceptions (or beliefs about the reality) do not match reality.

We have evolved in a world in constant change, and, consequently, our nervous system has adapted to detect those changes in our environment. In this constant flow of brain activity, our perception of the activities of others, and the perception that others have of ours, plays a central role in social dynamics. What we perceive as reality is our conception and subjective evaluation of what comes to us through our senses. In the Talmud, the book that compiles rabbinical discussions about Jewish laws, we can read the following sentence: *we do not see things as they are, we see the things as we are.*

The reasons for such human behavior could be related to the fact that if all the perceptive stimuli are ambiguous, then it is necessary to use the previous accumulated knowledge during the evolutionary and life process to convert the sensory energy into useful information to guide the behavior. It is also known that the expectations, knowledge, and demands of the tasks in execution can shape the perception. The degree to which perception is influenced by factors such as goals, beliefs, desires, and expectations is not

known. Anyway, it is known that such influence exists and for this the concept of *cognitive penetrability* has been defined.

Since our experience of other people is conditioned by our perception, it is natural that diverse human behaviors linked to empathy and social cognition are influenced by the way we perceive our fellow human beings. Given that, consciously or unconsciously, we are continually involved in cognitive cycles; the construction of what we call reality is strongly influenced by our perceptions and our predictions. The predictions that guide our actions are then contrasted with our particular perception of the results of that action. As the perception confirms our predictions, we are consolidating the mental models we are developing. Problems in prediction or in perception can alter the normal dynamics of the cognitive cycle, affecting behavior.

It is clear that behavior is strongly influenced by the subjective construction of reality, in the same way that any feedback loop is affected by the estimate (perceived) of the output variable. As we have seen, if the temperature sensor of a heating system is damage, it is not possible to reach the desired temperature level. If a music student cannot adequately perceive the music note achieved from his instrument, he will not be able to improve his performance.

One of the most important brain abilities is to discard and integrate information from different sensory modalities and combine them with memory traces and with information about the current state of the brain and the body with the purpose of predicting and making the necessary decisions for adaptive behavior [18]. We will focus now in such processes where perception and prediction are intrinsically involved, and in that sense, we need to deepen the analysis of them.

Perception

In the book *The Doors of Perception*, Aldous Huxley said: *There are things known and there are things unknown, and in between are the doors of perception.* Perception is the process in which the brain senses the stimuli it receives through the senses to form a conscious impression of the physical reality of its environment. From a simplified approach, in the personal process of perception, it can be appreciated two stages related to the treatment that our brain carries out with the stimuli that reach our senses:

1. **Fusion:** Process of integration of the different pieces of information coming from the different senses, in order to unify and complete them. The natural environment constantly produces multimodal information, and humans have developed sensory organs optimized to perceive the environment, as well as a brain to represent and reflect the multimodal nature of this information. For this, it has generated an unconscious sensory fusion system that allows it to rapidly represent its environment in working memory. The human being maintains an adequate static and dynamic balance, thanks to the continuous, simultaneous, and congruent fusion of all the information coming from three systems: the vestibular, visual, and proprioceptive systems. Alterations in the fusion of all that information can produce different conditions such as vertigo. It is not possible to arrive at a single and congruent information, crucial for the motor control system.
2. **Inference:** Process of evaluation of conclusions based on the information available. At this stage, conscious semantic information has its influence. If we imagine a scene where a clergyman walking through a meadow during the thirteenth century witnesses a flash, it would be natural for him to infer that it was a divine manifestation. If we imagine the same scenario but now Benjamin Franklin (inventor of the lightning rod) a few centuries later, we would conclude that his inference would have been completely different from that of the cleric, even if the fused information of his senses had been exactly the same.

The process of inference allows the generation of new pieces of information that are stored in the memory and are then used in new processes of inference, reinforcing or contradicting the exist-

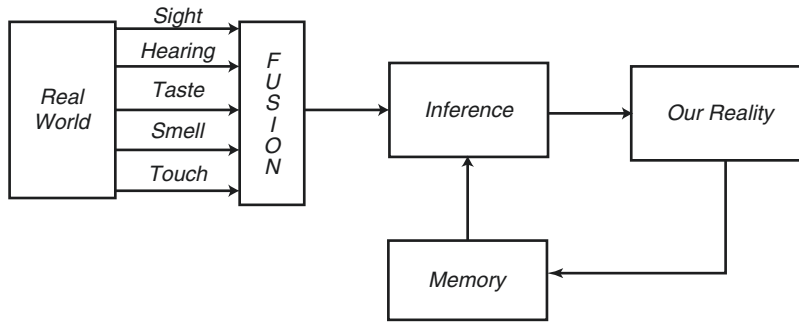

Fig. 5.7 Perception process. The multimodal sensory signals are fused into pieces of information. These are combined with those in memory through an inference process. Thus new pieces of information are generated that are, in turn, stored in memory, and so on

ing mental models. As can be concluded, the perception process also forms a feedback system and can be seen in Fig. 5.7. The importance of the quality of perception in behavior should be noted. It is clear that misperception contributes to the generation and reinforcement of mental models that differ from reality.

Given the dynamic nature of the stimuli that reach our brain, the perception of time is of great importance in human behavior. The ability to perceive time accurately is fundamental to control our movements and behavior. Different neuropsychiatric and neurological disorders such as depression, bipolarity, and schizophrenia show different alterations in the perception of time [19]. We use internal timing processes to measure whether we have enough time to cross the street before a car approaches the intersection or to maintain a regular conversation rhythm when talking to a friend [20]. Like other senses, our perception of time is modulated by changes in the environmental context. Emotions greatly affect the perception of time. We have all experienced that the passage of time is not the same during a pleasant situation as during another highly stressful one. This shows the dynamic nature of social interactions.

Prediction

The theory of the mind is an approach to describe the ability to attribute thoughts and intentions to other people. It is used to designate the ability to attribute mental states to other people and to interpret and predict their behavior. Predictions or expectations drive what we perceive and the way we integrate the perceived aspect of the world around us. The energetic brain efficiency and the adaptive behavior are the results of its predictive nature [21]. We are continuously using knowledge from previous experiences to produce predictions about the future and minimize the cost of the future uncertainty.

An interesting description of the predictive brain function is provided by Giovanni Pezzulo and Paul Cisek [22]. In a given situation, one of the possible options is chosen, which is modulated not only by the predictions of the immediate benefits that could be achieved but also by the predictions of the new possible benefits that could be obtained later. They summarize the same in the following way: *in its interaction with the environment, or because of it, the brain generates a set of desirable actions within the possibilities within the available possibilities (affordances)*.

Eliot Brown and Martin Brüne [23] analyze the importance of prediction in terms of individual and social behavior. They analyze a number of studies which demonstrate that bottom-up sensory input and top-down expectancies can be modulated by social information. They affirm that perception is more related to the construction of the environment predictive modeling; and predictive mechanisms are also at the core of the processes of evaluation of the outcomes of others'

actions and can be applied to both non-social and social contexts. They suggest that selection pressures operating on predictive mechanisms have made them more malleable by social matters. Individuals who can better predict the behavior of others may have been those whose genes benefited from a greater reproductive success.

The relationship between brain predictive mechanisms and social cognition also has implications in psychopathology. For example, there is good empirical evidence that the capacity to infer one's own and other persons' mental states is specifically impaired in schizophrenia [24]. False perceptions (hallucinations) and bizarre beliefs are characteristic symptoms of schizophrenia and other psychotic illnesses. An important number of results in the literature suggest that patients with schizophrenia are impaired at making predictions [25]. Some of the first studies were in the area of motor control. They suggested that the processes involved in predicting the sensory outcome of an action and comparing it to the real outcome are impaired. Recently it has been proposed that prediction impairments in patients may concern the prediction of time in particular.

Eliot Brown and Martin Brüne [23] conclude that the use of a predictive coding framework provides conceptual scaffolding to bridge different domains of cognition and different research disciplines. In exploring social neuroscience under the guise of prediction, a more integrated and inclusive approach is permitted to understand the brain as a whole and not just a sum of its parts.

Social Behavior as the Emergent of a Self-Organized System Dynamic

It is known that relatively simple self-organized artificial systems can exhibit complex behaviors [26]. Then, in a much larger structurally self-organized system, it is certainly to be expected that their behaviors are of such complexity that it is very difficult to estimate. This is the reason why the possibility of control of such a system is virtually impossible. Self-organization is a process in which the high-level functionality of the system arises only from the numerous interactions between the members that compose it without external intervention. These systems are able to adapt to changing environments and be resistant to faults and damages (resilience). However, in different circumstances, a small change in the environment can result in a big change in systemic behavior (chaos).

The microscopic structure of the circuits of the adult neocortex, considered as the basis of our highest cognitive capacities, is still very little known. Only some structural characteristics of such circuits are known, such as their capacity for self-organization, but it is not known how these characteristics are produced and how they are maintained. It is actually well known that a phenomenon at a given level of organization is a consequence of the mechanisms at a lower level. Self-produced molecular processes arrived at the conformation of simple cells, societies of simple cells produced eukaryotic cells, the societies of these cells emerged to produce multicellular organisms, and the societies of these arose to produce animal societies. Human evolution shows a similar pattern in which societies of increasing scale have gradually emerged from previous ones. In each of these cases, the new entities that arise in each step are formed by entities that previously lived independently and competed with each other.

At this point, it is appropriate to consider what is stated by Francis Heylighen [27] to explain the emergence of organization. We need to take into account that the outcome of interactions is not arbitrary, since they exhibit a preference for certain situations over others. The principle is analogous to natural selection: certain configurations are intrinsically fitter than others and will be preferentially maintained and/or multiplied in the evolution process. When the agents are goal-directed, they will prefer an outcome that brings it closer to its goals, i.e., increase the reward. For example, in a market, a firm will prefer the outcome that brings it more profit. In an ecosystem, an animal will prefer an outcome that brings it more food or that reduces its risk of being attacked by a predator.

In words of Heylighen, it is clear why an individual agent tends to organize itself so as to settle down in its preferred situation. The problem is that what is best for one agent is in general not best for the other agents. For example, more profit for a firm generally means less profit for its competitors, and an animal safe from attack by a predator means a predator that goes hungry. Individuals are independent and interact locally. Besides, they in general do not know exactly what will be the effect of their actions on the other individuals; therefore, the dynamic is unpredictable. They can choose plausible actions or even select it by random and compare which ones bring them closer to their goals. But each individual has its own goals. Even when they appear similar, small differences in a large collection of individuals can generate unpredictable patterns.

Recently, in 2016, John Stewart, in his work *The Self-Organizing Society: The Role of Institutions* [28], studies the conditions that should be observed so that, even when individuals pursue their own interests, they behave in favor of the interest of society as a whole, regardless of any intention to do so. Not only to go in pursuit the social goal but also to be able to withstand the swings, always present, in a society. From this study, he developed a model to study the evolution of self-organized societies. He begins by sketching a simplified model and then goes on to show how this simple model can be adapted to consider the key characteristics of the emergence of self-organized societies at different levels of organization. Of course, the use of a model is always reductionist, especially when we talk about people and societies. In any case, it should be analyzed if the behaviors obtained through the use of the model are correlated with the observations. That is, if the results are convincing as to transcend to a higher level, from individuals and their relationships to social behavior.

Stewart considers a population of agents that compete against each other to survive and persist. In addition, agents have the ability to develop various adaptation mechanisms. The model shows that the key condition for a society to self-organize is to capture the consequences. In general terms, this means that all agents of society must capture their profitable benefits from those produced by the impact of the actions that are carried out. If this condition is not met, the agents, who invest resources in actions that produce social benefits, tend to be overcome by those who do not. That is, there will be fewer and fewer interested in promoting and doing good deeds. This condition of capture of consequence can be fulfilled when a society is administered by adequate systems that establish certain restrictions to the individual agents and promote pro-social actions. I think the results are convincing enough.

As we have seen in the previous section, in the framework of the PCT, a personal conflict appears when two control loops attempt to manipulate the same variable with two different reference values. There is no possibility to converge to a stable situation. Similarly, in the social level, a conflict is promoted when the initiatives that are carried out do not foster the consequence capture for all the members. Unconstrained or inappropriately constrained self-interest can destroy a society. The conclusion of Stuart is intuitively understandable. The repeated failure of movements that have been directed at building a better world is due largely to their inability to envisage how society can be successfully reorganized so that it henceforth inexorably self-organizes "the good." A key element in behavior, at both the individual and the social levels, are the communicational skills, the ability to describe significant events and concepts in clear and precise messages that enable minimal perceptual errors. This is strongly linked with the concept of emotional granularity introduced by Lisa Feldman Barret [29], i.e., the ability to differentiate between the specificity of the emotional content. An adequate communication at a horizontal level not only facilitates self-organization but also strengthens the system against the negative effects of different perturbing actions. Let's go back for a moment to the cognitive cycle of Fig. 5.3 where two people interact on a given environment. Suppose the case of two people discussing about a given topic. It is clear that small deviations in the messages significances due an imprecise verbal description

or/and small perception errors can induce a growing unexpected dynamics as a result of the feedback loop.

These results of Stewart, obtained through simulation, are confirmed in the real world by Simon Gächter and Jonathan F. Schulz [30]. They present cross-societal experiments from 23 countries around the world, which demonstrate a robust link between the prevalence of rule violations and intrinsic honesty. They developed an index of the Prevalence of Rule Violations (PRV) based on country-level data of corruption, tax evasion, and fraudulent politics. They find individual intrinsic honesty is stronger in the subject pools of low PRV countries than those of high PRV countries. The results show that weak institutions and cultural legacies that generate rule violations not only have direct adverse economic consequences but might also impair individual intrinsic honesty that is crucial for the smooth functioning of society.

Conclusions

We are essentially dynamic beings, perceiving what has already happened and predicting our next actions, most of the time, unconsciously. Let's pay attention to how we move in a given environment. However, the statement is also valid for all kinds of actions. This fact is strongly reflected in our individual and social behavior, which is the result of emerging patterns of neurons' dynamic activity in a huge interactive feedback network system, our brain.

An increasing amount of evidence indicates that behavior and cognition are patterns of self-organized responses based on the adaptive and coordinative activity in a given environment. These patterns show high degree of sensitivity to the physical and social environment. However, the dynamic balance between the coordination of motor and sensory processes regulates the behavior. The dynamic approach of behavior allows to appreciate the effects of perception and prediction in our actions, as well as in various pathologies, that becomes more accessible to understand and address.

It is well known that the congruence between the emotional state of a perceiver and the emotional character of information received about others may play a significant role in how person-perception judgments are commonly made [31]. However, prediction was adequately considered later as an important factor in the feedback loop dynamics related *to* behavior [21].

In the case of an individual, there exist many different approaches in the literature to analyze the dynamic nature of behavior. Is clear in the context of the PCT that individual behavior is strongly guided by personal goals (motivations) that act as reference values to be achieved. In the case of social behavior, it is important to look for a wider frame in the context of self-organizing systems, since we need to consider the dynamic of a system with multiple different individual goal-directed control actions. In 1988, Wolfgang Hofkirchner outlined the social self-organization process. Through their interactions, individual agents promote the emergence of social structures that in turn shape the individual actions of agents. Regulations, values, ethics, norms, etc. frame individual and social dynamic and structures. There are numerous scientific works showing the dynamic processes that allow the emergence of activities that seek the "the good." However, we are needing to integrate all existing knowledge related to individual and social behavior and its implications in individual and social dynamics.

The main objective of the present work was to show that the study of both individual and social behavior can be framed in a broad context that allows analyzing its main dynamic characteristics. Within the present study context, it is evident to appreciate that a key element in the behavior, at both an individual and a social level, are the perceptive and predictive systems. Individual and social perception and prediction are modulated by beliefs and judgments. However, perception is a very sensitive aspect in the feedback loop, and this feature should be carefully considered.

As Anzola, Barbrook-Johnson, and Cano [32] affirm, there is a large theoretical-methodological gap between social complexity and mainstream social science. But in my opinion, it also extends to other areas of science. We have a very important amount of knowledge and tools, and we do not use them to their full potential.

Finally, it should be emphasized that the dynamic of a self-organized system is a direct consequence of the communications between their agents. It is important to note that an adequate social communication at a horizontal level not only facilitates self-organization but also strengthens the system against the effects of many negative disturbances.

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6

Dynamis, the Object of a Philosophical Medicine: An Epistemological Analysis of the Treatise *On Ancient Medicine*

María Teresa Gargiulo

Introduction

The International Colloquium on Hippocrates that was held in 2002 at the University of Newcastle and the recent publications that have appeared on his writings testify to the current flowering of studies around the Corpus Hippocraticum. Among the diverse works that compose the act of this colloquium stand out those that are dedicated to study particularly the treatise *On Ancient Medicine*.

One of them is the article by Mark Schiefsky who shows the author of the paper as a defender of an empiricist methodology. He argues that the author assumes the ideal of empirical verifiability and, consequently, he considers all that speculative philosophy absolutely irrelevant to medical practice [1].

Francis Dunn, for his part, identifies this writing with the proposal of an empirical model of medical practice, which certainly excludes any teleological reading of its object [2]. Both studies, in one way or another, put back into effect the hermeneutical line developed in the nineteenth and early twentieth centuries. Namely, an empiricist-type hermeneutics that we could exemplify with a study by Dolores Nava where she presents the writing in question as the "attack of a man of science who is literally opposed to the application of philosophical methods in medicine" [3, p. 136, n. 1].

Certainly there are passages in this writing that validate this analysis. The Chap. 20, for example, could be interpreted as a point of inflection with respect to the traditional attitude of unity that the preceding Hippocratic treatises maintained with philosophy. There it is said:

> Certain sophists *(sophistai)* and physicians say that it is not possible for anyone to know medicine who does not know what man is [and how he was made and how constructed], and that whoever would cure men properly, must learn this in the first place. But this saying *(lógos)* rather appertains to philosophy, as Empedocles and certain others have described what man in his origin is *(ex arkhés)*, and how he first was made and constructed. But I think whatever such has been said or written by sophist or physician concerning nature has less connection with the art of medicine than with the art of painting *(graphiké)*. And I think that one cannot know anything certain respecting nature from any other quarter than from medicine; and that this knowledge is to be attained when one comprehends the whole subject of medicine properly, but not until then; and I say that this history shows what man is, by what causes he was made, and other things accurately. [4, Chap.1, pp. 20–22]

This text seems to be enough to justify the hermeneutic tradition that present the aforementioned ancient text as the birth of a scientific medicine detached from all kinds of philosophical reflection. Since the author of the writing would seem to behave like an empiricist who

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seeks as a doctor to get rid radically of philosophy or, at least, of that ancient medicine that founds his medical practice in the philosophical theory of the elements. Lloyd specifically points out Empedocles and Filolao of Taranto among the interlocutors to which this text objects [5].

This treatise apparently denies that the philosophical explanation of the material origin and development of human nature can lend some utility to medicine. Everything that the philosophers have said about nature, says the author, belongs to the *graphiké*, that is, to the art of writing, and not to medicine.

However, despite the partial success of this hermeneutical tradition, we believe that it runs the risk of offering a fragmentary and incomplete reading of the new epistemological statute that the author seeks to assign to medicine. The characterization of medicine as a non-philosophical science is correct insofar as it only deals with the author's effort to refute some postulates of ancient medicine. But it is incomplete if one realizes that concomitantly with this criticism the author proposes a new way of doing medicine that is not detached, at all, from the philosophical questioning.

The positivist mentality of the nineteenth century was pleased to oppose Galen, with its constant propensity to teleological explanations, the figure and work of the "empirical" and "scientific" Hippocrates, apparently devoid of finalist prejudices. But several years ago, and in opposition to these hermeneutics in an empiricist key, an hermeneutic tradition has been developed that seeks to correct the empiricist key that has been assigned to our text. Jones [6], Diller [7], Kühn [8], Miller [9, 10], and Jaeger [11, pp. 31 y 800], among others, have pointed out that the author of the treatise *On Ancient Medicine* does not establish a dialectical split between medical practice and philosophy. Our study is inscribed in continuity with this line of interpretation.

Our purpose is to show how all the writing is vertebrate on the concept of *dynamis* and how it supposes the proposal of a teleological model of scientific explanation and the concretion of a new synthesis between medicine and philosophy.

In order to analyze the author's understanding of the medicine-philosophy relationship, we will study two theses that justify the division of the article. Firstly, we will present the *dynamis* as the new object of medical practice (1). We will argue that this new object supposes the postulation of a teleological model of scientific explanation that involves by itself the philosophical questioning. Secondly, we will analyze the methodological slogan of adhering to the sensations of the body. We will show how, far from being part of an anti-metaphysical restriction (typical of a specific type of empiricism), it represents a causal, metaphysical, and epistemically more perfect question than that proposed by the Ionian philosophers (2).

About the New Object of Medicine

In the first place, it should be clarified that the treaty is not a writing against ancient medicine – as one might think. On the contrary, the author shows that ancient medicine is presented in its beginnings as a scientific practice insofar as it seeks a causal and rational explanation about the order and proportion of the elements of the body through food. Certainly the author points out some errors and further delimits the questions that concern medicine. But this does not imply that he does not recognize that his ancestors were the ones who established this art in virtue of an object.

The purpose of the author of *On Ancient Medicine* is to give a scientific account of medical practice. He defends it against detractors who even deny its existence. The Hippocratic authors were concerned to establish medicine as an art or *techne* against those who held that medicine was only a matter of *tuche*. To prove the scientific status, it was necessary not only to show the success of their therapeutic results but also to show that the results were due to the causal knowledge that was possessed with respect to medical treatment. Hence, the author does not limit himself to justifying medicine according to the practices of his ancestors, but seeks to delimit these practices – as

pointed out by Laín Entralgo [12, p. 140] – by virtue of the causal knowledge of an object.

In the first part of the writing, the author describes the object of ancient medicine as the *metron*, the measure, the number, and proportion between elements. The good *kresia*. "In food – writes the author– we have to point to a certain measure" (Chap. 9, p. 146). The doctor must discover the measure of the food according to how it is each type of constitution.

When defining this object, the author manifests his assumption of the philosophical doctrine of the elements that predominated in the Ionian philosophers. Man is understood as part of a *kosmos*. And, in turn, he constitutes a microcosm. Now, just as those philosophers explain the appearance or corruption of all the beings of the cosmos through the association or dissociation of the first elements, ancient medicine defines the health or illness of man attending precisely to the dynamic balance between the elements that make up his body. Health is only an *isomoira*, that is, a proportionality between the elements that make up the organism. On the other hand, disorder, *apokrisis* (disintegration) or *dyskrasia* (bad mix), absence, or monarchy (*μουναρχίη*) – that is, the predominance of one of the elements over others – behaves as the cause of the disease (Chap. 14, p. 153).

But in Chap. 13, the author opens his critique of this ancient medicine. And, through it, he begins to show the need to reformulate or delimit even more the object of medical practice.

He assumes the doctrine of disequilibrium as the cause of illness, but rejects as an oversimplification the reduction of medical treatment to the search for the opposites. Ancient medicine in order to achieve balance between the elements that make up the patient's body uses the method of allopathy or treatments for the opposites. This type of therapy dictates that opposites are cured by opposites: *contraria contrariïs curantur*. Through the right medium between the opposites, the doctor restores the proper balance of the *physis*.

The author of the *On Ancient Medicine* shares with his opponents the doctrine that medicine should seek good mixing or balance. Nevertheless, he points out its limitation as so as that doctrine does not attend to the active principles or the *dynamis* of the elements. His argument is that the proportion or order of food cannot be determined if its active ingredients are unknown. He describes this situation not without a certain irony:

> What, then, shall we say? Whether that, as he suffered from cold, these hot things being applied were of use to him, or the contrary? I should think this question must prove a puzzler to whomsoever it is put. For whether did he who prepared bread out of wheat remove the hot, the cold, the moist, or the dry principle in it?; for the bread is consigned both to fire and to water, and is wrought with many things, each of which has its peculiar property and nature, some of which it loses, and with others it is diluted and mixed. (Chap. 13, p. 151)

The doctor does not cure simply by administering the element contrary to what apparently produced the disease. To heal, and to do so rationally, he must know the *dynamis* proper to each element or the combination or mixture of each of the components he administers to the human organism:

> I cannot think in what manner they who advance this doctrine, and transfer Art from the cause I have described to hypothesis, will cure men according to the principle which they have laid down. For, as far as I know, neither the hot nor the cold, nor the dry, nor the moist, has ever been found unmixed with any other quality; but I suppose they use the same articles of meat and drink as all we other men do. But to this substance they give the attribute of being hot, to that cold, to that dry, and to that moist. Since it would be absurd to advise the patient to take something hot, for he would straightway ask what it is? So that he must either play the fool, or have recourse to some one of the well-known substances. (Chap. 15, p. 154).

What damages a man is not the dry, the wet, the hot, or the cold. What makes him sick is "the strength of everything" and "what may be excessive about it" (Chap. 14, p. 152). Then, the remedy that is needed for a disease is not simply to counteract a symptom with its opposite element. "Men do not have a fever simply because of the heat" (Chap. 17, p. 157). The author argues that the hot could heal or become ill depending on the active ingredients of the hot substance. In themselves, hot substances can have "active principles opposite each other" (Chap. 15, p. 155).

It may happen that there are ills that are due to cold or heat without these being combined by any other quality.

In this case, they could be cured by the opposite element. But in the rest of the cases, the doctor must look for the cause of the disease in the active principles and in the mutual combination of the same (Chap. 19, pp. 158–159). Even more, he adds that "of all the active ingredients in the body, cold and heat are the least influence." Because "heat is balanced by cold and cold by heat" (Chap. 16, p. 155). The author begins to consider heat and cold as secondary active principles, while the acid, the salty, the sweet, and other humors, not defined in number, will be considered as basic principles.

Through various tests, testimonies, and comparisons, the author argues that ancient medicine is incapable of curing or giving a scientific or causal explanation of the disease. Instead he proposes to rethink the object of the medical art presenting the *dynamis* as the true causal explanation of the disease and health.

The powers of each, too, are great, and the one nowise like the other. Whoever pays no attention to these things, or is paying attention, does not comprehend them; how can he understand the diseases which befall a man? For, by every one of these things, a man is affected and changed this way or that, and the whole of his life is subjected to them, whether in health, convalescence, or disease. Nothing else, then, can be more important or more necessary to know than these things (Chap. 14, p. 152).

Here is the new object of medicine: the *dynamis* that each thing possesses and that is part of man by virtue of his own diet.

The term *dynamis* derives from the verb *dunamai* which means to affect. This verb can be substantiated as a quality, property, power, active principle. We will refer to it in this last sense insofar as it corresponds better to the idea of a simple and real entity, characterized by an activity that causes specific observable effects in the organism. The author recognizes qualities, such as heat or cold, as specific forces or abilities that affect the body. He understands *dynamis* as the characteristic movement of substances. Or conversely, the operational manifestation of these substances is recognized as their *dynamis*.

In Chap. 22, he writes: "By active principle I understand the maximum degree in intensity and strength of each one of the humors." This passage has opened among specialists the discussion about whether the author of our treatise presents the quantity or quality of humors as the new object of medicine. We believe that the notion of *dynamis* solves this dialectic by itself because it must be recognized as an active quality. Each thing has an operation, a specific behavior that is explained by virtue of its qualities and its morphological properties. Evidently, the greater the quantity of humors, the greater the intensity with which they operate their active principles. But the specific form of behavior or effect of a mood is determined by its qualities and not just by its quantity. The Hippocratic author does not establish a confrontation or dialectic between the quantitative or the qualitative. On the contrary, he establishes a true explanatory complementarity between these accidents.

The doctor must study the dynamics or virtue of the humors or of the disease because only then he will be able to discover the natural structure of the *physis* of the body which becomes the normative principle of his *téchnê iatrikê*. His therapeutic treatments consist of intervening respecting the order of nature. He must find out where he can intervene to help the natural process, directing it towards its healing. Hence, the author of our paper insists on presenting the *dynamis* as the true object of medicine. The doctor's task is not to understand human nature from its material origin but to infer the purpose of its development, that is, to study the dynamic development of the patient in order to achieve a healthy nature.

Now, this transition with respect to the object of medicine does not imply, at all, a prohibition or a renunciation of philosophical inquiry. On the contrary, in this new object, as we will see in the next point, the author seems to assume a causal model of scientific explanation that was later cataloged by philosophy as a more perfect way to interrogate about the world and *physis*. Namely, a model that requires accounting not only for the

material constituents of the object but also for its own nature and behavior. The author of the writing not only does not distance himself from philosophy, but in his very criticism of the materialist philosophers, he assumes and incorporates a new philosophical approach.

About the Method or the Teleological Model of Scientific Explanation

Throughout the writing, the author argues that the doctor must study the *dynamis* of three elements, namely, food, the various humoral constitutions, and the structure of internal organs.

In Chap. 3, the author explains that the doctor must know the interaction between the fluids or humors present in different foods and beverages. The foods contain different humors – salty, bitter, acid, etc. – with their respective active ingredients. Well, the good doctor must indicate to his patient a temperate diet where these active principles balance each other. In Chap. 4, he explains that wine and cheese do not affect human beings in the same way. The effect of drinking a lot of wine is different from the effect of eating a lot of cheese. Medicine must know what the respective effects of each one are and how they are caused.

In Chap. 14, the author argues that the same body – like food – contains different fluids, substances, or humors which affect the body in a certain way. These individual differences in the *physis* are understood as differences in the humoral constitution, that is, as a difference in relation to the quality, strength, and quantity of humors in the body. The author insists on the need to study the *physis* or the *dynamis* of each of the individuals to assimilate the diet. It is due to the individual constitution of each patient, and their peculiar state of health requires special treatment in each case. To illustrate this type of knowledge, in Chaps. 5, 6, and 20, the author appeals to the variation that exists between different individuals. Cheese when eaten in large quantities is not harmful in the same way for everyone. While for some it is beneficial for others, it is harmful – he explains.

The author refers here to the *physis* in its condition of formal cause. He understands it as the principle by which everything is what it is. The universal nature is realized and concrete in each thing according to its own *physis*, determining the specific and individual constitution of each thing, namely, the different notes that make up its morphological peculiarity and its operative properties (*dynamis*). There is a *physis* of man as such – the human *physis* – and within the common or generic nature is that which corresponds to each individual. Human nature is diversified into several types: the male, the female, the child, the old, the bilious, etc.

Finally, in Chap. 22, the author emphasizes the need for the physician to know the *dynamis* of the internal organs. That is, the doctor must know the capacity of the organs according to their shape, consistency, or texture to affect and be affected by fluids and body air. However, in this chapter, the author is not concerned about the strictly anatomical, such as the structure and consistency of the organs. His attention is once again placed on the *dynamis* of the organs, that is, on its behaviors or response to the substances of the body. An example of this is that the author does not describe the internal movement of organic liquids as purely mechanical and inanimate movements. On the contrary, he refers to these with the concept of *dynamis*, meaning that there are biological movements that externalize or manifest the vitality of the *physis*.

The food, the organism, and the structure of the internal organs possess – to the understanding of the author – their own *dynamis*. All of them are capable of producing a certain physiological reaction in the human organism. The important thing for medicine is not to resolve the question about the material origin of human nature but to know what human nature is in relation to diet, habits, or anything that interferes with it. The doctor must know the qualities and active principles of each element, habit, or diet and the different responses that human nature may have in their individuality to these principles.

Wherefore it appears to me necessary to every physician to be skilled in nature, and strive to know, if he would wish to perform his duties,

what man is in relation to the articles of food and drink, and to his other occupations, and what are the effects of each of them to everyone (Chap. 20, p. 162).

The one who possesses the authentic *tèkhnê iatrikè* focuses on the dynamic relations of interdependence that the part has with the whole. The doctor cannot limit himself to knowing, on the one hand, the *dynamis* of each of the humors and, on the other, the *dynamis* proper to human nature in its individuality together with the anatomical constitution of its internal organs. Its object is precisely at the intersection, in the combination and response of these factors (Chap. 24, p. 167). It must investigate not only the relationship that the humors reciprocally hold but also the influence exerted by each one of them in the organism. It must study how each part is ordered to what is convenient for the *physis* and how various active principles construct together more or less specific operative units with a common purpose that is or is not effective for health.

When replacing the object of medicine, the author is presenting –although perhaps in a tacit or implicit way – a new conception about what scientific explanation should be. It is not simply replacing the doctrine of the elements with the doctrine of humors. What he fundamentally changes is the causal question from which the object of medicine is analyzed. A disease, malaise, or illness must be explained – according to him – not so much by reference to material causes (such as the presence of pure elements in the diet and by the consequent administration of its opposite) but by reference to the proper ends that they determine the course and the effects they have on the organism according to its quality and quantity. The new model of medical explanation must replace the Ionic question about the *Arjé* (the question about the primordial constitutive elements of the universe) by question about the active principles or *dynamis* of the humors. In order to heal –says the author in Chap. 20– it is not important to answer the question of how (man) is formed and what is it composed of? but how do the foods and humors of the organism, and the internal organs behave reciprocally in relation to health?" – as it is said in Chap. 20 – but of "how do the foods and humors of the organism and the internal organs behave in a reciprocal way in relation to health?".

If we are allowed to go from a descriptive instance to a hermeneutical level, we can say that the concept of *dynamis* admits the presence of real ends in the *physis*. For if the *dynamis* is the strength of every food, humor, or organ to be able to do something, that something can only be defined in view of an end. Each humor, each organ moves towards an internal end to the very development of its form. Each one has its own mode of transformation by virtue of its form. The end of all *dynamis* is nothing but the actualization of a form. And, in this sense, end and form coincide.

From all this, it is inferred that each element corresponds to its own place, towards which it moves naturally. The *dynamis* proper to fire is to heat and the dynamis of water, to hydrate. The end of each entity is its own form. The movement of substances towards an end comes from the very development of their natures. In this way, nature operates as the internal principle of development and at the same time as the result or end of that movement. The *physis* has a *telos* that determines the path that must follow any natural process of the organism. This teleology is an intrinsic property of the structure of the internal organs and the humors of the body and food.

Certainly in numerous passages of Hippocratic treatises, *physis* is understood as the genetic principle of all natural things. The word *physis* has its root in the verb *phyein* which means to be born, to sprout, or to grow. In this sense, it is understood in its condition of *Arjé* or efficient and material cause of each of the things. However, since the first half of the fifth century – the time where our writing is historically contextualized – the word *physis* acquires another sense that is complementary to it. *Physis* is also understood as dynamic ordering to an end.

The teleological explanations are explanations about the existence of certain entities by virtue of what they do, that is, in terms of a purpose inherent to their processes and structures. In this sense, we believe that the treatise *On Ancient Medicine*

complies with the basic structure of a teleological explanation. Now, this model of teleological explanation is what in our opinion is in contradiction with the empiricist or anti-philosophical hermeneutics that have been tested around this writing.

The doctor does not study an amorphous series of empirical facts, but the dynamic structure of the humors and the organs of the patient insofar as they are ordered or not to the health of the patient. Evidently this *dynamis* is not discovered from philosophical speculations about abstract and generic postulates (*hypothesis*). This manifests itself sensibly in the patient's symptoms. Hence, the author insists that the correct method of medical research is in attending to the *aisthesis tou somatos*, that is, to the sensation of the body. Understand well, the author does not refer with such an expression to the sensitive subjectivity of the doctor or – what would be even worse – to the sensitive subjectivity of the patient. The author refers to the application of the doctor's senses to the knowledge of the reality of the patient. For it is precisely the physician's senses that can perceive through the corporeal signs the patient's own *dynamis*, by virtue of which he studies the prognosis, the therapy, and the most appropriate medication.

Now, it would be a mistake to decontextualize this methodological slogan interpreting it as an anti-philosophical empiricism and forgetting that it is inserted in a writing that advocates a teleological conception regarding the *dynamis*. The methodology is certainly empirical because the object of medicine is manifested and must be sensibly perceived by the doctor.

However, we believe that this methodological slogan should be interpreted in continuity with the thesis that the author develops throughout his writing. That is, within the framework of his proposal of a teleological model of scientific explanation that involves by itself the philosophical questioning. His empiricism is not at all anti-metaphysical. On the contrary, even if the *dynamis* is susceptible of empirical verification, the theoretical understanding of it is still informed by a particular metaphysical and epistemic conception of human physiology.

Conclusion

The treaty is not the claim of an empiricist against philosophy. On the contrary, it states the need for a new type of philosophical and epistemic reflection that seeks to define *physis* not so much because of its material origin but because of its formal or final causality. And this is far from claiming an independence of medicine with respect to philosophy; instead, it presupposes a deepening of the philosophical questioning as an integral part of the scientific discourse of medicine.

It should be noted that our author is not at all interested in creating a dialectic or dichotomy between two models of causal explanation (the teleological model and the explanation of material origin). He simply opens, as a new synthesis, a new explanatory model that deals particularly with the active principles of things. However, this model also assumes in its body of scientific explanation the possibility of also addressing the question about the material origin and the development of human nature. In Chap. 20, the author writes that such knowledge can be achieved over time through the study of medicine. Well, "only through medicine will it be possible to know something sure about nature." In this way, the teleological model of scientific explanation would seem to have a priority and the possibility of subsuming in a higher synthesis the explanations related to the material origin.

Through a new model of scientific explanation the author reinvents a new synthesis or relationship between medicine and philosophy. He presents a philosophical medicine that stops being a set of abstract and theoretical premises to become an empirical and finalist understanding of the same object and practice of medicine.

The novelty of the treatise *On Ancient Medicine* lies – in our understanding – not so much in the method but in the new causal definition that incorporates the scientific discourse of medicine. After all, the reader can only understand the scope of its methodological proposal insofar as he realizes the continuity and concordance that it has regarding the application of a new object and a new explanation model for medicine.

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7 Inserted Thoughts and the Higher-Order Thought Theory of Consciousness

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Introduction

Various psychopathologies of self-awareness, such as somatoparaphrenia and thought insertion in schizophrenia, might seem to threaten the viability of the higher-order thought (HOT) theory of consciousness since it requires a HOT about *one's own* mental state to accompany every conscious state. The HOT theory of consciousness says that what makes a mental state a conscious mental state is that there is a HOT to the effect that "*I* am in mental state M" [1, 2]. In a previous publication, I argued that a HOT theorist can adequately respond to this concern with respect to somatoparaphrenia [3]. Somatoparaphrenia is a "depersonalization disorder" which is characterized by the sense of alienation from parts of one's body. It is a bizarre body delusion where one denies ownership of a limb or an entire side of one's body. My focus in this chapter, however, is on "inserted thoughts" which is a common symptom of schizophrenia, although it will be useful to compare and contrast it with somatoparaphrenia. Schizophrenia is a mental disorder which often manifests itself through auditory hallucinations, paranoid or bizarre delusions, or disorganized speech and thinking. Thought insertion is the delusion that some thoughts are not "one's own" in some sense or are somehow being inserted into one's mind by someone else. Stephens and Graham, for example, have suggested that thought insertion should be understood as alienated self-consciousness or meta-representation [4]. I will argue that HOT theory also has nothing to fear from this phenomenon and can account for what happens in this admittedly unusual case.

Somatoparaphrenia and HOT Theory

Somatoparaphrenia is a pathology of self characterized by the sense of alienation from parts of one's body. It is a very odd body delusion where one denies ownership of a limb or an entire side of one's body. It is thus sometimes called a "depersonalization disorder." Relatedly, anosognosia is a condition in which a person who suffers from a disability seems unaware of the existence of the disability. A person whose limbs are paralyzed will insist that his limbs are moving and will become upset when caregivers say that they are not. Somatoparaphrenia is usually caused by extensive right-hemisphere lesions. Lesions in the temporoparietal junction are common in somatoparaphrenia, but deep cortical regions (e.g., the posterior insula) and subcortical regions (e.g., the basal ganglia) are also sometimes implicated [5]. Anton's syndrome is a form of

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anosognosia in which a person with partial or total blindness denies being visually impaired. The patient engages in rationalization in order to account for the inability to see. Patients with somatoparaphrenia utter some rather stunning statements, such as "parts of my body feel as if they didn't belong to me" and "when a part of my body hurts, I feel so detached from the pain that it feels as if it were somebody else's pain" [6].

There has been some question as to whether or not the higher-order thought (HOT) theory of consciousness can account for the depersonalization psychopathology of somatoparaphrenia. Liang and Lane argue that it cannot [7]. The HOT theory of consciousness says that what makes a mental state a conscious mental state is that it is the target of a HOT to the effect that "I am in mental state M" (see Fig. 7.1). When the HOT itself is unconscious, the conscious state is still outer-directed. When the HOT is conscious, we have *introspection*, and so the conscious thought is directed at the mental state [8].

HOT theory has been critically examined in light of some psychopathologies because the theory says what makes a mental state conscious is a HOT of the form that "*I* am in mental state M." The requirement of an I-reference leads some to think that HOT theory cannot explain some of these "depersonalization" pathologies. There would then seem to be cases where I can have a conscious state but attribute it to someone else. The "I" in the HOT is not only importantly *self-referential* but essential in tying the conscious state to *oneself* and thus to one's *ownership* of M.

Rosenthal responds that one can be aware of bodily sensations in two ways that, normally at least, go together: (1) aware of a bodily sensation *as one's own* and (2) aware of a bodily sensation *as having some bodily location*, like a hand or foot [9]. Patients with somatoparaphre-

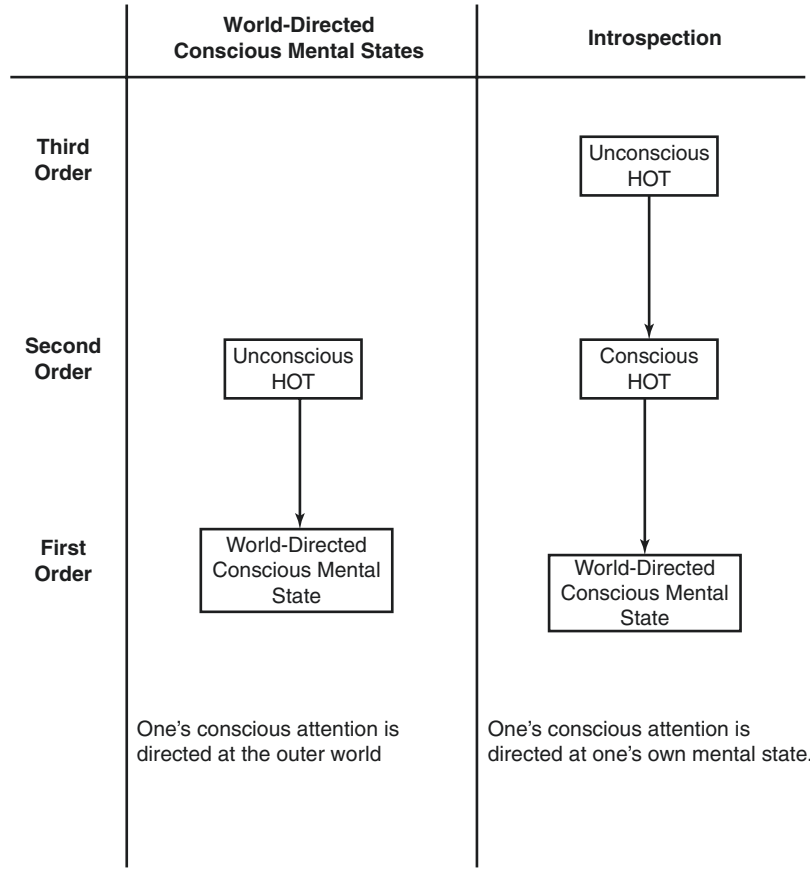

Fig. 7.1 The higher-order thought (HOT) theory of consciousness

nia still experience the sensation as their own but also as having a mistaken bodily location. Such patients still do have the awareness in (1), which is the main issue at hand, but they also have the strange awareness in sense (2). So somatoparaphrenia leads some people to misidentify the bodily location of a sensation as someone else's even though the awareness of the sensation itself remains one's own. But Lane and Liang are not satisfied and, among other things, counter that Rosenthal has still not explained why the identification of the *bearer* of the pain cannot also go astray [10].

I have replied that we can go further than Rosenthal in defending HOT theory [3, p. 57–8]. First, we must remember that many of these patients often deny feeling *anything* in the limb in question [11]. As even Liang and Lane point out, patient FB, while blindfolded, feels no tactile sensation when the examiner would in fact touch the dorsal surface of FB's hand. In these cases, it is therefore difficult to see what the problem is for HOT theory at all. Second, when there really is a bodily sensation of some kind, a HOT theorist might also argue that there are really *two* conscious states that seem to be at odds. There is a conscious feeling in a limb but also the (conscious) attribution of the limb to someone else. It is crucial to emphasize that somatoparaphrenia is often characterized as a *delusion* of *belief* often under the broader category of anosognosia [12, 13]. A delusion is often defined as a false belief that is held based on an incorrect (and probably unconscious) *inference* about external reality or oneself that is firmly sustained despite what almost everyone else believes and despite what constitutes incontrovertible and obvious proof or evidence to the contrary [14, 15]. Beliefs, generally speaking, are themselves often taken to be intentional states integrated with other beliefs and mental states. They are typically understood as caused by perceptions or experiences which then lead to action or behavior. Thus, somatoparaphrenia is much closer to self-deception and involves frequent confabulation. If this is a reasonable interpretation of the data, then a HOT theorist can argue that the patient has the following *two* conscious states:

S1: a conscious *feeling* (i.e., a tactile sensation) in the limb in question, and
S2: a conscious *belief* that the limb (and thus sensation) belongs to someone else.

Having both S1 and S2, especially if conscious at any given time, is indeed strange and perhaps even self-contradictory in some sense, but the puzzlement has nothing to do with HOT theory.

A similar critique of self-representationalism [16] based on somatoparaphrenia might also be posed to what Billon and Kriegel call "subjectivity theories" which say that "there is something it is like for a subject to have mental state M only if M is characterized by a certain sense of 'mineness' or 'for-me-ness.' Such theories appear to face certain psychopathological counterexamples: patients appear to report conscious experiences that lack this subjective element" [17]. Patients with somatoparaphrenia seem to be cases where one has a conscious state without the "for-me-ness" aspect and thus not experienced as one's own [7, 18]. However, Billon and Kriegel counter that "none of the patients that we know of claim feeling *sensations that are not theirs*. Rather, they say that they feel touch in someone else's limb. This does not yet imply that they feel sensations that are not their own -- unless it is analytic that one cannot feel one's sensations but in one's own body, which we have phenomenological and empirical reasons to deny" [19]. Again, many disorders, including somatoparaphrenia, involve delusion or self-deception. A delusion is distinct from a belief based on incorrect or incomplete information, poor memory, illusion, or other effects of perception. Self-deception is a process of denying or rationalizing away the relevance, significance, or importance of opposing evidence and logical argument. Self-deception involves convincing oneself of a truth (or lack of truth) so that one does not reveal any self-knowledge of the deception. Delusions have certainly received extensive treatment from philosophers in recent years, sometimes in connection with self-deception [20–22].

Liang and Lane tell us that "what seems to be happening is that these tactile sensations are *represented as belonging to someone other than*

self' [7, p. 664], and that is problematic for HOT theory. But this is at best highly ambiguous because another way to represent sensations as belonging to someone else is via a propositional attitude such as a belief. And there would be no problem for HOT theory, as such, as to whether or not these patients can have the conscious belief in S2. That is, a patient with somatoparaphrenia would still represent that *belief* and report it as her own. Still, Lane is not satisfied with these responses [18].

Thought Insertion and Schizophrenia

Another much discussed depersonalization disorder can be found in those with schizophrenia, which is a mental disorder characterized by disintegration of thought processes and emotional responsiveness. It most commonly manifests itself as auditory hallucinations, paranoid or bizarre delusions, or disorganized speech and thinking, and it is accompanied by significant social dysfunction. Thought insertion, a common symptom of schizophrenia, is the delusion that some thoughts are not one's own and are somehow being inserted into one's mind. In some particularly severe forms of schizophrenia, the patient seems to lose the ability to have an integrated or "unified" experience of her world and self. The person often speaks in an incoherent fashion and is unable to act on simple plans of action.

Stephens and Graham suggest that thought insertion should be understood as alienated self-consciousness or meta-representation [4]. They think that schizophrenics make introspective inferential mistakes about the source of inserted thoughts based on delusional background beliefs. Some bodily movements can of course be movements of *my* limbs without counting as *actions of mine* or as caused by me. Perhaps someone else is controlling my movements, or perhaps they are entirely involuntary such as the physical tics and vocalizations in Tourette's syndrome. Another relevant disorder is anarchic hand syndrome which is a neurological disorder in which individuals frequently perform seemingly voluntary movements that they do not consciously intend and cannot directly inhibit. But in these cases the bodily movements are still self-attributed to the person with the disorder, so something else must be going on to explain attributions to others in thought insertion. If a song spontaneously runs through my mind, I still think of it as an episode in my mind. But it may not count as *my* mental activity in the same way as when I am thinking through a math problem or trying to plan a trip. The latter, but not the former, involves intentional thought that expresses my agency. There seems to be something special going on when I consciously engage in some activity which involves mental effort and voluntariness. Stephens and Graham call the feeling of *having* a mental state the "sense of subjectivity" and the feeling of *causing* my mental state the "sense of agency." They urge that these two can come apart in unusual cases so that thought insertion involves the sense of subjectivity without the sense of agency, which accounts for the curious "passivity experience" of schizophrenics. So attributing thoughts to someone else under these circumstances does make some sense since the mental state must be caused by something or someone (see also [23–25] for much more on the above line of thought).

Gallagher makes a similar distinction between a "sense of ownership" and a "sense of agency" [26] but, in contrast to Stephens and Graham's "top-down" approach, argues instead that the primary deficit regarding thought insertion is more of a "bottom-up" problem with the first-person experience itself rather than a self-monitoring abnormality. What happens at the introspective level is not erroneous but rather a correct report of what the schizophrenic actually experiences, that is, thoughts that feel different and externally caused. Gallagher also points to some preliminary neurological evidence which indicates abnormalities in the right inferior parietal cortex for delusions of control and ownership.

It is worth mentioning again that in the background is the related issue of whether or not there is "pre-reflective," or implicit, self-consciousness in all conscious states, which is often associated

with a so-called sense of ownership or sense of mineness [27–29]. According to self-representationalism, for example, a fundamental aspect of all conscious experiences is that they seem to be *mine*. In being aware of any thought, action, perceptual experience, memory, or bodily experience, I am aware of it as being *my own*. Perhaps this is one reason why it seems difficult to understand how one could attribute a pain as located in another's body or a thought caused by someone else or even located in another's mind. Perhaps a sense of ownership explains why some mental states seem to be "immune to error through misidentification," that is, impossible to be mistaken about with regard to mental state ownership (more on this below). Perhaps lacking this sense of ownership in abnormal cases can explain the odd and delusional thinking in such rare cases.

But even within the context of an implicit account of self-consciousness in experience (or a "sense of ownership"), there is significant disagreement between robust and deflationary accounts. For example, Zahavi and Kriegel defend a more robust understanding of the sense of ownership as a distinct aspect of the phenomenal character of all conscious states and as a necessary feature of all experience. By contrast, Bermúdez argues in favor of a deflationary account of the sense of ownership over one's own body, according to which it consists in nothing more than the phenomenology of the spatial location of bodily sensations together with our tendency to judge the body in which they occur to be our own [30]. So perhaps there is no "sense" of ownership which accompanies all conscious states.

I have used something like this line of response against Ford and Smith's argument in favor of the so-called "self-representationalist" theory of consciousness, whereby first-order conscious states are always accompanied by an inner-directed peripheral conscious awareness [31]. Ford and Smith contend that cases of depersonalization show that something like Kriegel's view is correct. But again, just because the removal of something – for example, normal proprioception – causes deficits in one's conscious mental states, it surely does not follow that the awareness of that thing is part of normal conscious experience. The relation could be causal instead of constitutive. That is, the typical abilities and awareness in question might merely, in the normal case, causally contribute to the phenomenology of one's conscious mental states without being part of the conscious state itself. There are many ways that normal consciousness can be disturbed or impaired (e.g., being unable to breathe), but surely we shouldn't conclude that every such disturbance shows that the ability in question normally shows up regularly in our phenomenology [2, p. 127–9]. (See [32] for another deflationary account from an Eastern philosophical viewpoint.)

Notice that this fits nicely with HOT theory, which can explain why there is a phenomenological sense of myness when one *introspects*, namely, that the HOT is itself conscious, whereas no such sense is present when one has an unconscious HOT. The concept "I" is part of a *conscious* thought in the introspective case but part of an *unconscious* thought in the first-order case. Nonetheless, it is certainly true that when there is a disturbance or abnormality in one's I-concept, such as one's bodily representation, one's consciousness will be altered and result in some odd feelings of body *dis*ownership (or thought disownership for that matter). But, like Bermúdez, I do not find it compelling to argue that if a deficit of bodily awareness is manifested in consciousness, then that aspect of bodily awareness is always or even normally part of our consciousness.

Thought Insertion and HOT Theory

The puzzle of thought insertion is thus perhaps somewhat analogous to what we have seen with respect to somatoparaphrenia. For example, just as we might distinguish between experiencing a sensation as one's own as opposed to its *bodily location* in patients with somatoparaphrenia, so we might distinguish between experiencing a thought as one's own as opposed to its *causal origin* in patients with schizophrenia. Those with

somatoparaphrenia attribute a limb and/or a sensation to someone else, whereas some schizophrenics attribute thoughts (or at least their causal origin) to someone else. If this analogy is correct, then a HOT theorist might argue that the schizophrenic patient has (at least) the following *two* conscious states:

S1: a conscious *thought* in one's own mind, and
S2: a conscious *belief* that the thought has been inserted by someone else.

Having both S1 and S2, especially if both are conscious at any given time, is indeed strange and perhaps even irrational in some sense, but the puzzlement again has nothing to do with HOT theory. In S1, we can suppose that the patient has a HOT about her own thought, i.e., she is aware of the mental state as occurring in her mind. In S2, the patient *also* has a conscious belief that S1 has been inserted into her mind by someone else. Once again, and analogous to somatoparaphrenia, there is an essentially delusional element involved in schizophrenia and inserted thoughts. Why or how it is that S2 is generated is of course interesting in its own right. For example, as Stephens and Graham believe, perhaps the best explanation is indeed that it is inferred from a lack of sense of agency (or "passivity" experience) or perhaps it is inferred from a perceived lack of causal origin. But none of these explanations cause trouble specifically for HOT theory's ability to explain S1. Just as HOT theory says about all conscious states, when a patient is aware of having a (first-order) conscious thought, there is a HOT about that thought. Once again, we must also recognize the delusional element involved in S2. There are of course situations where those with thought insertion might even realize that they have come to believe contradictory things based on their abnormal experiences [33].

It would again be highly ambiguous if Liang and Lane were to say that inserted thoughts are "represented as belonging to someone else" as if that is problematic for HOT theory. This is partly because another way to represent such thoughts as belonging to someone else is via a propositional attitude such as a belief. And there would be no problem for HOT theory, as such, as to whether or not these patients have the conscious belief in S2. That is, a schizophrenic patient with thought insertion would still represent *that belief* as her own while still having S1.

In some ways, then, I am somewhat sympathetic with Parrott's account of thought insertion [34]. Parrott first notes that prevailing views in philosophy and cognitive science tend to characterize the experience of thought insertion as missing or lacking some element, such as a "sense of agency," found in ordinary first-person awareness of one's own thoughts. However, in another sense, it might be that rather than lacking something, experiences of thought insertion have an *additional feature* not present in ordinary conscious experiences of one's own thoughts. So thought insertion consists of two distinct elements: a state of ordinary first-person awareness of a thought and the thought that this state of awareness is highly unusual. The latter leads via delusional thinking and inferential reasoning to the belief noted above in S2. So it is really the additional S2 which creates most of the puzzle.

Smith makes a further distinction and explains that "we might…wish to make a three-way distinction between the sense of *agency* (the sense that one is the *author* of a mental state), the sense of *ownership* (the sense that one is the *owner* of a mental state), and what we might call the sense of *location* (the sense that a mental state is located *within* one's own mind). The sense of location might be understood as being possessed if one is aware of a mental state in the ordinary way, i.e., introspectively. Crucial, it would seem, for evaluating the significance of thought insertion and related cases…will be determining which, if any, of the senses of agency, ownership, or location remain intact" [35]. Smith notes that it might be argued that what such patients retain is in fact the sense of location, rather than the sense of ownership. That is, it may be possible to take their descriptions at face value when they deny, in thought insertion, for example, that the thoughts are their own (or were thought by them) while still accepting that the inserted thought occurs within the boundary of their own mind. The explicit mention of location is reminiscent of

patients with somatoparaphrenia who arguably still experience the sensation as their own but also as having a mistaken bodily location, as Rosenthal had initially urged.

One difficulty of course is how literally to take reports of patients with schizophrenic inserted thoughts. Should we take them at face value or at least treat them with some skepticism given the delusional aspect in question? Consider, for example, some statements of those suffering from thought insertion. Frith quotes one patient as saying that "thoughts are put into my mind like 'kill God'…They come from this chap, Chris," but then the patient says that "they are his [Chris'] thoughts" [23, p. 66] which is a further stronger claim. So there is also some ambiguity in patient first-person reports. In a standard textbook on the topic, we are told that "in thought alienation [i.e. thought insertion] the patient has the experience that…others are participating in his thinking" [36]. It is difficult to know when to take such patients literally, but it does also seem that they sometimes report that the inserted thoughts themselves are someone else's.

Thus, let us go further and consider the following three statements in this context:

(1) I am having (experiencing) a conscious thought T, but it was inserted into me by someone else.

In (1), we might say that the location is in my mind, the ownership is mine, but the origin is elsewhere.

(2) I am having (experiencing) a conscious thought T, but it is in another's mind.

For (2), we might say that it is my ownership but not my location or origin.

(3) I am having (experiencing), or "aware of" in some sense, someone else's conscious thought T.

In (3), it seems that T is actually someone else's thought and so that even my ownership appears to be in doubt. The thought is "in my mind" in some sense (location), but I also think of it as someone else's, perhaps analogous to having someone else's furniture in my house. The notion of "ownership" might also sometimes be confused with location because there is a sense in which location would normally imply ownership. Is it "my chair" if I am renting a furnished house? Well, yes and no. Is it "my chair" if I have someone else's chair temporarily in my house? Well, yes and no. Grammatically, there can also be some confusion or ambiguity. When I say that I am home sitting in "my chair," it would perhaps be more accurate to say that I am sitting in a chair that is in my house.

Still, with regard to (1)–(3), it would seem that there is still at least a grammatical reference to oneself, and thus at least some sense of ownership, in each case. For one thing, each starts with "I am having (experience)…." This is especially clear with respect to (1) and (2) and clearly reflects the typical content of a HOT, that is, something like "I am in M" or "I am aware of M" or "I am experiencing M," as we have seen. Even in (2), the additional and puzzling belief that T is in another's mind can be the result of delusional reasoning as described above with respect to S2 earlier. Still, there is nothing in (2) itself which automatically rules out my *ownership*. At most, perhaps the location and perceived origin is different or unusual. It may of course be false or delusional to think so, but that is a different matter and does not threaten HOT theory.

In addition, the more radical notion that "I am having a thought that is in your mind" is perhaps not impossible in some hypothetical or empirical instances. For example, if telepathy is real (or just possible), perhaps person A can cause person B to have certain thoughts, or it is possible for person B to gain access to some of person A's thoughts. Hirstein goes further and describes in some detail how what he calls "mindmelding" might be empirically possible [37]. He argues that it is indeed possible for one person to directly experience the conscious states of another. This would involve making just the right connections between two peoples' brains. He then considers the consequences of the possibility that what appeared to be a wall of privacy can actually be breached. In line with the analogy above, I might also say that I am sitting in "my chair" but in

"your house" in a case where I have lent you my chair temporarily.

Relatedly, Langland-Hassan discusses an actual case of craniopagus twins – twins conjoined at the head and brain – where each twin seems to know what the other is seeing or feeling, and perhaps even thinking, in a way others cannot [38]. Interestingly, each twin (Krista and Tatiana Hogan) would seem to know these things through introspection. Perhaps the twins can *introspect* the same shared mental state, that is, have a kind of mental state *co-ownership*. If this is granted as a possibility, then mental state ownership need not be understood as an either-or matter. Perhaps the typical underlying assumption that each mental state only has one owner is false in some admittedly rare and bizarre circumstances. These considerations would also help to make sense of (3) above. Perhaps I can experience someone else's thought T, but T is *also* my thought because we can co-own T (and so T is still in my mind, in some sense). Alternatively, Langland-Hassan considers the more radical possibility that one person may be said to be introspectively aware of only another's mental state.

There are also numerous other very strange and related delusions discussed in the literature, such as Cotard syndrome where people hold a delusional belief that they are dead (either figuratively or literally), do not exist, are putrefying, or have lost their blood or internal organs [39]. But notice that even here the statement that "I think I don't exist" or "I think I'm dead" still at least contains an initial reference to oneself (as a grammatical fact). It seems to me that there must always be some reference to oneself when expressing any kind of thought or belief (even if delusional, irrational, or self-contradictory in some other way). But this is precisely what leads to a contradiction. Imagine: "Who thinks that you don't exist?" Answer: "I do"? "Who is dead?" Answer: "I am"? Again, perhaps this is a misleading way of speaking, but the same might be true of many patient reports.

In all the above cases, there is a conscious thought which can be explained by a HOT theorist in the usual way, but there is *another* belief or thought which contradicts the first one or is delusional in some other way. It is also very important to recognize that HOT theory comes with a well-known noninferentiality condition, such that a HOT must become aware of its target mental state noninferentially, that is, in an unmediated way. As Rosenthal repeatedly emphasizes, the point of this condition is mainly to rule out certain alleged counterexamples to HOT theory, such as cases where I become aware of my unconscious desire to kill my boss because I have consciously inferred it from a session with my psychiatrist, or where my envy becomes conscious after making inferences based on my own behavior. The characteristic feel of such a conscious desire or envy may be absent in these cases, but since awareness of them arose via conscious inference, the HOT theorist accounts for them by adding this noninferentiality condition. Thus, HOT theory requires that the HOT arises in an unmediated manner. This is also important in this context because if there is any kind of inference to a belief, such as in S2, then the HOT would not arise in the requisite manner. Also, and perhaps more importantly, using inference and reasoning to become aware of a mental state is much more likely to occur via introspection, that is, by *consciously* thinking about one's own mental states. This, in turn, increases the likelihood that delusional thinking will generate further false beliefs. Another way to think of this is that there is "introspective access" to a mental state M which involves a kind of "ownership" as well. Normally, at least, M is assumed to be mine in the sense that it is the object of my introspection. In cases of thought insertion, however, the patient sometimes seems to be calling that assumption into doubt.

Immunity to Error Through Misidentification (IEM)

I mentioned above the much-discussed "immunity to error through misidentification" (IEM) principle [40]. According to Shoemaker, a certain subset of thoughts about oneself is immune to error through misidentification [41]. As Shoemaker makes clear, one can think about one-

self under any number of descriptions. But only some I-thoughts are immune to error through misidentification – namely, those I-thoughts that are directed at one's mind and mental life, as opposed to one's body and corporeal life. Wittgenstein observed that I can see in the mirror a tangle of arms and mistakenly take the nicest one to be mine [42]. I may think to myself, "I have a nice arm." In that case, I may be wrong not only about whether my arm is nice but also about who it is that has a nice arm. Such an I-thought about my body (or body part) is not immune to error through misidentification. In extreme abnormal cases, such as in mirror self-misidentification, one might even believe that *one's own* reflection in a mirror is some other person. However, one cannot be mistaken that it is I who is having the experience in question. I cannot be mistaken that I am perceiving the reflection in the mirror. In a sense, then, I can be wrong about what my mental state is directed at (the object) but not about who is having the mental state in the first place (the subject).

Let us return to the above statements (1)–(3):

(1) I am having (experiencing) a conscious thought T, but it was inserted into me by someone else.
(2) I am having (experiencing) a conscious thought T, but it is in another's mind.
(3) I am having (experiencing), or "aware of" in some sense, someone else's conscious thought T.

Recall that HOT theory has been critically examined in light of some psychopathologies because, according to HOT theory, what makes a mental state conscious is a HOT of the form that "I am in mental state M." The requirement of an I-reference leads some to think that HOT theory cannot explain these "depersonalization" pathologies. There would seem to be cases where I can have a conscious state but attribute it to someone else or possibly even introspect another's mental state. Thought insertion seems to threaten HOT theory because it contradicts the notion that the accompanying HOT is "I am in mental state M." Recall that Lane and Liang protest that Rosenthal has still not explained why the identification of the bearer of the pain cannot also go astray in somatoparaphrenia, especially since Rosenthal clearly holds that misrepresentation can occur between a HOT and its target. But whatever one thinks of standard cases of misrepresentation between the first-order and second-order level on HOT theory, it is not clearly relevant here because those abnormal cases involve differences in the *contents* of the two respective states. Although Lane and Liang claim that there should be equally the possibility of a mismatch between the "I" in the HOT and the "I" in the first-order mental state, it is unclear how this could be so.

Notice again that (1), (2), and (3) still all start with what Wittgenstein called the "I-as-subject" and thus there seems to be some kind of minimal sense of "ownership" which remains even in the most extreme cases of thought insertion. This minimal sense can be the extent to which a HOT theorist allows for a HOT that "I am in mental state M" with regard to S1 even if the patient also has another delusional conscious state which runs counter to S2. Wittgenstein usefully distinguished between the "I-as-subject" (e.g., "I have a pain") and the "I-as-object" ("I have a broken arm"). Crucially, however, there is never an I-as-object in the *content* of the *first-order* conscious state, but there is an implicit (and unconscious) I-as-subject at the second-order level *as well as* an I-as-object in a typical HOT. According to HOT theory, there would only be an I-as-subject concept in a first-order state, whereas the content of the state refers to the outer world. This would be a kind of "raw bearer" of the state, as Rosenthal calls it [43]. After all, if we assume that any mental state must have a bearer, then even first-order states should involve some primitive concept of I. The same is true for the unconscious HOT that accompanies a first-order conscious state, but here there is *also* an I-as-object referenced in the *content* of the HOT (i.e., "I think that *I am in M*"). Still, these I-concepts are normally parts of unconscious thoughts, and so there is little reason to suppose that there is any phenomenological sense of "myness" in these cases. However, when one *introspects* and has a conscious HOT directed at a mental state, there is not only a conscious

I-as-subject concept but also a *conscious* I-as-object concept in the content of the HOT that may account for any subjective sense of myness. Further, there would be no mismatch between an I-as-object in the content of a mental state M and its HOT because there isn't an I-as-object concept at all in the *content* of M itself. (Caruso also presents an interesting discussion of thought insertion and IEM in the context of HOT theory. I do agree with him that the overall sense of mental state "ownership," to the extent that there is one, comes more from our ability to *introspect* our mental states [44]).

Seeger also discusses IEM and several psychopathologies including somatoparaphrenia and thought insertion [45]. He is very careful to clarify and define some of the claims at issue. For example, he describes the "Self-Awareness Thesis" as the claim that "if one is introspectively aware of a mental state, then one is necessarily aware of that state as one's own state" but then convincingly argues that the self-awareness thesis is not quite the same as what he calls "The Immunity Thesis" which says that "if one self-ascribes a state of which one is introspectively aware, then that state is one's own state." So it may be that thought insertion is a counterexample to the self-awareness thesis but not to the immunity thesis since, as we saw in the previous section, the schizophrenic patient may indeed assert that an introspected thought is someone else's. Seeger explains that the pathologies show that it is possible to be introspectively aware of a mental state without self-ascribing it. However, when a patient does self-ascribe an introspected thought, the thought must be his own. Self-awareness and self-ascription can come apart in unusual circumstances. In any case, my main concern here is that thought insertion is not a threat to HOT theory as opposed to arguing for a particular preferred definition of IEM.

In any case, I conclude overall that HOT theory can withstand the alleged threat from the phenomenon of thought insertion in a way somewhat analogous to somatoparaphrenia. Indeed, I think that HOT theory can even help to explain what happens in these cases, especially when one is clear about the nature of delusions and is careful about the concepts in question, such as the complex nature of self-awareness and the more subtle details of HOT theories. It is important to recognize the difference between the content of first-order mental states and the content of HOTs (including introspective states).

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8 Relationship Between Body and Soul According to Saint Thomas: An Obsolete Issue?

Francisco Rego

Position of the problem:

> "Professor, what is the soul?", asked a fourth year high school student at the beginning of the class.
>
> "To begin with," I started to answer, "It is about a reality of spiritual order whose existence and nature can be known through its effects…"
>
> "A reality of spiritual order?" interrupted the student in an exultant and sharp manner, "Then, it does not exist!"

As seen in the above dialogue, it seems that, at least nowadays, a time of essentially material urgencies and needs, to speak about a relationship between body and soul, was an open nonsense, only belonging to a mind whose clock, now rusty, stopped beyond repair in the past, because the question now asked is the very existence of one of the terms of the relationship searched for in the present paper. Then, what relationship are we talking about if the soul, truly, does not exist? Then it is clear that under that tight materialistic premise which in advance denies the very existence of the soul, the issue should be reduced to no longer establish the relationship between body and soul in the heart of human nature but between the body and certain phenomena which could be considered, if it were the case, as a mere collateral or residual manifestation of brain processes ruled by laws and principles of purely organic character.

All things considered, a so-called materialistic explanation about a subject that, in spite of its old age, it is still present among the specialists of all times would give way to an urgent question of the following tone: Is it reasonable to suppose that only the reality of that presented under the light of the empirically observable can be admitted? Of course, that is the way the representatives of the scientist and materialistic currents understand it because they depart from assumptions that cannot give way to another conclusion. But if we accept this premise, would we have to deny the realities which are not seen at first sight like life, health, illness, pain, or inner acts like knowing, believing, waiting, or loving? How could the life of living people be explained? It is the case of mental facts like thinking, understanding, loving, being aware, and deciding. Should they be understood like forms derived from processes of purely organic character? And what explanation should there be for experiences such as the conscience of moral responsibility and the search for wellness, truth, and justice? What about acts of self-denial and heroism in extreme situations? If the existence of the soul is denied, what would be left of the human being and under what foundation could his universally admitted dignity be defended?

In spite of the opinion of materialistic thinkers, from ancient times, the soul was understood as the principle of life, and far from restricting its activity to purely vegetative and sentient

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functions, it was extended to the rational field as well [1]. For better understanding, see what happens to a tree leaf, when at the end of its cycle of life, it falls and changes color from bright green to grey and turns brittle. It happens because it is a leaf deprived of life. And the same thing happens with the human body when it stops having the vital impulse of its own soul, initiating an irreversible corruption process.

This is a point of view that gives way to the reasonableness of the human existence and to the justification of the question because of the relationship that soul and body have between them.

Assuming as admissible the question about the relationship of body and soul, it is considered timely to examine the following points:

I. Some history.
II. A reasonable answer: the soul is the formal principle of the body.
III. How do we get to know the soul?
IV. The steps to the knowledge of the soul.
V. Definition of soul.
VI. Its subsistence and immateriality.
VII. Its union with the body.
VIII. Effects of the union.

Some History

Concerning the relationship between soul and body, Beuchot [2] presents the main background.

The clearest initial position was that of Plato, who, in the frame of his doctrine of ideas, understands man as two substances of different attributes [3]. Though, anthropological dualism explicitly appears with René Descartes in his anthropological doctrine of the two substances: res cogitans-res extensa [4]. This dualism later adopts the form supported by Malebranche's occasionalism [5] and Leibniz's preset harmony [6].

A derivation of the Cartesian dualism, according to Beuchot, is present in the parallel and monist doctrines.

Parallelism can adopt the form of substantial monism as it is the case of Baruch Spinoza, who assumes parallelism of extension and thought as modalities of a unique real substance that identifies itself with God. In turn, among the negative currents of substantial order, there appears a psychophysical and a psychophysiological parallelism.

With Haeckel, dualism will assume the form of a materialistic monism that will understand thought as a phenomenon derived from the cerebral activity.

As a reaction, Hegel affirms an idealistic monism where only the psychic is the real thing, thus reducing the material to be a mere epiphenomenon of what is spiritual.

Being against these two positions, Bertrand Russell holds as real the corporal as well as the psychical order, considering them as manifestations of an unknown "X."

What can be said about this? To begin with, it can be said that along history there are biased interpretations of the relationship between soul and body where one or the other extreme of the relation is overrated. And what today seems to have a major weight is a rather scientist reading of the same that postpones the spiritual part of the relationship. No doubt it can be thought that some kind of relationship exists between the organic and animic order and between the cerebral and mental process. That is, the decrease of the motor activity corresponds to a decrease of the conscience which fluctuates between the estates of obtundation, sleep, and coma. And when there is an acceleration of the motor activity, we can observe an upsurge of the conscience which rocks between excitement, mania, and delirium. We can also notice the incidence of wishes or passions in the body and the influence that psychotropic drugs or cerebral injuries or cerebrovascular accidents have over the psychical states.

Nevertheless, a man's activity does not always reflect the influence of the organic over the mental, because there are faculties that operate on an extrinsic dependence from the body, that is, when the activity presupposes the participation of the body only at an accidental level not an essential one. That would be the case of the rational activities, because although they can imply some organic activity of neuronal character, which

would explain the conservation, transmission, and limitation of mental processes, they would not imply the generation of, so to speak, mental processes.

Well, if acts like thinking and loving, being of immaterial nature, radically exceed the possibilities of a purely organic nature, then there would be no way of justifying in what way an inferior cause could explain a superior effect. Then a sufficient reason is necessary, as can be an immaterial soul, to explain the genesis of mental processes: "the bigger the nobility of the forms, the more its virtue exceeds to such point on the corporal matter, that it has an operation and virtue in which no mode participates the corporal mode and this virtue is the intellect" [7].

Thus, although the materialists Smart [8] and Place [9] hold a certain identity between the mental and cerebral processes. Other authors, like Kenny [10] and Malcolm [11], with different arguments, reject the reducibility of the mental processes to the cerebral processes. Beuchot, following Malcolm, answers that there is no way of empirically verifying the relation that stands between both spheres of the human activity, simply because there cannot be a sensitive experience of the genesis of a mental process. "We can only attend to certain effects to determine mental states. But this is 'conceptually independent' of the investigation of cerebral processes. It will never tell us anything about the bodily location of that mental event. We would not know what the meaning of a thought being localized in the brain is. We would not even understand what that means" [12].

A Reasonable Solution: The Soul Is the Formal Principle of the Body

According to Beuchot, the stance of contemporary authors confirms the reasonableness of Thomas Aquinas' position: "Henny's reflection helps us see the coherence and the adequacy of the notions used by St. Thomas in his doctrinal construction" [13]. And it is Thomas Aquinas who, following Aristotle, affirms that the rational soul is the substantial formal principle that is united to the body like form to matter [14]. This means that the soul is the principle of being (first act or perfection of man) and also the principle of acting (act or second perfection of man).

First argument: *the first thing for which a being acts is the form.* The first thing for which a man lives is the soul. Intellection is an *expression of life.* Thus, the principle by which man understands is the form of the body.

Second argument: the act of understanding is convenient to man by essence. The essential act is founded in the substantial formal principle, which is the intellective soul. But it is the same man *the one who perceives understanding as well as feeling.* Then, the formal principle that founds him as capable of understanding is the same one that founds him as capable of feeling.

Third argument: the third argument says that the nature of anything is manifested by its operation. The specific operation of man is the intellectual one. But the species is founded on form, and then through the form, the human species performs the specific operations.

But how do we hold the existence of one vital principle when life is manifested in diverse degrees? Against Plato's doctrine of the three souls, Thomas insists that man cannot have more than one soul because he must have one substantial form which is the one that makes him a human being. Then the soul, as vital principle, is such in all the levels of the spiritual potential activities, *anima ut spiritus*, and of the organic powers, *anima ut anima* [15]. "The rational soul is soul and spirit" [16]. And although it has multiple and diverse functions, it can be defined because of its superior activity, that is, as an intellective soul, and because of its inferior functions, which is only understood as virtually vegeto-sensitive. It may be sustained assuming that "whatever can do more, can also do the least" [17], the vegetative and sensitive functions are also attributed to the soul.

Its unity with the body is solved by affirming it as formal principle of the body, that is, not because of the union of two by means of a third party but by the immediate union of two co-principles of a unique reality: the whole being. "The principle by which we understand, whether

it be called intellect or intellective soul, is the form of the bod." [18].

The "plus" of actuality or perfection that the soul has respective of the body is the consequence of its not being totally immerse in the matter, the reason why it is distinguished of any other substantial form.

What follows will try to clarify all this.

How Do We Know the Soul?

Which is the most convenient attitude and method to know the soul and its relation with the body? Answer:

> Attitude with which such investigation must be assumed: against the defence of the *"post true"*, or of relativism at all cost- because, such attitudes despair from the knowledge of the real order- it becomes reasonable to assume an integral and realistic attitude, because, although imperfectly, *a parte homini,* a knowledge of the real order is admitted in all its dimensions, then, *a parte rei,* it is assumed that everything that is, as far as it is, is intelligible. [19]

Method of investigation: the assumption of a realist attitude supposes that nature be the thing which determines the method of its investigation [20]. Thus we have to overcome the concept that the scientific data be limited to the horizon offered by the empirical data to admit also the transempirical or metaphysical order. Because while the empirical data allows to answer the external question about how something is, it is the metaphysical data the one that will allow to answer the questions about the what, the why, and the what for something is [21]. Taking all this to the anthropological field, we can see that while the empirical data hardly gives account of the external manifestations of the human reality, and for this reason, it will not be able to reach the reality of being, that is, of an immaterial nature, as for the metaphysical data, it will be able to offer data about the essential constituents of man and the relationship that they have among themselves.

The metaphysical data is obtained by starting from a confused consideration of what is human so as to then analyze its constituents parts, starting with its more visible external manifestations, paying particular attention to the human acts, to sequentially reach the nearest and farthest intrinsic principles of operation, to finally ascend to the extrinsic or transcendent causes of the human whole. This is what doctors know when they ask about their patient's illnesses, starting by the knowledge of the symptoms:

> *When a doctor invokes to his senses, together with the sensitive intuition which captures the external manifestation of the illness there are intellectual intuitions at play by means of which such manifestations as symptoms of a given illness are related. By any chance could these things take place if some force [not sensitive but intellectual] perceived by the mind and under the action of the intelligence, were not present [intimately accompanying] the instruments of the senses?* [22]

The method of investigation of a mood order is characterized by being a posteriori, analytical, and ascendant. It is *a posteriori* or inductive because it starts from the immediacy of the data of the experience, whether it be external or internal; it is *analytic* or resolution based because it knows by explaining through its principal parts; and it is ascendant because it goes from the effects to the intrinsic – near and far – causes, to reach finally the extrinsic causal principles, that is, to its efficient and final causes [23]. This way establishes a sequence that goes from the act to the operational force and from the operational force to the soul.

Steps That Lead to the Knowledge of the Soul

Because of its spirituality, the soul cannot be known immediately, but – following the principle "the operation follows the being" – in an indirect way through its own operations: "The essence of things are unknown to us, we know their virtue through the acts" [24]. Then the consideration of its intelligible object expresses the nature of its cognitive act and through this the cognitive potential: "It is evident that, since it knows the intelligible, it understand its own act of understanding, and through this act gets to know the intellective potential" [25].

(a) The acts of man and the human acts

Man can perform two kinds of acts: the acts of the man and the human acts. The acts of the man, shared by other beings, are done in an unconscious and involuntary way, such as breathe or digest; while the human acts, proper to man, depend on a deliberate will, they lead to a goal and look for happiness [26], thus reflecting the richness of the inner soul. It is the case of activities such as studying, reading, talking. Then, not every act of the man can be called human, rather, "those which come from a deliberate will" [27]. Consequently, if the nature of anything is manifested by its operation [28], and such is the case of the intellective operation, then she will be the one who will better help know the nature of the soul and of the man.

(b) The powers or faculties as near principles of operation

Once the acts of man and the human acts have been examined, we inquire about their near principles of operation, which are the powers that make them.

The powers are possibilities or "near principles of operation" [29] which we know indirectly through their own operations: "Our understanding knows itself not because of its essence but because of its acts" [30] – whose function is to complete the perfection that man possesses in an innate way, because through them, he acquires and develops new physical and spiritual capabilities.

"The power as such, follows the act. Its nature must be determined by the act it follows and therefore, it is necessary that the diversity of nature in the powers be established according to the diversity of the acts, which, in turn, is established on the basis of the diversity of the objects" [31].

Such powers may or may not have an organic base, thus the distinction between the organic powers and the non-organic [32], that is to say, between the powers that are vegetative, sensitive, appetitive, locomotive, and the intellective and the will faculty. Some suppose an intrinsic dependency of the body and others only the extrinsic one. The spiritual activities are the work of the soul, because such operations are done without the intervention of a corporal organ, but the somatic activities are the result of the work of the soul and the body:

> Certain operations of the soul are done without the intervention of the corporal organ. For example, understanding and loving, then the powers that are the principles of these operations are in the soul. Instead, there are other operations of the soul that take place by means of the corporal organs. For example, to see through the eyes and to hear through the ears. The same can be said of all the other, the nutritional and the sensitive. Then the powers that are the principles of such operations are in the composite and in their own subject and not only in the soul. [33]

Then, while the sensitive knowledge knows the external aspects of something through the sensitive representation – *species sensibilis* – the intelligible knowledge reaches its essential aspects through the intellectual representation, *species intelligibilis*.

(c) The soul as subject and remote principle of operations

The soul is the principle of life, but properly, who operates is the man: "The action, as well as the being, is the composite, because operating is inherent of the one who exist" [34].

A real distinction between the soul and the powers must be established: "The essence of the soul is not its power, because nothing is in power concerning an act given that it is in the act" [35]. The distinction between the soul and its faculties lies in several reasons. First, the soul is a substantial form that is the principle of the being and of the essence; the powers are accidental forms, and they are principles of operation that complete the perfections of the soul, because they imply the capacity of doing and receiving. Second, the soul is one because the real individual supposes a unique principle of subsistence; and the faculties are many because they multiply according to their operations directed to different specific objects [36]. Third, the soul is always in an act, because it founds the thing in the order of the being and of the essence, while the powers are not always in the act: "If the very essence of the

soul were the immediate principle of its operation then anyone who has a soul would be always performing in act the vital actions." [37]. Fourth, the soul orders and coordinates all the powers or faculties, while the faculties perform their action only over one part of the body. Two examples could be cited. They are the active faculty of audition of the ears, and the faculty of sight of the eyes.

(d) The transcendent cause

The human reality explains itself by its intrinsic principles and also by the extrinsic ones. Because if what is contingent supposes imperfection, then what exists in an imperfect way, such as the case of man, cannot have the being and its perfections by itself but having received them in an imperfect or limited way from someone else who possesses them in a perfect or absolute way [38].

But the existence of man demands some distinction. Because if the corporal part of man can be explained through the existence of his parents, it does not happen the same thing with the part of the soul, given that its spiritual nature, which is simple, cannot be originated by engenderment but through creation, because only one cause that possesses the being by itself can give its being to someone else [39]. As the human soul is not deductible from matter, it does not arise from what is contributed by the parents. Thomas Aquinas argued here that arises from an act of creation. "The human soul is produced by God" [40]. Being this so, we can admit the ontological dependence of man concerning that transcendent last cause. And this is so, not only because of the contingency itself –"What have you got that you have not been given?" [41] – but also because God can freely and completely produce all his creatures, "The will of God is the cause for the things," [42] because He can do everything [43] and He is the final cause: "There is someone intelligent by means of whom all the things are directed to the end" [44]. And that end, the last one, coincides with the own principle of all the things: "He himself is the last end of all the things" [45]. It is a transcendence that is not detrimental of his presence in the innermost part of the human being: "You were inside me, more innermost than the most intimate part of myself" [46].

As from here, we can answer not only the question for the nature and existence of the soul but also the relation that the soul holds with the body. The soul, although it is not understood as a sensible reality, does not have to be considered as a non-existent or mythological reality. The soul also belongs to a real order that links the body as a substantial formal essential principle. This determines the body in the order of being and mode of being. In other words, the soul makes man be and what he is and, at the same time, animates him and founds all his spiritual and organic activities.

Definition of Soul

Aristotle defines the soul as "the first act of an organized natural body that has his life yet to be" [47], so that it is understood as "the beginning of life" of the body. This life is manifested in terms of "movement" or, more properly, of "growing," "generation," and even "sensitivity" and "understanding." The term "first act" invokes the foundation in the order of being and the way of being. "Among all the acts, the first one is the being" [48], "the first perfection is the form each thing has through which it has the being" [49], which implies that it may also be in the order of the three levels of its vital activity. The vegetative level, which comprises the reproductive and nutritional powers, is common to all the living beings; the sensitive level, which comprises sensitivity and movement, is common to all animals; the rational level that includes thought and reasoning belongs only to man. When saying "over an organized body that has his life yet to come," we indicate that the soul must not be understood as a separate relation from the body as would be something that is accidentally added to something else but as an essential, encouraging, perfective, optional part of the human whole, which corresponds itself with subordinate essential part constituted by the organic part of the human being. Then such

soul must be understood as the founding principle of the human whole – "formal principle of the body," "forma corporis, physici, organici" [50] – which performs a sustaining and guiding function concerning the body and shaping with it a distinct or substantial whole. With this in view, Saint Thomas defines the soul as the first vital principle and act of the body. "The soul, first vital principle, is not the body but the act of the body" [51].

The Nature of the Soul: The Soul as an Immaterial and Subsistent Reality

(a) The immateriality of the soul

If the nature of the soul is known by its operations, then, to know it, we must determine the nature of its proper activities, which distinguish it from other forms. But the proper operations of the human soul are its superior activities, regarding which we may ask: Are they organic or spiritual activities? It is difficult to determine through the natural sciences because its method has no access to the immaterial order; thus we can only expect a metaphysical explanation.

True as it may be, the materialists may think that mental activities can be understood as belonging to the organic sphere, because the unity between body and soul could suppose an intrinsic dependency in the order of human knowledge: "All the ideas come from the sensation or reflection" [52]. In this sense, it could be thought that the rational activity intrinsically depends of the neuronal activity. And what we can see in the cognitive field could also be understood in the same way in the willingness and affective field; also, it could be thought that the affective and willingness activities could only answer to instances of material subsistence, thus being of an essentially organic character.

But favoring the affirmation of the spirituality of the rational acts, important arguments appear. Concerning the intellectual activity: First, the soul is immaterial because it can know the nature of all the bodies, "because to know everything it is necessary that none of these things be contained in the nature proper, because if it were like that, it would prevent knowledge" [53]. Second, the soul performs some operations without the assistance of the body: "certain operations of the soul are executed without the intervention of the corporal organ. For example, understanding and loving" [54]. Because if man understood through the corporal organ, the nature of the organ would stop the knowledge of everything corporeal. Third, because only one principle that surpasses the ontological level of the matter could give place to an operation disconnected from the matter: "the nobler the form the more dominated the corporal matter is and the less immerse is in it... […]" "...of all the forms, the noblest is the human soul". "That is why its power surpasses the corporal matter which has a power and an operation where there is no participation of the corporal matter" [55]. Fourth, the intelligence, being different from the material beings, has the capacity of performing a complete reflection over its own act by virtue of which it knows the intelligible, its own act, and the power that executes it: "It is evident that if it knows the intelligible, it will understand his act of understanding, and through the act, know the intellective power" [56]. Fifth, the reflection over its own act is not possible in the order of the sensitive knowledge: "No sensitive power reflects over itself" [57]. Sixth, knowing supposes the possession of the thing known, and there seems to be no limit for the knowledge of all the things; this is possible by way of the immaterial: "In a certain way all the things can be done because through the sensitive everything is sensitive and by way of the intellect everything is intelligible" [58]. Seventh, man can know universal, necessary, and abstract realities, as it is in the order of the essence: "The intellectual soul can comprehend the universal, it has the capacity for what is infinite" [59].

About the volitional activity: First, although the volitional activities can pursue subordinate aims, like satisfying the needs for subsistence and organic well-being, they can also attend the needs of a superior order such as contemplative activity or personal self-sacrifice. Second, the

rational activities are far from being submitted to the determinism that suits the organic activities. They overcome them giving place to the free determination. The election, which is the proper act of freedom, supposes the participation of the intelligence and the will: "the wisdom of the reason over particular actions can follow several directions, without being determined to only one" [60].

We also have to affirm the immateriality of the object of the intellect. The object of the intellect is the quidditas or the nature of the material thing: "The proper object of the human intellect which is closely joined to the body is the quidditas or the nature that exists in the matter" [61]. The mental content or concept by means of which the nature or essence of the things is known requires two conditions, that it be representative of the thing known and that it be immaterial: "For an idea to be a means to knowledge two things are required: the it be the representation of the thing known, which is convenient for its proximity to the knowable; and the immaterial or spiritual being" [62].

But the content of the concept, before being in the intellect, is in the thing, in which, because it is immerse in the matter, cannot be the object of an immaterial faculty, because, if "what is received, is received according to the way of the recipient" [63], it is necessary to adapt the way of the nature of the singular things to the way of the immateriality of the intellective faculty. Then, because of this, it must absolutely know nature – *the intellective soul absolutely knows something in its nature* [64]. Hence the need to abstract the nature of things from the conditions of matter to make them intelligible in act: "The forms existing in matter are not intelligible in act, […] some virtue must be asserted over the part of the intellect that it could make them intelligible in act by means of the abstraction of the representations of the conditions of the matter" [65]. And in this way, the material things can be known by the human intellect: "Nothing forbids material things from being known by the forms that exist in an immaterial way in the mind" [66].

The soul in which such intellective faculty lies is also immaterial because a vital principle cannot be a body but an "act of the body" [67].

Since that it is a faculty that performs acts detached from the matter, the soul is understood as a foundation which is intrinsic and far from the spiritual activity, and then if there is a proportion between the doing and the being, "the doing follows the being" [68], then there is a spiritual correspondence between the doing and the being.

Because of this spirituality, it cannot be considered neither as made from matter because in this case it would be corporeal nor from a spiritual being, because the spirituality is not transferable; thus it must God's direct creation: "It is not done but by creation" [69].

And because of its relative immateriality, the human soul can entirely be in all the body and in each one of its parts: "In the same way as each act is in that of which it is act, it is necessary that the soul be in all the body and in all and any of its parts" [70].

(a) The subsistence of the soul

Once admitting the spirituality of the soul, its own subsistence must be supposed. If the doing of a being must be proportional to its nature, and what it does for itself, it must be by itself – "Nothing can operate by itself if it does not subsist by itself" [71] – then the soul, being capable of doing independently from the matter, must also have a being by itself: "The intellectual principle called 'mind' or 'intellect' has an operation by itself independent from the body and nothing does for itself if it is not subsisten" [72].

The subsistence of the soul can be affirmed, because, according to the principle "the doing follows the being" [73], the perfection of the doing of the soul is proportional to the perfection that is convenient to its own being:

> *The human soul […] has an operation that completely supersedes the matter and it is not done through a body organ, the understanding. And because the being of the thing is proportional to its operation, given that all doing as far as it is an entity, it is necessary that the being of the human soul surpasses the corporal matter and that it be not confined in it, although it might reach it somehow. Consequently, because it supersedes the being of the corporal matter and it is capable of*

> *subsist by itself and do, the human soul is a spiritual substance.* [74]

But the subsistence that suits the soul is not simpliciter, but *secundum quid*; this is not because it possesses a "complete nature" but because it is "almost subsistent," that is to say, "existing as part of the human nature." And if "the complete nature" is the "composite of the soul and the body", then the soul possesses the existence only to be part of the concrete human being:

> *Something concrete may be considered in two ways: whether it be something subsistent or, as long as it is subsistent with the complete nature of some species. The former excludes the inherence of the accident and the material form, the latter, excludes the imperfection of the part […]. Because the human soul is part of the human nature, it can be said that it is concrete in the first way, that is, it is almost existing, but not the second way, that is as the composite of soul and body.* [75]

So the soul is not entirely subsistent, because it does not exhaust but it is only part of the specific essence, that is, "that by which and in which the entity has its existence" [76].

(a) The soul as co-principle

Because it exists as part of the human essence, the soul must be understood as an active co-principle of the human nature in the same way in which the form is respective of the matter or the act respective of the capacity [77].

The distinction between matter and form originates in the necessity of explaining different attributes of each thing given different principles that underlie them, for example, the matter underlies the attributes of quantity, passivity, and multiplicity, while the form underlies the attributes of quality, activity, and unity. But, above all, assuming its creation, the form is distinguished as principle in the order of the being: "the substantial form makes it be in an absolute way" [78]. Because if its own, the matter lacks of all actuality, then "the matter acquires the being in act because it acquires the form" [79]. And over this hilemorfism base, each corporal entity could be known according to its near gender and specific difference. In turn, the determination of the matter by a determined matter – *materia signata quantitate* [80] – expression that redirects to a real not abstract matter, constitutes itself in the principle of individualism of the hilemorphic entity.

Under the distinction "matter-form," we understand the essential constitution of all the corporal reality so as to explain its substantial change. The form operates under the matter that receives it as a determinant principle. Then, as far as the act through which one thing is what it is and not another, the form determines in its species a given subject. Whereas the *prime material*, is that from which something is made, because it is a susceptible permanent element which receives successive determinant forms.

"In the substantial changes – Aristotle says – there must be a subject of generation and corruption, that is, a subject that receives the substantial form or is deprived of it" [81]. But for the matter to receive a determined form, it is necessary that it, by itself, lacks of all formal determination, that is, that it be pure capacity. And both, matter and form, are joined in such an endearing way like a rubber seal over soft wax [82].

The implementation of the hilemorfic doctrine to the order of the human reality.

The human "matter" is given by the capability that the matter has to adopt the form that upgrades it to give place to a human being.

The soul, as a substantial form, is a vital principle that manifests itself in the body it inhabits giving place to an immanent activity to that same being, because it is born from itself and remains in itself. But its encouraging function does not exhaust by its relation to the body, because it is complied in two levels, the animal and the spiritual. At the animal level, it is fulfilled, by relation to the body, in the vegetative and sensitive. At the spiritual level, the soul operates founded in a "plus" of actuality that supersedes the capacities of the corporal matter giving place to the rational activities: "So that the man can understand with his intellect all the things and for him to understand the immaterial and universal it is enough that the intellectual faculty is not act of the body" [83]. Then because of its essence, the soul, without ceasing to be one, is "forma sui corporis y spiritus" [84].

That is why man is said to possess a nature that, although being an animal, he also participates of the rationality or spirituality. Because if the vegetative and sensitive activities are performed with the dependency of the body, that does not prevent other specific activities that are performed without depending on an organ, in virtue of which a rational or spiritual nature can be attributed to the soul. In this way, the fact of conceiving an idea supposes a suitable proportion between the spiritual nature of such concept, the act of conceiving, the faculty that conceives, and the soul that possesses such faculty. From all this, it follows that it is not possible to understand the soul and the body as separate realities, because they behave as material and formal co-principles and we are speaking about a soul that defines itself as an encouraging or vital principle of the body and of a body that is understood as animated or alive. Then, we cannot understand the soul without referring it to the body that it encourages, "It is proper of the soul to be the form of some body" [85], nor can the body be conceived without supposing the soul that animates it. A body without a soul is reduced to lifeless matter; and a soul that is not referred to the body that it animates is meaningless, because its essential function is to enliven the body [86].

Unity with the Body

In which way can two distant realities such as the soul and the body be united? The Aquinate clears up the difficulties. The soul and the body are united "without blending," because there cannot be a mixture between two that "have nothing in common." But what is between them is a certain unity "by contact." A contact that can be established through action or passion:

> *The contact of the virtue proper of the intellectual substance touches the intimacy of the body. And thus the tangent substance is inside of that it touches and incorporates itself without any obstacle. In this way, the intellectual substance can join the body through a virtual contact.* [87]

And this contact happens in the way of the matter and the form. Because, in such a way as the substantial form immediately joins the corporal matter [88], so does the human soul immediately join the raw material [89]. And as the bright color of the skin depicts a healthy human body, the vivifying function of the soul extends over all the body [90]. And if the vital signs also belong to the subsistence or the real existence, then it can be thought that the same principle that enlivens it must be the one that grants its perfection of being subsistent [91].

But the unity in the order of the being is only possible if the soul acts as a substantial form of the body, which demands two conditions: first, that "the form be the principle of the substantial being of which it is its form" and, second, "that the matter and the form converge in only one being and in such being, the composed substance which is one as far as its being and consists of matter and form must survive" [92].

And that union in the order of the being supposes the simultaneity, *a parte ante*, of the soul and body, taking into account that the body does not precede the soul, because if the matter receives its being from the soul – "the [substantial] form gives the being" – then the matter cannot be or exist without the form [93], because its reason for being is the form [94]; neither does the form precede the body, because it was not produced but to animate a body: "The soul, being part of the human nature, does not have its natural perfection except when joined to the body. That is why it would not be consistent that it be created before the body" [95], but "when they are imbued in the bodies" [96], because it does not receive the being but in the body [97]. Thus, the soul is present in the body since the beginning of the human gestation.

This simultaneity is also exhibited *a parte post*, because once all the manifestation of life is extinguished, a body is no longer considered "body" but simply "remains" or "mortal remains." Because of this, the difference between a living body and a dead body has to be searched in a principle of life, recognizable by its vital effect. And as life is also the one that allows to warn its subsistence or real existence, it can be thought that the same principle that enlivens it must also be the one which, in its condition of "first act,"

confers its perfection of subsistent being. Thus, it can be confirmed that the soul, as the principle of life, is "the formal substantial principle" of the human whole [98].

Effects of the Union Between the Soul and the Body

In its condition of substantial formal principle, the soul is understood as the optional or active or decisive part of the body and the body as a subordinate or passive or determinable of such distinct whole. Consequently, the soul determines the body as regards the being, the union, the truth, perfections, operations, and relations.

First, the soul is the formal principle of the body in the order of being and the way of being, because it is proper of the form to give the being to each thing: "The first perfection is the form of each thing, by which it has the being" [99]. And it grants the human whole the subsistent being: "The substantial form is the one that gives a thing its substantial being" [100]. *Because* "The being corresponds substantially to the form which is act. Therefore the [corporal] matter acquires the being in act once it acquires the form" [101]. In this way, man receives the being in an integral way by the soul: "And all the body and all its parts have the specific and substantial being by the soul" [102]. Because although the essence receives the being – which is created – it does not keep it for itself but, after receiving it, ipso facto, grants it to the body to constitute with it only one subsistent entity: "The soul communicates the same being with which it exists with the corporal matter and from this and the intellective soul only one entity is formed, then the being that has all the mixture is also the being of the soul" [103].

And, at the same time, the soul is what determines a man to be a man, and it does so since the beginning of his conception to the last instant of his existence. Because if we cannot find an essential difference between his initial zygote state and a further state, then how can we justify that what in the beginning was not admitted as a human person could later be recognized as such? "After the conception, no moment of radical change can point out authorizing to say that there, and not before, human life starts?" [104], which is in accordance with the evidence that in an initial stage, the cells are already marked by a genetic code that is different from the parents. It follows then that the soul is present in the body since the beginning because the human germ pre-contains all the man.

But why is it necessary that the soul be the formal principle of the body in the order of being? This is due to the fact that when we speak about the being, we are not only speaking of the existence of an entity but also of an absolute perfection that is the base of all perfection. That is why we speak of the "act of being" as "the act of all the acts": "The being [ipsum esse] is the most perfect of all things, for it is compared to all things as that by which they are made actual; for nothing has actuality except so far as it exists. Hence being [ipsum esse] is that which actuates all things, even their forms" [105].

No creature, including man, can receive perfection in an absolute way because in that case it would be entirely perfect. And in spite that the man does not stop wanting it, nothing indicates that he actually achieves it. This reveals that the being is given to the creature in a limited or partaken way. And the constraining principle of the being is precisely the essence, but not because of its material principle – because as it is undetermined, it does not set limits – but because of the formal principle of the essence, because when determining the being to be this or that, it limits the being to the being of man. "Because the form created as subsistent has a being and it is not its being, it is necessary that its own being be received and acquire [or enclosed] to a determined nature" [106].

Second, it can also be said that the soul operates as formal principle on the order of the unity, "Anything is one by its essence" [107]. Its last foundation, though, is in the act of being, given that all that is, cannot have more than one *act of being*. The soul unites and organizes all the parts of the human whole. And this happens because in "any totality there must be a formal and predominant part in virtue of which the unity of the entita-

tive whole is founded" [108]. And this is what happens with the rational soul when it complies in it all the vegetative, sensitive, and rational functions: "The most perfect form makes in a unitary way all the things that the inferior makes in a diversified way: for example, if the form of the animated body gives the matter the being and the body, the form of the plant will give it that and also life; and the sensitive soul will give that and also the sensitive being; and the rational soul will give that and the rational being" [109]. That is why in the man, the substance form is the rational soul: "In this man there is no other substantial form than the rational soul by which this man is not only man but also an animal which is alive, has a body and substance and entity" [110].There is a function that the soul complies with in its highest part which is the intellective soul: "in this way the intellective soul contains in its virtue what the sensitive soul has from the brute and the vegetative soul from the plants" [111]. This assumption can be made because "what can the most also can the least." And the soul can also be considered principle of the same unity that it has concerning the body: "As the soul is the form and gives the being to the matter, it is the one that joins the two orders of the being and constitutes itself in horizon and boundary of the bodies and the spirits as it is an incorporeal substance and is the form of a body" [112].

Think, for example, in the extreme complexity of a human body. A body is constituted by millions of cells – near 37 billions – each one of them has an inner activity similar to that of an industrial city; the number of neurons can vary between eighty six thousand millions and hundred thousand millions, and they perform the most essential functions of human life, which demands a principle of unity to integrate them. This unity is lost when the soul stops its enlivening function on the human organism, because when the living person losses his life, ipso facto, his body – which implies composition of matter and form [113] and parts *extra partes* – initiates an irreversible process of division, decay, dissolution, disintegration, and corruption.

Third, the ownership of things has to do with the "intelligibility" or "aptitude that things have to be known" [114]. The truth as property of the intellectual knowledge supposes the actual possession of knowledge. And in the case of man, the soul constitutes itself in the foundation of the truth, and it is so twofold because the man knows and is known in virtue of the nobility of his substantial form, which is identified with the intellective soul.

Fourth, the soul also performs a formal function in the order of perfections. To say that the soul operates over the body in the order of being implies that the soul, as formal principle of the body, operates on it providing it all its perfections, in such a way that without soul, the body would not exist. This is explained because if the matter is defined in terms of pure capacity, then it does not possess any perfection because without the substantial form, the matter would not exist, so all the perfections that the body has, beginning by the perfection of being, are possessed because they are received from the soul which is the formal principle. The vegetative, sensitive, and rational perfections are sustained in it [115]. Therefore, if the soul enlivens matter in an integral and finished way, then there is nothing in the body that the soul that animates does not receive entirely, metaphysically speaking. Then all the ontological kindness of man, that is, his appetizingness and his level of perfection, have their intrinsic principle in the soul as a substantial form. It is so because the soul in respect of *form of the body* – "the soul is the act of the body" [116] – is a perfective act of man. And if the man, because of his corporal attitudes, seems to be the less endowed among all living creatures, because of his rational aptitudes, he is not because through his cognitive he can possess the forms of all the things.

Fifth, the soul, which constitutes itself in foundation of all the powers of the man, is also, in a remote way, foundation of all his operations, starting by the vegetative activity, then the sensitive, the emotional, the mnemonic, and the rational, as they are assumed by the principle of the superior activity. "It is necessary to affirm that the intellect, starting point of the intellectual operation, is the form of the human body. Because the first thing for which a being pro-

ceeds is the form of the being to which the action is attributed" [117].

Then, if the soul is admirable because of its perfective action on the body, it is much more so because of its superior activities which are of a rational order and are related to the order of knowing and loving. In this sense, think of the grandeur and nobility of all the attributes that suit the man as such, in the capacities that he naturally possesses and in the ones he can obtain through his personal development. And in this way, we can see in which way all the doing of man is naturally organized to look for the fullness of all his capacities: "all the beings have their reason to be in their operations" [118].

Sixth, the soul is also the formal principle in the order of the relationships. They are presented in a pluralistic and exuberant way because they fundamentally contribute to the development of the human person. Man is not reduced to being a relational entity, because the relation does not exist by itself but supposes the related extremes that make possible and found the relationship [119]. These relationships are given as follows: regarding the world, with their equals, by affiliation, by conjugal unity, by friendship, and by procreation, and even regarding the last responsible being of his own existence [120]. This last relationship is discovered when the man, knowing that he is temporary, at one point wonders about his origin and, above all, his final destiny. In this sense, some people think that the end of life has to coincide with its own beginning, with the Author of all life: "You made us Lord, for you, and our heart will be restless until it rests in you" [121]. In the order of the faith, as a last instance, it supposes the beatific vision, which, in a supernatural way, allows its union with God: "For a perfect beatitude it is required that the intellect reach the essence of the first cause. And thus its perfection is reached by the union with God as its object" [122].

Importance of the Body

The nobility and subsistence of the soul must not lead to thinking that the body does not comply with an important function even to benefit its own soul. And this is so, because the fact that the soul is subsistent does not make it a separate being, as would be an angelic being or even God. It is so because its condition of "form of the body" implies that the soul is not "purely immaterial" but "simply immaterial" [123], because "as it is reached by the matter which communicates its being, it is form of the body" [124].

The body complies with a notable function given its passivity, so in the same way the soul sets limits to the absolute perfection of the being. Because it determines to the act of being to be, in each case, this or that, according to its species, in a similar way, the body also sets limits to the perfective action of the soul over the matter, because this receives its perfections in a singular way according to the measure of its capacity: "[the defects of the body are] a necessary consequence of the matter that are needed so that the right proportion is given between the body and the soul and its operations" [125].

And in fact, the body does not properly suppose a threat against its own nature but rather a benefit, because its union with the body responds to its nature: "The soul, as part of the human nature, does not have its natural perfection as long as it is joined to the body" [126]. And this is also valid for its way of operating, because it knows appealing to the sensitive images: "It is joined to the body to exist and do in agreement to its nature" [127]. In such a way that the soul is more perfect when joined to the body than separated from it: "Being united to the body and understanding through images is better for the soul" [128].

For this reason, even though the specifically human is the intellective part, its nature supposes the composition of the soul and body. Hence, its definition is formed as from the corporal and rational parts: "The nature of the gender is taken from the material in the corporal thing, the reason of the species, of what is formal; the reason of the animal comes from the sensitive and the reason of the man from the intellect" [129]. Hence, it is the compound not the soul that has the dignity of person. That is why many of its functions are achieved with the help of the body. This is clearly manifested in the traction order. Because the trac-

tion activity, on the one hand, is ordered by an appetitive power that is manifested through some corporal mutation, for example, in the state of anger and of joy, and on the other hand, it is executed by the members of the body which are apt to obey. It follows that "moving is not an act of the sensitive soul done without the body" [130].

Does this mean a devaluation of the body in relation to the soul? On the contrary, because if the soul is act of the body, then the body, upgraded by it, becomes participant of its same dignity by having in it its own intrinsic end: "The forthcoming end of the human body is the rational soul and its operations, because the matter is ordered to the form and the instruments to the actions of the agent" [131]. And as regards the last aim, the body could reach, after death, a glorious destiny thanks to a divine intention: "In the last state, after the resurrection, the soul will communicate to the body that which is peculiar as spirit: the immortality to all; the impassivity, the glory and the virtue of the good, whose bodies will be called spirituals" [132].

Having said that and by way of conclusion, we see the firm and healthy equilibrium that the Aquinate finds between the soul and the body avoiding the excesses before and after it took place regarding its relation. He does so affirming the existence and nature of both components of the nature of man, the double condition of the soul as spirit, and form of the body.

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84. S. Thomae Aquinatis. S Th., I, 76, 3: cf. I, 76, 3, 3m; I, 76, 4; I, 76, 5; I, 77, 2; I, 79, 2 fi; I, 89, 1 fi.; I-II, 50, 6; C. G., II, 68 me°; C. G., III, 25, 3m; III, 61, 4m; III, 80, prin°; III, 81, prin°; Uer. q. 5, 8; Uer. q. 10, 6, De Malo, q. 1816, 10, 2m; cf. GARCÍA LÓPEZ, Metafísica tomista, op. cit. p. 256.
85. S. Thomae Aquinatis. S Th. I, 75, 5.
86. Aristotle, De anima, III, cap. I, Bk 412 b 7.
87. S. Thomae Aquinatis.CG, II, 56.
88. S. Thomae Aquinatis. S T., III, 76, 6.
89. S. Thomae Aquinatis. S Th., I, 76, 4, 3m.; –I, 76, 4, 2m; I, 76, 6, 2m; C G lb II, cap. 56, 57, 68, 69, 70.
90. S. Thomae Aquinatis. De spiritualibus creaturis, a. 4.
91. S. Thomae Aquinatis Th, I, 76, 1. Cf. –I, 76, 4, 2m; -I, 76, 6, 3m; -I, 76, 7; -I, 76, 8; -I, 90.4; −110, 2, 1m; -I, 117, 3, 3m; -I-II, 83, 2, 3; -III, 8, 2; C.G., II, 56, 57, 68, 69, 70; II, 73, 1m; II, 93, 2m; De Uer. q. 26, aa. 2, 3, 8.
92. S. Thomae Aquinatis. C G II, 68.
93. S. Thomae Aquinatis. De spiritualibus creaturis, I, 6m; 5, 1m; De ente et essentia, 5, prin° 3.
94. S. Thomae Aquinatis. S Th, I, 47, 1.
95. S. Thomae Aquinatis. S Th., I, 90, 4.
96. S. Thomae Aquinatis. S Th., I, 118.
97. S. Thomae Aquinatis. S Th. I, 90, 4. Cf. S. Th., I, 91, 4, 3m; -II-II, 164, 1, 4m; -III, q. 63; C.G., II, 83 -84–85, 1m; -IV, 33, fi; Uer. I, 19 1.
98. S. Thomae Aquinatis. S Th., I, 76, 1.Cf –I, 76, 4, 2m; -I, 76, 6, 3m; -I, 76, 7; -I, 76, 8; -I, 90.4; −110, 2, 1m; -I, 117, 3, 3m; -I-II, 83, 2, 3; -III, 8, 2; C.G., II, 56, 57, 68, 69, 70; II, 73, 1m; II, 93, 2m; De Uer. q. 26, aa. 2, 3, 8.
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100. S. Thomae Aquinatis. S Th., I, 76, 4.
101. S. Thomae Aquinatis. S Th., I, 75, 6.
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106. S. Thomae Aquinatis. S Th., I, 7, 2.
107. S. Thomae Aquinatis. S Th. I, 6, 2.
108. S. Thomae Aquinatis. S Th., II-II, 49, 6, 1m.
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123. S. Thomae Aquinatis. I-II, 3, 8.
124. Cf. García López J., Metafísica tomista, op. cit., p. 92.
125. S. Thomae Aquinatis. De spiritualibus criaturis, a. 2.
126. S. Thomae Aquinatis. S. Th., I, 90, 4.
127. S. Thomae Aquinatis. S. Th., I, 89, 1.
128. S. Thomae Aquinatis. S Th., I, 89, 1. Cf. –I, 89, 2, 1m; -I, 90, 4; -I, 118, 3; -I-II, 4, 5; De Pot. Q, 5, a. 10; −q, 5, a. 10, 5m.
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130. S. Thomae Aquinatis. S. Th., I, 75, 3, 3m.
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9 The Conception of Psychosomatic Medicine in Spain: From Neurology to the Person

Consuelo Martínez Priego

Psychosomatic pathology is not only a new way of practicing the medical profession with more depth and scope, but it also opens new horizons in the issue of man. ([1], p. 2)

Introduction

Justifying a Historical Perspective

The topic I am proposing, the role of neurology in the conception of psychosomatic medicine in Spain, requires immediate ***justification***. In the context of a study update, a historical framework may seem out of place. Nevertheless, finding out how psychosomatic medicine developed and under what scientific input is currently of interest for at least two reasons:

1. The conception of psychosomatic medicine, and more specifically of "psychosomatic pathology," shed light on the physician's occupation and provided insight into the ill (and healthy) person [2]. Currently, such enlightenment seems relevant and even urgent. Indeed, our inspirational reference occurred almost a century ago; if we, however, consider, for instance, that psychosomatic medicine enabled an approach to "each man," it might inspire the current technification of medicine and the quest for human enhancement, which tend to de facto question the "individuality and oneness" of each subject-mind, thereby replacing the "person" [3, 4].
2. In view that neurology played a significant role in the conception of psychosomatic medicine and the current development of this field (and of neuroscience in general) seems never-ending and continuously poses new questions, we believe the knowledge of man can be updated as it is under the scrutiny of medicine (and of other related fields such as psychology) [4, 5].

There is a clear underlying interest in psychosomatic medicine as a means to access the human being considered as neither dualistic nor monistic. Indeed, this field of medicine attempts to overcome the material monism and mentalistic monism of certain neuroscientific pursuits that have marked the history of neuroscience [5] and have become commonplace in many neuroscientific studies [4]. Also, the dualism that values the mind and the brain as different entities can be construed as the questions raised and the response attempts of psychosomatics [6]. On the other hand, psychosomatic medicine has had its moments of splendor and has been linked to medical anthropology (its academic recognition began with the publication of *Psychosomatic Medicine* in 1939). This is not to say that it has not had its critics who view it as irrelevant or

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merely temporary: an author thinks that when our biological knowledge increases, psychosomatic medicine will become superfluous or sheer mysticism [7].

Objective of Study

The purpose of this study is to clarify the role of neurology – together with other fields – in the expansion of medicine, in order that, by becoming psychosomatic, medicine will be capable of broaching how each man becomes ill as opposed to the mere deliberation of illness as such. In this way, science – and not just an ambiguous humanistic desire – will bring us closer to man, every man. This breakthrough was analyzed, described, and performed by the physician Juan Rof Carballo (a physician and humanist educated in the most conventional anatomic-physiological medicine, with a mechanistic approach, and is also the father of psychosomatic medicine in Spain) [8]. He has recently been acknowledged as being fundamental to the development of this medical perspective [9]. Together with the contributions made by another physician, Pedro Laín Entralgo, we will outline the conceptual history behind the expansion of medical horizons we view as relevant in the twenty-first century, including other fields such as psychology, social science, and the science of health.

Why Rof Carballo

Focusing on the contributions of Juan Rof Carballo to the scope of this study may be sufficiently justified if we mention a few biographical details: Rof Carballo was born in Santiago de Compostela in 1905 and acquired a multidisciplinary vision of scientific and cultural reality in his early education (Table 9.1).

We have pointed out that Rof read Freud in the 1920s. However, it was in the 1940s that he dis-

Table 9.1 Main teachers of Juan Rof Carballo

Period	People and type of influence	Source
Schooling	Juan Vicente Viqueira, philosopher and psychologist. Comes from The Free Institution of Education (philosophy of K. C. Friedrich Krause)	[10]
Early university studies (1922)	Meets Ortega y Gasset (philosopher) Reads Freud Universities at Madrid and Barcelona	[11]
University teachers	Mira y López (psychiatrist) Cuatrecasas Rumí (physician, psychobiology) Pittaluga (hematology and parasitology) Jiménez Díaz (medical pathology)	[12] [13] [14] [15]
The 1926–1927 academic year	Nóvoa Santos, familiar with Freud's work. Most distinguished personality at the time	[16]
Board scholarship for study expansion directed by Ramón y Cajal. Moves to Vienna and Cologne	Sternberg (attends autopsies) Jauregg (1927 Nobel Prize)	[11]
1936: Spanish Civil War	Ángel Garma: Prominent figure in institutionalizing psychoanalysis	[17]
1940–1945	Learns about *Psychosomatic Medicine*	[11]
1949	Writes *Psychosomatic Pathology* (first edition). First book in Spanish of this nature [18]	The 6 most quoted authors: Wolff, H. G. 70 Hochrein, M. 58 Kretschmer, E. 49 Friedman, M. 48 Weizsäcker, V. 43 Freud, S. 39

Prepared by author

covered the journal *Psychosomatic Medicine*, and according to Laín, "it shook the foundations of his technical and applied habits" ([19], p. 43). With this new medical insight, he began working with Gregorio Marañon at the General Hospital of Madrid, and a small department of psychosomatic medicine was established. Also, during these years, he came into close contact with the Spanish philosopher Xavier Zubiri who was a very influential figure together with his personal friend, Laín Entralgo. About the former, he wrote: "his way of understanding philosophy requires knowing biology to such an extent and depth not seen to date in any philosopher (…) his concern for biological issues is at times far deeper and outreaching than in most "professionals" and experts" ([20], pp. 219–220). As we will see later, it seems interesting that the scientific knowledge of a philosopher is emphasized in a way that philosophical influence in no way diminishes the value of scientific data, but rather enhances it.

Rof Carballo is a good example of major transformation: coming from a rigorous anatomical and pathological background, clinical in the most conventional sense of the word (he was an internist physician and endocrinologist), he embraced the psychosomatic perspective through his contact with scientists who deeply impacted the history of science in the twentieth century. Therefore, it seems appropriate to study the following issues from Rof Carballo's viewpoint: (1) Which was the framework, *humus*, or fertile soil that made this new perspective flourish? (2) What were neurology and other sciences like at the time and what did psychosomatic medicine contribute? (3) How did those contributions expand the analysis of an ill (or healthy) person? The final point of analysis will be to acknowledge and discuss how this historical event sheds light on our times and the questions raised today.

The "Humus" That Enabled Change

The scientific groundwork that surrounds the conception of psychosomatic medicine was developed in the early twentieth century. We could say there was an ideal *humus* or fertile soil in which a new way of clinically studying and approaching human issues was able to flourish. On the other hand, the authors who developed this groundwork shared, to an extent, three basic concerns or theories.

Background and Scientific and Philosophical Context

In terms of the *humus*, three major elements stand out. Firstly, psychoanalysis was already a well-established psychological and psychiatric train of thought (anthropological also) and provided important findings to the medical profession: considerations of the constitutive process of the subject and the progression of illness, in such a way that the individual's story must be taken into account; the importance of the person's word, his narrative, which becomes revealing and healing; the relationship between the physician and the patient which has specific characteristics, not comparable to almost any other situation; and so forth [21].

On the other hand, Max Scheler had already published the content of his philosophical anthropology, including the issue of man within the framework of a natural and cultural cosmos [22]. After having determined that man is not just one more living being, that he is not an animal among other animals (quantitative difference), human existence is emphasized as a fundamentally distinct object of study (qualitative difference); he is a person. The human spirit, free and capable of shaping its own biography, is not limited to organic life. Precisely because Scheler included culture in anthropology, the human issue is always viewed in social contexts, coexisting in fact.

And lastly, neuroendocrinology. Once the studies of Cajal and others have been consolidated [5, 23], and the visceral or limbic brain is discovered and enhanced [24, 25], consideration of the nervous system does not juxtapose the endocrine one (after all, hormones and other similar substances of the endocrine system appear at the synapse) [26]. These are pillars that enabled modifying the preceding medical framework.

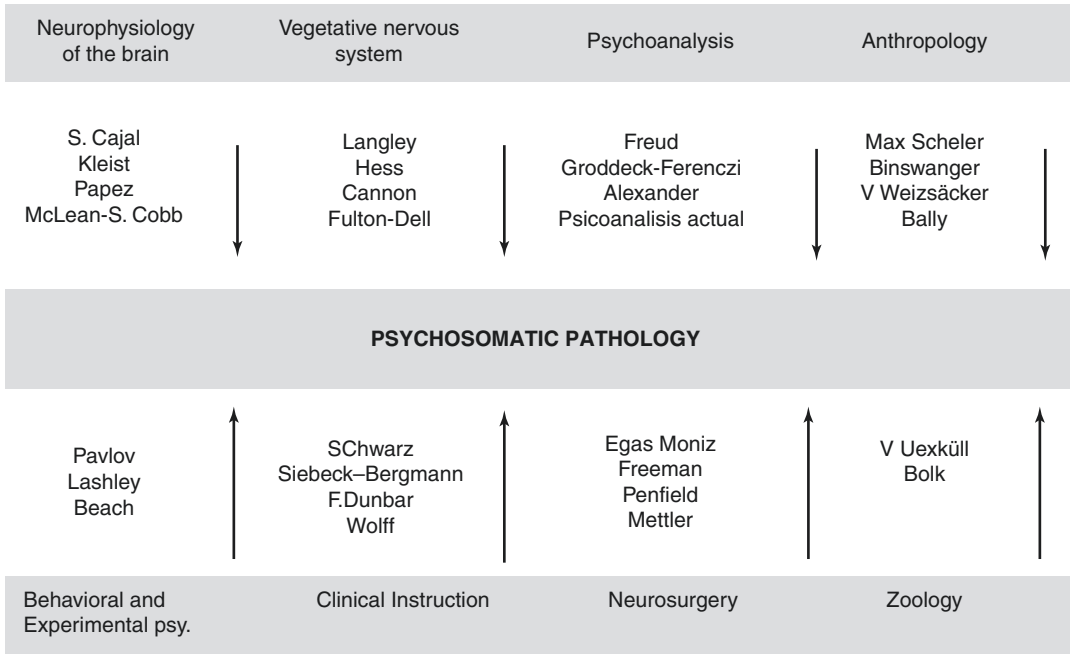

Fig. 9.1 According to Rof, the development of psychosomatic medicine is due to the concurrence of several fields of learning. (From Rof Carballo [27])

However, according to Rof, the development of psychosomatic medicine is due to the concurrence of several fields of learning (Fig. 9.1).

Along the same lines, the number of writers he met in 1949 was considerable [18], as well as the number of times he quoted them [28]. Specifically, he quoted 1837 different authors in 4838 references. Below is a list of renown writers (Table 9.2) listed according to the number of quotes (at least 12).

Common Criteria of the Psychosomatic Perspective

Together with this substrate, psychosomatic developments share certain concerns (derived to an extent from the above), a "*convergence of opinions*" both in Europe and within the United States, which also impacted the theoretical and practical development of medicine. There is the criticism of causality in the first place. Citing Galton, Rof wrote that it is a major mistake to "… view causality according to a temporal sequence […] We must eliminate from our minds the notion of a causal relationship between the psyche and the soma" ([29, p.404]). Thus, emotion becomes one of the "causes" for illness. It becomes necessary to resort to "transactional" concepts, i.e., of reciprocal influence, as opposed to the mechanistic perspective that reaches no further than the "interactional."

The second element with which writers converged was *disagreement with the term emotion* because it was too vague and disguised the true psychosomatic issue. In fact, "it is common to think that the psychosomatic perspective in medicine means that emotions can generate illnesses. But this misconception stems from not recognizing precisely what emotions really are" ([30], p.7). Indeed, understanding emotion should entail *clarifying the psychic-somatic duality*, by considering that to gain insight into emotions, it has been necessary to include new realities, recent findings about physiopathology, and specifically those related to the "internal brain," construed as a functional unit.

Table 9.2 Main writers listed according to the number of quotes

Wolff, H. G.	70
Hochrein, M.	58
Kretschmer, E.	49
Friedman, M.	48
Weizsäcker, V.	43
Freud, S.	39
Jung, C.G.	37
Bilz, R.	32
Dunbar, F.	32
Lopez Ibor, J.J.	32
Jimenez Diaz, C.	30
Alexander, F.	28
Grinker, R.R.	27
White, B.V.	27
Massermann, J.H.	26
Alvarez, W.C.	24
Brun, R.	23
Jahn, D.	23
Murphy, G.	23
Jones, M.	22
Cannon, W.N.	20
Cobb, S.	20
Müller, L.R.	20
Wittkower, E.	20
Bockus, H.L.	19
Portis, S.A.	19
Hurst, A.	17
Menninger, K.A.	17
Selye, H.	17
Deutsch, F.	16
Künkel, F.	16
Mittelmann, B.	16
Monakow, C.	16
Weis, S.	16
Hoff, H.	15
Lashley, K.S.	15
Fulton, J.F.	14
Heyer, G.R.	14
Watts, J.W.	14
Wolf, St.	14
Beach, F.A.	13
Bergmann, G. v.	13
Goldstein, K.	13
Hess, W.R.	13
Lange, J	13
Schindler, R.	13
Bleuler, M.	12
Gantt, W.H.	12
Lain, P.	12
Lewis, J.M.	12
Livingston, W.K.	12
Mayer, A.	12
Pavlov, I.P.	12
Richter, C.P.	12

The third point is the issue of coexistence as the various psychosomatic, philosophical, and psychiatric schools all agree on the need to consider interpersonal relationships to comprehend man: the issue of the "person" leads to "the issue of neighbors" ([29], p. 406). That is why "the aspiration is to see man as a whole, with a personal destiny, as a connection of psychosocial networks" [31]. This way, "the rich underlying life story re-emerges now as a social issue" ([11], p. 36). The contributions of Buber, Heidegger, and Marcel from a philosophical standpoint and Trüb from psychoanalysis all point in this direction. From a purely psychosomatic perspective, this same notion was emphasized by Halliday, Lindemann, and Engel and Sullivan in psychiatry, while in Spain, Laín is one of the most prominent advocates of this topic.

Based on these elements, medical practice acquires a new dimension. Let us not forget that "natural" medicine stems from the study of a human corpse during the Renaissance, turning the human being into an object, under the Cartesian and mechanistic influence [1, 5]. In the nineteenth century, the:

> «anatomical clinical» method "basically asserted that medicine would acquire scientific rigor when a true relationship was determined between clinical observation of the ill and anatomical injuries revealed by an autopsy after death. Clinical symptoms must be subject to anatomical injuries, which are the cornerstone of science and medical practice." ([23], p. 97)

Without undermining the importance of anatomic pathology, it becomes obvious that with this *modus operandi*, *a* substantial part of the pathological process remains hidden, unknown. The procedural correctness required of every physician, i.e., the need to acknowledge the patient and his/her entire morbid process, was limited to displaying "courtesy" with the ill person and the family. In the end, procedural correctness did not "scientifically" include the personal dimension. Indeed, the medical practice that disregards aspects of reality cannot be called "scientific," including those that a rigorous anamnesis puts forth, which must incorporate – if the physician has been adequately trained – disruptions of a biographical nature [32]. In sum,

"aware that illness is almost always a 'personal story,' a deep biographical connection, and that this biography is derived from human interrelations, psychosomatic medicine becomes *dialogical and socio-genetic*" ([33], p. 235).

However, what does it mean that medicine evolves into a dialogical practice, and what about socio-genetics? Mostly, the answer lies in understanding man himself as someone who is constitutive "with the other" and that this constitutive process, his "origin," is not merely a biological and autonomous event. Just like there is genetic heritage, we will find, if we carefully analyze human growth from its inception, a far more important and instrumental heritage than the former one, which could be labelled "socio-genetic heritage" [34]. The representativeness of "affective warp" is key to understand this process and also to turn medicine into something different to what has been established to date. The neuroendocrine system will play a major role.

Psychosomatic Medicine and "the Constitution" of Man: Affective Warp

The basic condition for the psychosomatic analysis of illness is founded on understanding the way of being and constitutive process of man. If the formation of man is the autonomous organic development of each individual, becoming ill, as many physicians point out, is per force an anatomical-functional imbalance. Now then, if the constitutive process is something more complex in which the person's biological conditions intervene, developed in terms of the person's immediate surroundings due to the epigenetic zones; if primal relationships are involved not only in the formation of the psyche but of the organism itself in view of plasticity and prematurity; and if the person's decisions impact and leave their mark on his/her development, the morbid processes and their healing will also become involved in the elements that the constitutive process incorporates. The psychosomatic study, in any case, is not distinctly psychological or corporal, particularly if we consider the role of the internal brain, of man's affective background. Science itself forces us to reconsider the constitution of the human being. That is why the question and corresponding answer given by Rof Carballo, making biology a necessary element to study man, are very significant:

> To what extent does the increasingly more perfect understanding of the ultimate structure of life: mitochondria, lysosomes, ergastoplasm or the chemical substances that intervene in the endocrine and cellular nerve mechanisms, increase our comprehension of man? ([33], p. 238)

A negative response would be as inadequate as ignoring the biographical dimension. However,

> The main characteristic of contemporary medicine is derived from pondering the issue of the "psyche" within the clinical practice, in other words, the conviction arising from experience that the physician does not fully satisfy his/her objective if man is not treated as an entire entity. This fundamental focal point of medicine has been called psychosomatic medicine. ([27], p. 7)

Indeed, psychosomatic understanding does not deny, but rather incorporates these contributions as comprehensive elements of human reality, which are an integral part of man's "constitutive process" ([35], p. 100). The most outstanding element of the human being in terms of its constitution – its being in the world – is the previously mentioned neonate's prematurity and defenselessness [36, 37]. From their perspective affective warp is readily comprehensible.

Some antecedents to this notion are symbiotic relationship [38], primal relationship [39], pre-object relations or "a particularly favorable environment" [40], primal love [41] and the lack thereof (basic fault) [42], attachment [43], epigenetic process [44], "imprinting" [45], etc. These are not *exactly overlapping* terms or synonyms. It is clear all of them lead toward common ground, toward a *primary fabric* that becomes whole within a *social weave*. At their presentations, Rof established relations with almost all of them, but he always pointed out that his "warp" concept was more convenient and better adjusted to human reality, due to its wider spectrum and because it emphasized the dialogical nature from the biological root to social development [28]. To the same extent that all these concepts are related to prematurity, defenselessness, and lack of "completion" of the child's somatopsychic configura-

tion on a neurological, endocrine, and enzymatic level [46], that "primary fabric" is the constituent element of the infant's personality. But what does Rof mean by "affective warp"?

The first thing affective warp intends to signify is the importance of the first, immediate learnings that leave a lasting impression on the child (and also on the mother or guardians as it is a transactional relationship). That impression is decisively neuroendocrine. The situation that ensues is as follows:

> «Imprinting» or minting, instant learnings, teaches us, in the first place, that many of the newborn's activities *seem innate* due to the quickness with which they take place but are only partially so as they follow a learning so unexpected and fleeting that it *may go unnoticed to the observer*. Secondly, substituting the convenient scheme: on the one hand, innate behavior or *instinct*, and on the other, *learning*, separated by an unsurmountable barrier, we witness something much more complex take place: *the purpose of innate or instinct behavior (…) is to enable the formation or initiation of learned behavior*. ([47], p. 50)

Therefore, the *warp* can be described as follows: the special nexus between transactionally linked invalidity and diatrophic[1] love, a harmonious growth condition for the child's personality; a fundamental structure of human existence, the one used for its constitution, shaping the ultimate biological structures via interaction with the child's parents, and through them, with the cultural norms and basic guidelines of the society to which the child belongs, in order to come to terms with it [47]. This reality is *indispensable* for the completion of the person precisely due to its nature as a *constitutive foundation*, programmatic, and implying a *substantial* anatomic-physiological, immunological, enzymatic, and biochemical *modification*. It has the tendency of being *transmitted* again to new generations, as well as the *summation* capacity of the hominization process, that is, a most unique ontogenetic abbreviation of a lengthy phylogenetic process in the midst of which man has been able to emerge. This way, its close link with heredity processes is highlighted. Ultimately, it is a *quasi-hereditary* process, a *socio-genetic inheritance*, *or a historically conditioned heredity*. This primary transactional relationship is of paramount importance in the establishment of the world of perception, as a basic programmatic activity [48]. Lastly, this relationship implies not only the mutual dependency between child and mother but also the tendency to be free of her, thereby establishing an "independent and autonomous life" ([21], p. 463).

This insight into the origin of man leads us to assert that a morbid process is of such *organic*, *transactional*, *biographic*, and, in the end, *constitutive complexity* that it requires us to scientifically approach the entire man. In keeping with Grinker's ideas, Rof states that the net distinction between individual heredity and learning patterns and their integration within the personal system is, on the one hand, difficult to determine due to the depth and quickness of what was learned or acquired from the surroundings, and on the other, the period of such integration will determine the development of a healthy, ill, or potentially ill organism [46]. But if we look for the primary concept derived from the fields of biological anthropology and ethology, it would have to be prematurity, the incomplete nature particularly of the neuroendocrine dimension, which renders the primary transactional interaction, i.e., the warp, as paramount and prominent. That is how neurological findings will lead to focus on aspects that until now were superficially or dualistically understood. Such is the case of emotion or the affective setting. The affective warp is, in fact, an enhancement of these bonds, and its center of development is the emotional brain itself, and with it, the structure of personality.

Neurology and the Psychosomatic Perspective: The Internal Brain

Psychosomatic Medicine and Psychosomatic Pathology

Contrary to the determinations of Descartes and assumed by traditional medicine in the way it analyzes the human body, life is not mechanical [49] but rather "systemic": everything has to do

[1] "δια" refers to two, duality; "τροφοσ" is the dimension of nourishing and caring for. Diatrophic particularly refers to maternal-paternal love.

with everything else, and elements or "parts" cannot be disregarded without affecting the whole. In this sense, the analytical method, which seeks clarity and distinction, simplicity, is not adequate to consider the human issue at all times [50]. That is the reasoning behind "the relationships of live reality are 'transactional'" ([46], p. 765). This does not mean, however, that the analytical way of thinking has not provided major achievements in medicine; it means, as we shall see, that it disguises other facts. In keeping with numerous studies of the time [2], Rof Carballo points out that medicine has always been psychosomatic, even if the physician's mentality has been natural-scientific. In fact, the first action of any treatment is to shake hands with the patient and treat him/her personally as a unique being according to Von Leiden.

> Whatever the scientific approaches, principles or theories that any prominent physician claims to believe, the truth is that objectively, as *a good clinician*, i.e., *abiding by and addressing the entire reality* in front of him, one way or another that physician will necessarily face the «personal» aspect of the human being, in other words, the patient as a person. ([47], p. 3)

The natural and personal condition of the human being is always asserted. Compared with the undeniable demand of treating the patient as "a whole man" – not merely a sick body – in pathology it has not always been this way: pathology has not always been psychosomatic.

> The expression «psychosomatic pathology» has two complementary meanings. One direct and superficial: the study of human illness attending to its two aspects of realization, the psychic and the somatic; another intermediate and plenary: the scientific consideration of man's morbid conditions according to the personal nature of the psychophysical reality. ([2], p. 6)

In theoretical interpretations that are most distant from psychosomatic pathology, either only the organic dimension has been considered (natural medicine or material monism), or every pathology has been reduced to imbalances of psychological mechanics pursuant to the psychoanalytical school of thought [27]. From the patient's perspective, Rof understands that many cannot accept that:

> these anatomical adjustments have a «soul», or that the soul is in the person's instinctive impulses, because then, the illusion of being entirely free, the unrestricted owner of an instrument: a body, vanishes. If the body breaks down, the person wants the reason to be a random event not concerning him/her, and the only obligation is to take it where it can be repaired or wait until it fixes itself. ([29], p. 423)

Ultimately, including the biography and life's disruptions as a relevant variable in the development and way of becoming ill involves the patient, the person, both from the etiological standpoint and in terms of the therapeutic process. The ill person is committed with his/her illness and healing processes. Undoubtedly, the Hippocratic convention of this medical perspective is reflected; however, in doing so it does not lose its medical scientific nature. On the contrary, as we have seen, it is a requirement of the scientific consideration of the patient himself. That is why a writer states, and Rof reclaims, "internist physicians do not believe in psychosomatic pathology, but one day, we neurophysiologists will hand it to them done and completed, and they won't even realize it" ([46], pp. 771–772). Thus, "the incapacity of clinicians to see psychosomatic reality is not derived from an excess of pathological anatomy, but from an insufficient understanding of the anatomy of the nervous system" ([51], p. 239).

New Neuroendocrine Perspectives: The Internal Brain and Emotion

The neurological endocrine knowledge, the discovery of the internal brain, the areas affected by the child's constitution highlight the affective warp and the anthropobiological insight of writers such as Zubiri regarding the unity of the living being, radically changing the way emotion is understood and its biological and biographical place.

The first issue is to overcome Cannon's theories according to which the vegetative system has its own homeostasis, in addition to other biological consistency levels. Conscious life also has its own sphere. Well then, unity in its dual sense

(temporal homeostatic and homeorhetic) is essential in the living being [26]. The quality of the living being of continuing to be itself throughout its biological-biographical disruptions is what we call the "vital tone" [52]. To "be itself" would be the psychological dimension of this same reality, the vital tone, and means "the comprehensive totality of the psyche" [18]. In biological evolution there is a progressive functional and cellular differentiation and, simultaneously, in virtue of the nervous system, a most unique unity similar to the one in unicellular beings. As we have said, this is so due to the nervous (and endocrine) system. Zubiri calls such unification the "formalization of the vital tone" (of the homeorhetic and homeostatic unit that could take place at various levels).

In this sense, the reptilian brain, the vegetative system, is an integration level of the vital tone. But it is not the only level; there is a higher one. At this superior level, more information is integrated at the internal brain. There, pursuant to well-established findings of neuroscience, the world of memories, the sphere of the senses, one's own self-image, etc. come together, including the revelation to the individual of his neighbor's world [27]. We must bear in mind that the stimulus to which the subject is more emotionally sensitive is the psychosocial stimulus (the others).

If we were to itemize the formations that are primarily responsible for the inflow of all this information, we would see that (1) they meet or coalesce in the internal brain, (2) numerous connections unite them, and (3) the thalamus, hypothalamus, hypophysis, fornix, hippocampus, the association areas of sensitivity and motor functions, the reticular formation, the frontal area of the cortex, the amygdala, etc. are not unidirectional [1]. At this special "organ of emotion," due to the type of connections established for such unification (e.g., the cortico-hypothalamic connections), information flows from bottom to top and from top to bottom. Emotion can coalesce the cardiac rhythm and conscious judgment.

When superior integration, the emotional one, is possible, the dynamics of integration prioritization enable more flexible stimuli responses, adapted to the subtleties of reality, whereas when this level is missing due to pathology or an immature brain, as in the case of children, responses become more rigid, stereotyped, and inflexible.

Therefore, the importance of emotion as a psychosomatic reality, with its own organ that integrates cognitive and conative processes in a very close relationship with the condition of basic biological systems, has been summarily described, and all of it takes place following associations or connections that cannot be labelled "causal-lineal." Ultimately, emotion from a biological standpoint is not "an 'ornament' of life; rather, it is in its most intimate substrate […] It is not ludicrous to think that emotion is found not only at the noble pinnacle of the tree of life, but also in its humble roots, as something essential" ([53], p. 51).

From Illness to the Ill Person: Our Need

If the affective warp possesses the depth and altitude described, if emotion is so fundamentally human and psychosomatic, and if man is so biographical (temporal) and dialogical (related transactionally to others), illness belongs to "each one" in the most profound sense. That is why Rof Carballo applies Siebeck's unerring words:

> The direction an illness adopts and what it implies for the destiny of a man, depends not on the "illness" itself but, fundamentally, on the man, on his attitude toward life and his situation within it. The ill person not only *has the illness*, but the man himself and his destiny *make up the illness*. Morbid history is always biographical. ([47], p. 19)

Rof finds four levels in the biographical and personal nature of the illness process that should be part of the medical history (anamnesis) [47]. Indeed, illness is just an episode in life, but it has internal and external roots.

1. The first stage: the history, the totality of personal life: an allergy or a trauma, all of it is prehistory. Man is more than "bio."
2. Man is also a compilation of obligations, renunciations, anxieties, guilt, and regrets.

This level has an anthropological ranking: it is not an individual's history, but *my history*, the person's: the totality of a life project.

3. Because an ill person is not someone who lives isolated, the subjective historicity mentioned previously must be expanded: the person's world is a world of coexistence, something constituent that is neither transient nor minor. It is also a source of corporal illnesses.
4. The last stage of the ill person's personal historicity is found in the spiritual being: the person can progress and become more perfect. The ill person already in a timeless situation is faced with the last questions of existence, including the person's personal sense and attitude toward death. Evidently, what is made patent here is the ability and certainty of the person's "reconnective" nature (connected to transcendence).

Medicine's recent history has set forth the importance of certain structures of the nervous system called the "internal brain," human prematurity and its malleability, the truth behind the affective warp as designer of the human being's psychosomatic structures, and with it the place emotion holds in human life and in the illness process itself. All this clearly shows the insufficiency of traditional or purely organ-oriented medicine. It is also clear that the entire morbid process "belongs to one person" and it is "dialogical and socio-genetic," highly individualistic, and never solitary in terms of isolation from the other, as isolation can also be a cause for illness. The analytical treatment of an "analytically" considered illness is certainly possible. However, scientifically speaking, we would be disregarding (as mentioned above) a whole array of founded assertions related to the morbid process.

In keeping with Rof's proposal in terms of the anamnesis, Laín Entralgo [54] points out that, in connection with the ill person's life, the following must be taken into account if we consider the ill person and not only the illness: (1) the patient's "non-truths" that the illness hides/shows, (2) the somatopsychic alteration it entails, and lastly (3) the spatial-temporal structure of the process: the where, when, what, why, and what for. "Why and what for, precisely here, precisely now and precisely this way?" ([54], p. 172). The patient is someone who "suffers" need and seeks help whether he/she is fully aware of it or not. Thus, every alteration holds its own entity (nosology), a cause (etiology), and a meaning (semiotics) ([2], p. 12). Ignoring this complexity in the twenty-first century is, we might say, renouncing the use of scientific facts, not just lacking a "humanitarian attitude."

The customization of products – either goods or services – is the trend in the twenty-first century: to improve or make the life of people easier through state-of-the-art advances. Two situations immediately stand out as paradoxical after we have seen the meaning behind the fact that it is the person who becomes ill or remains healthy (not the corporal organic machine).

1. The customization of the digital world, enabling it to enter our private world, and as we use it, it offers us what best suits our tastes and interests.
2. The quest for human enhancement to the point of overcoming every limitation, including illness and death.

In terms of the first situation, it would be worth analyzing whether satisfaction of a person's needs regardless of how personalized it becomes also results in the well-being and happiness of that person. Is maternal affection truly replaceable by "technological affection?" What does neuroscience have to say about emotions associated with personal bonds? [55] Are these superior or inferior in terms of their contribution to human happiness as compared to emotions derived from "customized" pleasure for instance? Likewise, we could question whether the technically mediated physician-patient relationship truly replaces the benefits of one person (the physician) knowing another person (the ill person) [56]. To what extent does entering the symptoms in a computer program and establishing a diagnosis replace the decisively biographical nature of the anamnesis we mentioned above? We understand the questions raised are proportional to the environment we are in.

The second situation could seem to some people as something closer to fiction than to reality.

But there are a large number of physicians focused on the study of human enhancement. It so happens that the limitations of a human being are derived almost completely (as far as science can attain) from the human being's organic, corporal condition. Therefore, science together with technology would be able to overcome those limitations. In other words, if it encounters no organic limitations (*res extensa*), the unlimited and perfect mind (*res cogitans*) is seeking a "space-time" in which to place itself that does not limit its possibilities and is not reachable by illness and, ultimately, by death.

Psychosomatic medicine, knowledgeable of the specific man, understands that disincarnation of the mind means losing the true man. As we pointed out [6], the key is not so much in the distinction of mind and brain, but rather in the relationship, interrelation, or duality of the *psyche* and *soma*. It therefore follows that the mind, the *psyche* rather, is not a constituted entity but one that always comes into being through an affective relationship with others. Perhaps overcoming the limitations imposed by the soma (man is "subject to illness") would be the annihilation of the human essence (to some, exceeding the human essence). But it is also possible that such a situation would generate forever unhappy beings, existing with a basic fault [42] or without primal love [41], that which allows them to know and feel goodness within themselves [57].

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10 From Phenomenological Psychopathology to Phenomenological Psychiatry: The Cases of Schizophrenia and Substance Misuse

Guilherme Messas and Melissa Tamelini

Introduction

Psychopathology is a foundational science of psychiatry, born with the task of assessing the patient's abnormal experiences from their own perspective and transposing such forms of consciousness into general and intelligible categories, to be shared as a body of knowledge [1]. This body of knowledge will be the basis of the different moments of the psychiatric act, and it is possible to point out a relationship of determination and mutuality in the implications between the psychopathological discipline and the psychiatric practice. Psychiatry is the raison d'être of psychopathology as a science, once it cannot ignore at its birth a conceptual understanding of the forms of consciousness that will be characterized as mental pathology [2].

There is a plurality of methodological proposals in the field of psychopathology and in the history of psychiatry. Different views of psychiatric illness have oscillated regarding their acceptance in culture [3]. Phenomenological psychopathology is one of them and its project of psychopathology began in the 1920s of the last century, based on Husserl's philosophical elaborations, which started to be drawn a few decades earlier and had a broad repercussion on the cultural context of the time. Eventually Husserlian methodology was incorporated into several disciplines, including psychopathology. In this specific field, it had a significant impact, as its epistemological foundations were a close match to the rigorous purposes of a scientific investigation of mental disorders [4].

The fundamental epistemological assumption behind phenomenological psychopathology is that mental disorders are a transformation of all existence – and not just of isolated psychic functions – or a disturbance of the experienced world rooted in transformations of the aprioristic structures of consciousness. In other words, phenomenology provides the elucidation of psychopathological conditions as modifications of the main dimensions of the life-world, such as temporality, spatiality, corporeality, and intersubjectivity. These structures are the underlying constituents of experience – its conditions of possibility – inherently vulnerable to pathological modifications [5]. Their examination marks a stage of greater maturity of psychopathology as an autonomous science [6].

Over almost a century, the phenomenological contribution in psychopathology has elaborated a unique conception of the psychiatric object [7]. Among the authors of the phenomenological tradition are names such as Minkowski, Binswanger,

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Von Gebsattel, Blankenburg, and Tellenbach, whose works have contributed to the construction of a sophisticated form of empirical science that aims to provide the basis for pragmatic objectives without failing to consider intimate links with anthropological reflections [8]. In a first moment, this pragmatic role of phenomenological psychopathology was more restricted to the diagnostic sphere, but, more recently, we can see the expansion of the frontiers of phenomenological research agenda in psychiatry towards the development of therapeutic strategies that target the whole of existence in their actions.

The transposition of scientific findings of phenomenological psychopathology to psychiatric therapeutic is more precisely a new feature in contemporary literature. Two complementary historical contingencies, one external and the other internal to the phenomenological field, contribute to the expansion of the epistemological scope already rooted in the psychiatric tradition, but still largely unexplored. On the one hand, psychiatry is compelled to reflect on its own foundations, given the explicit conceptual weaknesses of the neopositive model in driving clinical practice. At a time like this, not exactly a novelty in the psychiatric field, marked by the instability of the dominant paradigm, there is always a window of opportunity for dissonant views. In the midst of this scenario, phenomenological program returned to focus, in a "unique position" [9], capable of fostering the emergence of an alternative epistemological project that allow psychiatric praxis to be based on more solid foundations [10]. On the other hand, internally to the phenomenological field, since their scientific findings make up an advanced form of psychopathology, there is no reason to dissociate it from the conception of therapeutic strategies. The elucidation of the essential modifications underlying mental disorders traditionally linked to diagnostic purposes [4] can contribute with the same competence in conceptual and therapeutic debate. That is, the transposition of scientific findings of phenomenological psychopathology to psychiatric therapeutic field is a feasible and opportune attempt "not only because of the historical legacy of phenomenology but above all its living potential to deal with the dilemmas and challenges still in force in psychiatry today" [11].

Even though any psychopathological enterprise establishes its project of treatment intrinsically, only recently it can be observed less hesitancy to proceed phenomenological oriented studies focused on therapeutics, especially on psychopharmacology. There are recent articles proposing hypotheses in line with the fundamental understanding that "core insights into the disorder result as well as core treatment implications" [12]. In order to illustrate the viability of a psychiatric praxis of phenomenological orientation, from the diagnostic act to the design of therapeutic guidelines, and highlight its relevance to clinical psychiatry today, we will present the case of schizophrenia and substance misuse. The choice of these two conditions does not obey any specific logic; it only refers to fields in which such an attempt was preliminarily explored.

Schizophrenia

Psychopathology becomes, in fact, phenomenological when taking advantage of methodological alternatives that allow the investigation of the aprioristic structures of consciousness (Minkowski, 2000) [13]. These fundamental constituents of experience are inherently vulnerable to changes and the different psychopathological entities derived from prototypical kinds of disarticulation of such structures [5]. It is within the aforementioned aprioristic structures, like temporality, spatiality, corporeality, and intersubjectivity, that, in fact, the schizophrenic fundamental modification will be established, providing coherence to the multiple phenomena typifying this psychopathological entity [14].

Although there is no essential notion capable of satisfactorily summarizing the entire phenomenological understanding of the schizophrenic condition in the literature, there is a conceptual affinity between the classical views (of authors such as Minkowski, Kimura, and Blankenburg) and contemporary views that allow us to have a

"reasonably coherent vision of schizophrenic disorders" [15]. The convergence of these psychopathological investigations was synthesized by Sass and Parnas [16], in the ipseity-disturbance model. According to it, schizophrenia is characterized by instability and transformations in the most primordial and irreducible dimension of selfhood (ipseity), and these transformations allow an integrative understanding of key phenomena in the schizophrenic experience, such as hyper-reflexivity, diminished self-affection, and lack of common sense [17, 18].

Regarding schizophrenia as a particular disturbance of aprioristic structures of consciousness, the clinical targets for antipsychotics can be considered in terms of their impact and modification also in this fundamental dimension of consciousness. Empirically, we know that schizophrenia notoriously has a lifetime course, even when appropriate therapeutic measures are in place, which indicates that these drugs are limited in terms of the integral restitution of the modifications of the aprioristic structures of consciousness. Moreover, antipsychotics are often effective in reducing manifestations like delusions in schizophrenics. In phenomenological terms, delusion is seen as a response to the ontological need to stabilize the field of experience at the imminence of its full-blown disintegration or an immanent possibility of providing "a new foundation for the aprioristic sense of continuity, familiarity and stability of the field of experience" [10]. Therefore, it is reasonable to assume an equivalence between the phenomenological compensation given by the emergence of delusion and the action of antipsychotic drugs, even by different paths.

We can hypothesize that this therapeutic action can take place in the field of corporeality, more particularly in the lived body. In schizophrenia, there is a "disembodiment of the self in the sense of losing one's implicit body functioning, and with it the prereflective, questionless being-in-the-world that is mediated by the body" [19]. Among these radical disturbances in the consciousness, corporeality is an immediate counterpoint to the disintegration tendencies inherent to schizophrenia. The prominence of corporeality induced by pharmacotherapy can forge a new stability to ipseity and thus guarantee a primary point of articulation in the experiential field, since being a body is to be already necessarily connected to a world [20].

The possible benefits of replacing an intrinsic restorative element (delusion) with a supplementary element (pharmacological action) include the possibility of an earlier and more controlled process of restructuring consciousness in a field of instability. In theory, antipsychotics could minimize deleterious repercussions, well demonstrated by the existential price arising from the typically ubiquitous invasion of delusion into the whole of experience, and also mitigate other developments typical to the prolongation of unstable existential conditions, such as dissolution into fragmented forms (severe hebephrenia) or suicide. Thus, pharmacological treatment would represent an anthropological advantage over the schizophrenic natural forms of recomposition. Its major purpose would be the anthropological restitution of consciousness by means of a path, at first, less pervasive and less paralyzing.

Substance Misuse

Substance misuse is a highly complex condition, which comprehends a wide range of behaviors, from acute misuse to severe substance dependence syndromes. Though this is a still mostly unexplored field of research in phenomenological psychopathology, some recent contributions have been offering a frame for a clinical care based on phenomenological psychopathology [21]. As we will develop in the following, a comprehensive care for substance misuse relies on the recognition of the main anthropological modifications, crucial for all the deleterious effects of substance misuse. In order to better understand this contribution, we shall first shortly present two core characteristics of substance misuse and, having this in mind, summarize some consequences of this finding for clinical care.

Anthropological Hyperpresentification

Anthropological hyperpresentification means an excessive participation of the present dimension of temporality in human consciousness, whose consequence is the reduction of the participation of the past and the future in consciousness. This restriction of existence to the present makes consciousness unstable, which may produce many psychopathological experiences, as, for example, mood oscillations, or even contribute to hinder the existential ability for projection into an authentic future.

As a consequence, we propose that the aim of a substance misuse care is to help patients to avoid getting dominated by the present dimension of the temporality. The psychiatrist/clinical psychologist should strive to prevent the patient from being held hostage by a cycle of continuous present.

Excessive participation in the present can be tackled, for instance, by augmenting engagement with the future, by a psychotherapeutic process. This process must follow the psychopathological characteristics of the temporality of the patient. It must draw on the patient's hyperpresentification to establish some meaningful contact point with him or her. The clinician must act on immediate temporality, taking an intense and direct contact with the patient.

Treatment of substance misuse is therefore a process that gradually establishes a broader temporality, enabling the emergence of a kind of biographical "becoming."

Anthropological Plenitude

Anthropological plenitude depicts the experience of substance misusers of seeking substances to produce an experience of wholeness. Substance misuse consists of a surrender to the absolute, a merging with a one-dimensional experience of reality, that distorts the characteristic profile of human existence. This dominance of the absolute may be manifested in different ways in substance misuse, like the complete indifference regarding the world, typical of heavy cannabis users. From an existential point of view, one could say that cannabis use promotes a state of satiated plenitude in the consciousness, from which not only conflicts are expelled but also all personal desires and anxieties.

This state of satiated plenitude prevents the necessary existential confrontation with its life contradictions. Rather than a life in which decisions and renouncements constantly present themselves, the world appears to be full and doubt-free. Consequently, the aim of the clinical care is to reinstate in the patient the capacity to experience the different perspectives that exist in each experience. Treatment consists of reinstating the ability to doubt, to inhabit an essentially complex world, where a subject's experiences are, by definition, intersubjective and multifarious.

Conclusion

The immediate challenge of phenomenology in the current scenario of psychiatry is to intellectually help overcome the narrow biological vision of the patient and to promote the resumption of the humanist tradition historically established in the field. Since its incorporation into psychopathology in the 1920s, phenomenology has been the vigorous antithesis of reductive tendencies in the field, and the move from phenomenological psychopathology to phenomenological psychiatry can be a resumption of psychiatry on more complex bases. Once phenomenology observes the human being as a whole existence, it presents all the conditions to assume a central role in the contemporary psychiatric scenario.

What seems to be at stake in the present conjuncture is not only the resumption of the emphasis on pure psychopathological research, but the broad integration of phenomenology with clinical praxis as a whole. Especially attuned with current aspirations for change in psychiatry field, we present two brief examples of how phenomenology can contribute to the productive development of therapeutic strategies and, thus, make modern psychiatry effective as a practice that

allies strong humanist appeal to the technological apparatus in the care of mental pathologies.

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11 The Notion of Person in Neuroscience: From Cognitivism to a Comprehensive Phenomenology

Javier Bernacer and Francisco Güell

Introduction

Neuroscience is committed to change our lives – hopefully for the better. The main goal of neuroscientists is to unveil the extreme complexity of the brain in order to understand the material correlates of the mind: this would be the final step to cure – and not only alleviate – devastating mental disorders such as schizophrenia, autism, addiction, or depression, for instance. In recent years, this thirst for knowledge is also intended to *enhance* human beings, that is, to push evolution forward and improve our abilities (i.e., memory, intelligence), reaching a *post-human* species. Further, extravagant non-academic voices assure that imminent advances in neuroscience will end up with *mind uploading*, that is, transferring memories and consciousness to an artificial device, so "we" could live forever (see [1] for a critical view on this issue).

The core of our text is the *we* between quotes in the previous sentence. The most common term to refer to human beings from a holistic point of view is "person," which is a synonym of "human being" according to most dictionaries: Cambridge Dictionary, "a man, woman or child"; Merriam-Webster, "human, individual"; Lexico, "a human being regarded as an individual"; and Spanish Royal Academy of the Language, "individual belonging to the human species". The Collins dictionary includes an interesting third sense: "if you talk about someone as a person, you are considering them from the point of view of their real nature." It might be clear for everyone that any man, woman, or child is a person. However, what about someone whose abilities have been enhanced with a microchip? Or a mentally ill patient who cannot distinguish good from evil? Or an individual in an irreversible coma? Using the fictional scenario mentioned in the previous paragraph, we could also wonder whether an artificial device that contains someone's consciousness is a person. The concept of person is easily grasped by lay people. However, it becomes more obscure when it is object of research. The attribution of personhood to an individual is not trivial at all, since it confers a set of rights and duties that are fundamental: for instance, the word "persona" is used 32 times in the 30 articles of the Universal Declaration of Human Rights of the United Nations in its version in Spanish.

How can neuroscience contribute to the concept of person? As we will briefly explain in the next section, neuroscience aims to study the nervous system, at both a morphological and a functional level. Thus, its field is quite reduced. In fact, searching the terms "person neuroscience," "neuroscience of the person," and "neuroscience and the person" in PubMed does not yield any entry. Personhood is a rare topic of interest in

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neuroscience. However, the recent field of neuroethics is entering into the debate of which human beings should be granted the attribute of person and which should not. This discipline intends to merge the findings of neuroscientific research with certain philosophical interpretations to reach a definition of person that can be useful for neuroscience itself. Therefore, we will explain the techniques that are at neuroscience's disposal to explore the possible neural substrate of personhood. With respect to the philosophical interpretations of the concept of person, we will summarize how this notion has been understood by the main currents of thought. This is necessary to put into context the view held by neuroethics. Next, we will show how neuroethics may draw dramatic conclusions from poorly interpreted neuroscientific results and a restricted philosophical interpretation of personhood. It should be considered that decisions arising from neuroethics committees are very influential, since they advise governments and international boards to regulate medical and scientific practices. Finally, we will present a different interplay between philosophy and neuroscience to reach a comprehensive notion of person, promoting interdisciplinary research from a phenomenological perspective.

The concept of person in neuroscience is an extremely important topic. Even though most neuroscientists may believe that the attribution of personhood to human beings is irrelevant for their research, they must keep in mind that psychiatrists, neurologists, and psychologists, for instance, are *taking care of persons*. For that reason, a careful interdisciplinary research on the concept of person should be considered as an important piece of translational research.

Current Neuroscience Techniques to Explore the Human Brain

On July 18, 1990, President George Bush formally proclaimed the "Decade of the Brain," a 10-year period aimed to boost research on the nervous system, neurological, and mental disorders. Currently, optimism and interest on the neurosciences are experiencing a new breakthrough after the announcement of Obama's BRAIN Initiative (https://www.braininitiative.nih.gov/) and the European Commission's Human Brain Project (https://www.humanbrainproject.eu/en/). The ultimate goal of these gigantic projects is to give economic support to emerging and promising techniques to study the anatomy and physiology of the brain, in both humans and non-human animals. This would allow us to *start* understanding how this complex organ works as a whole and its relationship with cognition. Before explaining the available techniques to explore the human brain, we would like to clarify what neuroscience is.

Neuroscience, also used in its plural form, is the study of the nervous system of human and non-human animals. Even though some disciplines within this field of knowledge may seem purely descriptive, such as neuroanatomy, they all have a functional focus and are oriented to a better understanding of neural activity. In other words, neuroscience studies the nervous system as the material substrate of the cognitive, motor, and motivational aspects of behavior. Nowadays, medical doctors, psychologists, biologists, biochemists, geneticists, engineers, and computer experts, among many others, combine efforts to push neuroscience forward. With respect to the nervous system, it is organized in a central – the brain and spinal cord – and a peripheral-nerves and ganglia distributed throughout the body-component in humans, as well as in many other species. Neurons are the individual cells that capture most of neuroscience's attention, although they are outnumbered and crucially supported by glial cells, in both the peripheral and central divisions of the nervous system. In turn, there is an increasing interest in glia, since several studies have highlighted their importance in neuronal communication (see, e.g., [2]). In any case, due to the relevance for the arguments presented below, we will briefly explain why neurons are so special.

Neuronal membrane has a negative electrical charge because their inner milieu has a different composition than the surrounding extracellular liquid. This is maintained at a high energetic cost through transmembrane channels and ionic

pumps. In fact, membrane potential (i.e., electrical charge) may change after the opening and closing of these channels. These phenomena are not unique to neurons. The distinctive feature of neural cells, what makes them different to others, is the ability to communicate this membrane potential change between each other through a chemical signal. This process is called chemical synapse. A prototypical neuron has a soma that contains the nucleus and main organelles, a dendritic tree where the electrical change is received from another neuron or an external stimulus, and an axon or thin "biological cable" through which the electrical impulse is transferred to another neuron or a muscle fiber. When this impulse reaches the final part of the axon, certain calcium channels open and result in the release of a chemical substance: the neurotransmitter. This is specifically received by some receptors in the dendrites of nearby neurons, which entails the opening of ionic channels and the membrane potential change in the postsynaptic neuron. Hence, the electrical signal has been converted into chemical by the presynaptic neuron, and the chemical signal has been converted again into electrical by the postsynaptic cell. This electrical current traveling through and between neurons is the neural impulse or "neural information." This is, in a few words, the basis of neuronal communication and, in some intricate way that we could never grasp, the material substrate of cognition and behavior.

This extremely brief neuroanatomical and neurophysiological description is necessary to support the following assertion: *neuroscience does not have a proper technique to study the functionality of the nervous system in normal conditions in humans*. We use the term "normal" in two senses: (1) as non-pathological, that is, healthy conditions, and (2) as realistic situations that are similar to everyday behaviors.

Below, we describe the main techniques to study the function of the human brain: functional magnetic resonance imaging (fMRI), positron emission tomography (PET), electroencephalography (EEG), and intracranial recordings. Currently, fMRI is the most frequently used neuroimaging technique [3]. Its outcome is the widely disseminated photo of a black-and-white brain with red blobs, showing which brain areas are "active" in a particular situation. The reader may find surprising that this is an erroneous statement: as a matter of fact, our whole brain is active at any single moment of our lives. The idea that we only use 10% of our brains is a *neuromyth* [4]: if it were true, 90% of our brains would be dead tissue due to the "use it or lose it" law [5]. Then, what do we measure in an fMRI experiment? In a nutshell, we assess where in the brain there is an increase of oxygen supply in the event of interest of the task that our volunteers are performing within the scanner. For example, if we want to know which brain area is involved in looking at an angry face, we will sequentially show angry and neutral faces to our participants while being scanned. Then, after processing the signal, we will evaluate where in the brain oxygen supply is statistically higher when they looked at angry faces with respect to when they looked at neutral faces: in this case, we will probably find a red blob in a brain nucleus called amygdala. Therefore, that red blob is not a *direct* indicator of neuronal activity, but the result of a statistical analysis on the oxygen that reaches brain tissue through blood vessels. Obviously, rigorous studies carried out in laboratory animals have demonstrated that this indirect signal is a proper indicator of direct measures [6]. The technique is reliable – but it does not record neuronal activity.

PET is also a neuroimaging technique, although its basis is utterly different: first, a radioactive substance (also known as radioligand, e.g., a radioactively tagged glucose) must be intravenously injected in the participants [7]. Then, they are asked to perform a task (e.g., memorizing words) outside the scanner. Finally, they are scanned and the brain regions where the radioligand has accumulated are located. In this case, we assume that the brain areas more active while memorizing words had a higher energy expenditure, and they needed a higher glucose supply. Hence, those parts of the brain with larger quantities of radioactive glucose would be more active during the task. Note that whereas fMRI provides a set of images in time, as in a movie,

PET imaging offers a single image, like a snapshot. There are several radioligands that provide a better temporal resolution, but the final result turns out to be the same: an indirect measure of neural activity.

EEG had its inception in 1924, many decades before the development of fMRI and PET. In this case, however, it is a *more direct* recording of brain activity, since we can measure on the scalp the subtle electrical changes produced by small group of neurons that are jointly activated [8]. The main limitation of this technique is spatial accuracy: since we record the signal on the top of the head, we do not know exactly where in the brain it is coming from. Intracranial recordings, however, allow the researcher to directly measure the activity of small groups of neurons or even individual cells [9]. This technique is frequently used in laboratory animals, from rodents to non-human primates: after neurosurgery, a thin electrode is introduced in a specific brain region and the activation or inhibition pattern of individual neurons can be assessed in different experimental conditions. Given this, how is it possible to carry this out in human beings? This technique is exclusively used in patients that need a neurosurgical procedure due to a neurological or mental condition – epilepsy, Parkinson's disease, or depression, for example. At the time of surgery, and obviously after providing informed consent, the patient may be asked to perform a task while the researcher records neuronal activity in those brain regions exposed for the intervention. Therefore, the great advantage of directly measuring neuronal activity goes hand-in-hand with two important limitations: to be restricted to pathological samples and to have access only to those brain areas where surgery will take place.

In conclusion, despite the optimism emanating from neuroscience and the enormous resources invested in its development, neuroscientists are in a precarious position with respect to the grandeur of the object of their research: it is not just the challenge of reaching a comprehensive vision of the 86 billion neurons contained in the brain, but also the impossibility of studying directly their activity in normal conditions. It is plausible that the capital invested in the flagship projects about the brain could finally provide us with an ideal technique to analyze neural function, but nowadays this is a wish rather than a distant reality. When the moment comes, we should decide what aspects of cognition or behavior can be studied with this ideal technique, and we will need to keep thinking in depth whether neuroscience by itself can fully answer the big questions that human beings can ask. Let us suppose that we already have such technique and we want to study *personhood*: what notion of person shall we take as starting point? Being humble and honest, we will have to turn to sources of knowledge that have been studying this problem for centuries.

Concept of Person in the History of Philosophy: An Overview

An exhaustive description of the notion of person throughout the history of philosophy is clearly beyond the scope of this text. In this section, we will present a brief overview of the main traditions that have dealt with the issue: ancient Greek philosophy, Christian Medieval thought, rationalism and empiricism, German idealism, personalism, phenomenology, and current bioethics, which is focused on materialism (for a deeper analysis on the topic, we recommend the recent excellent book by Amengual [10]). Even though we will overlook those trends lacking of a fundamental contribution to our topic of interest – that is, the neuroscientific study of the person – the historical evolution of the concept will be essential to understand the concept of person underlying mainstream neuroscience.

In general terms, two parallel lines – with some overlapping branches – can be traced in the philosophical notion of person: on the one hand, there is a metaphysical approach on the concept, which aims to clarify who bears which distinctive features to be considered as a person, and, on the other hand, the moral approach assumes that the person is the agent of moral decisions [10]. In Greek philosophy, the term is not present as such, since the main focus was on the universal rather than in the particular aspect of beings. However,

Romans start using the term to refer to legal subjects; this is still in use: persons are carriers of rights and duties. Boethius (circa 480–524) was the first philosopher to clearly define the person as "individual substance with rational nature" [11]. He, as well as his scholastic Christian successors, faced a mystery that went well beyond the human mind or brain: the possibility of a single God existing as three persons and the fact that Christ could be one person with two different natures – human and divine. Since this discussion is theological rather than anthropological, we will ignore it. Before that, it is worth noting that influential scholars such as Augustine (354–430), Richard of Saint Victor (?–1173), and Thomas Aquinas (1225–1274) stress the relational character of persons or intersubjectivity: the distinctive feature of persons is to recognize themselves and, immediately, to recognize others as such. Modern philosophy identifies personhood with consciousness. For Descartes (1596–1650), the body – *res extensa* – is a mere burden, and it is the thinking substance, *res cogitans*, including perceptions and desires, that identifies with the subject [12]. According to him, there are no persons, but subjects and objects. John Locke (1632–1704) embraces this change of perspective and elaborates the notion of person that is currently assumed by neuroscience.

According to Locke, person is "a thinking intelligent being, that has reason and reflection, and can consider itself as itself, the same thinking thing, in different times and places" [13]. Hence, the defining features of a person are consciousness and memory: it is necessary self-consciousness at a given time point, but also the assembly of these experiences by memory. Persons are nothing but this. Thus, the notion of person distances from that of human being. This puts into question that a mentally ill human being, whose consciousness or memory is impaired, should be considered a person. Furthermore, following the Cartesian heritage, the relational aspect of personhood is overlooked. German idealism takes and enriches this supremacy of consciousness. Kant (1724–1804) initiates the interpretation of persons as moral agents. He starts divorcing the concept of person from classical notions such as substance and soul, setting it apart from theoretical reflection. According to Kantian philosophy, the person should be studied under the scope of the metaphysics of freedom: moral law is a fact of pure reason that does not need demonstration, and its manifestation is freedom. It is expressed as individual free actions, carried out by a concrete agent: a person. Besides, since this is a universal law, it entails the starting point of intersubjectivity. Hence, the person is the moral agent [14].

German idealism was argued by personalism and phenomenology. The former highlights again the role of relationships at the core of the person, as scholastic did before, although setting aside theology. Furthermore, it stresses the importance of an embodied existence and the dynamic development of human beings: we are configured through the development of interpersonal relationships [10]. Edmund Husserl (1859–1938), founder of phenomenology, reaches the concept of person after a systematic reflection on subjectivity and begins with the experience of the own body. By doing so, he is radically departing from cognitivist interpretations such as the Cartesian or Lockean. Being a person is primarily recognizing oneself as subject of a world of perceptions, memories, and thoughts – a world that we share with other persons [15]. Max Scheler (1874–1928) begins with the question about why humans are different to other beings [16]. He establishes different degrees of life according to interiority and the way to grasp the environment. The highest degree of life is obviously that of humans, since we have a spirit. The spirit allows us to make decisions by ourselves, to be open to the world, and to be able to objectify. The person would be the singular case of the spirit. Scheler assumes that this is a very strict conception, and probably none of us is a person during our whole existence. Consequently, he attributes a gradual character to personhood: human beings can be persons at a higher or lower degree, assuming that *all human beings are persons*.

Analytic philosophy is going to be a bridge between Locke's proposal and current materialism. It attempts to specify the list of conditions that a being needs to fulfill in order to be

considered a person, which are always centered around self-consciousness and decision-making. Strawson (1919–2006) states that the person is the subject whom material and mental predicates can be assigned to. Whereas Ryle's logical behaviorism assumes that there is no mind – and hence person – beyond observable behavior, Strawson advocates for a primitive and simple concept of person [17]. His contribution, which will be followed by authors such as Peter Singer, opens the door to the attribution of material and mental predicates to non-human beings. Harry Frankfurt (1929–), however, considers that Strawson has not been able to find a defining feature of persons; his proposal is that this feature is the ability to put second-order desires before first-order desires [18]. In other words, persons can be on a diet and put physical wellbeing – second-order desire – before hunger, first-order desire. Free will is doing what one wants to do, and this comes marked by second-order desires. Daniel Dennett (1942–) agrees with Frankfurt's view, but he still does not find the list of features to fulfill for being considered a person. Thus, Dennett clearly states six characteristics or themes: (1) being rational; (2) being attributed states of consciousness; (3) taking an attitude towards it; (4) being able to reciprocate such attitude; (5) verbal communication; and (6) self-consciousness [19]. After analyzing in depth all these themes, he concludes that they are necessary, but not sufficient, features; in other words, there can be no person without the six characteristics, but having them does not guarantee that *something* is a person. Therefore, he assumes that personhood cannot be explained by any empirical science or theoretical knowledge, and it has to be treated in a practical sense: personhood is a moral construct, and hence something will be a person if at certain moment we convene it to be a person.

This is the spirit underlying the concept of person in mainstream current bioethics, which reflects on the rules to be followed when a human being intervenes on another being. The global position of the following authors is that the concept of person is primal and easy to grasp in healthy and fully developed human beings, but it becomes tricky when applied to humans both at extremes of their lifespan – unborn children and elderly people – and in certain pathological conditions. For example, Tooley (1941–) argues that a person is a being that can express his or her desire to live. In the case of human beings, this happens in a non-verbal manner about 12 weeks after birth. Until then, infanticide is acceptable [20]. This is also the case when the human being loses this ability at old age. Eventually, from this perspective, being a person depends on certain degree of brain development. Peter Singer (1946–) exploits this interpretation under the scope of preference utilitarianism: we should always seek for the greatest good for the largest number of people. The defining feature of personhood is the capacity for suffering: we should give preference to those with a greater capacity for suffering with respect to desires for the future: "Killing a snail or a day-old infant does not thwart any desires of this kind, because snails and newborn infants are incapable of having such desires" ([21], p. 90). Engelhardt (1941–2018) substitutes suffering for permission: person is the subject that can give permission or consent [22]. Vegetative human life, which still does not have or has lost the rational capacity for consenting, has value, but lacks of rights. Despite this, he admits a "social concept of person" for those to be considered as persons by consensus even though they do not fulfill the requirements that he proposes. In conclusion, materialist bioethics shows a failure of the concept of person, moving from a metaphysical or moral conception to a merely practical utilitarian view.

On the other hand, within bioethics, there are utterly different interpretations to relaunch the notion of person from a non-materialistic point of view. For example, Theo Kobusch (1948–) argues that a modern concept of person cannot overlook its metaphysical heritage. According to him, the current understanding of person is impoverished due to materialist reductionism, which entails abandoning the moral character of the term, as well as the importance of biographical history and interpersonality [23]. The greatest current contribution to restore the notion of person is that of Robert Spaemann (1927–2018). For him, since Boethius, philosophy has attempted to distinguish

persons according to a set of characteristics; from this starting point, there is a "rational," Locke, and a "social" – German idealism – way. However, according to Spaemann, there are no features to be fulfilled: every human being is a person because he or she is always recognized as part of a moral community [24]. We get to that recognition because we identify him or her as a member of a species that have a set of features. In other words, we should not ask which human being fulfill certain properties, but assume that the species has those properties, and therefore every single individual within the species must be recognized as person. The person is the holder of the features, but is not defined by them. Being a person is a way of existing that is mainly defined by two characteristics: self-difference and interpersonality. The former is based on Helmuth Plessner's contributions, who concludes that only human beings can have an "eccentric position" with respect to their lives: by being in charge of their own lives, they can contemplate it from an "external position." This is related to free will, since by means of the exercise of freedom we can go beyond the homogeneity of the species and become individual persons. Spaemann clarifies that if a human being does not have the capacity for self-difference, he or she is not a mere animal, but an ill person with respect to others. On the other hand, interpersonality underlies a respectful relationship and mutual recognition. However, being person does not emerge from having the feature of being recognized; rather, one is recognized because he or she is a person. Even though a patient in a minimally conscious state does not manifest as person, he or she appears as such in the relationship with others. In fact, the embryo is not a *potential*, but an actual person due to the continuous interaction with the mother from the very moment of conception. Underpinning the primal character of the person beyond the assignment of properties, he concludes that being person is not the result of certain development, but the very structure that allows development.

Through this historical journey, the reader can realize that the concept of person has followed a tortuous pathway, where the tightest turns ended up with its separation from the notions of human being, corporality, and intersubjectivity. Starting from Locke and merging with utilitarian ethics, the person is reduced to the mind (i.e., consciousness and memory) as observed from the third-person perspective. Moreover, since the mind is hard to objectify, it is naturalized, and the brain becomes the new object of interest. Although there are other contemporary proposals that attempt to relaunch the concept of person, as we mentioned above, the naturalized standpoint is pervasive in neuroscience, as we will show in the next section.

Neuroscience Needs a Limited Concept of Person

Going back for a moment to Amengual's book, he states the following with respect to scientific materialism:

> If we start from the distinction between human being and person, it should be said that natural sciences, neurosciences included, deal with human beings, that is, humans as members of a species and with the conditions, characteristics and features that make possible and differentiate human life and, more precisely, the life of human beings as persons. Thus, they are about what humans are, and not *who* they are. ([10], pp. 289–290)

Interestingly, this poses the question whether neuroscience must be materialist by definition. Inasmuch as it is defined as the study of the nervous system, that is, the biological bases of behavior, our response is affirmative: neuroscience is a materialist discipline and, furthermore, it *must* be so. The following question to be posed is whether it has to be reductionist or eliminativist, that is, to overlook the conclusions coming from other sources of knowledge. Our response to this question is negative, although the actual prevailing attitude in neuroscience is eliminativist. For example, this is the case of the famous Libet's experiment – and the subsequent interpretations – about free will [25]. In this case, neuroscientists decide to study free will in the laboratory, which is completely legitimate. To do so, they assume one among many philosophical interpretations of free will, ruling others out, and they take certain selected features to design an

experimental paradigm to be tested in the laboratory. At this point, the notion of free will has been pruned in two different ways – theoretically and in the development of the experimental task. Then, the experiment is conducted, which entails a series of intrinsic methodological limitations that researchers must take into account to be aware of what conclusions can be drawn from the study. Finally, after analyzing data, the main result of the experiment is that certain brain area shows a peak of activity before the sample of participants felt the conscious desire to move their hands. The widely disseminated conclusion is that our sensation of freely choosing to move is fictitious, since our brains – the material bases – *decide* to move well before our desires, mental experiences, have even appeared. Related commentators state that Libet demonstrates that free will is an illusion [26]. These conclusions and statements overlook methodological limitations and the unavoidable bias to transfer a philosophical concept into a laboratory. This is the most popular case of reductionist materialism applied to the issue of free will. What about the notion of person?

There is not a clear definition of person in neuroscience, due to the lack of a *corpus* of neuroscientific studies about this matter. In any case, due to the legitimate materialist aspect of neuroscience, the definition should be adequate to study its biological substrate. Furthermore, the obvious link between neuroscience, psychology, and philosophy of mind supports a cognitivist interpretation of the person. For example, the neuroscientist and philosopher Georg Northoff [27], when asking the relationship between the synchronic aspect of personhood (i.e., the conditions that make *something* to be a person) and the diachronic aspect of personal identity (i.e., what makes *someone* be the same person through time), answers as follows:

> What do persons have that non-persons don't have? The philosophical goal has largely been to identify a set of mental features possessed by all and only persons. These features, both traditionally and in recent philosophical discussions, are determined first and foremost by higher-order cognitive functions. It is fairly agreed upon the view that a person is someone who acts from reasons. This conception of personhood has a long tradition, reaching back to John Locke…. ([27], p. 2)

Northoff is a prestigious neuroscientist and philosopher who has proposed that several midline brain structures are the biological substrate of the self. Interestingly, in a recent study [28], he concludes that most of these "self-related" areas overlap with the well-known default-mode network – those brain areas significantly active when volunteers are at rest. According to Northoff and his team, these are the neural correlates of self-consciousness. Note the contradiction: whereas the person is explicitly defined as the being that possesses higher cognitive functions, it is stated that one of the main characteristics of the person, self-consciousness, manifests when the subject is mentally at rest.

Some authors within the field of neuroethics have followed the same train of thought of Libet's experiments and have emphatically stated that, according to neuroscientific findings, personhood is an illusion [29]. First, like many researchers did before, they ask about the characteristics that a being should have to be considered a person. Through an extremely short philosophical overview, they summarize the interpretations of Locke, Kant, and Dennett, highlighting intelligence and self-consciousness as the main characteristics of the person. In addition, they mention the criteria by Joseph Fletcher [30], one of which is based on IQ: "Below IQ 40 individuals might not be persons; below IQ 20 they are definitely not persons." Farah and Heberlein acknowledge the relevance of personhood for bioethics in order to assign moral responsibility. However, they find difficulties to clearly determine if *something* is a person, especially in the beginning and near the end of the human lifespan. Likewise, in accordance with Steven Wise, they believe that nowadays we can make the terrible mistake of refusing personhood to animals as it was done to slaves in the past [31]. Based on the multiple lists of characteristics for personhood that have been created throughout the history of philosophy, the authors conclude that it is impossible to define the person from a metaphysical point of view, so they propose the search for a biological marker to clearly differentiate persons from non-persons.

Their starting point is that, like biology could find an unquestionable physiological trait to differentiate plants from animals (i.e., photosynthesis), there may be a biological trait to define persons. Considering the cognitivist approach mentioned above, the obvious choice to find that trait is neuroscience. They start from a clear-cut assumption: persons must have cortical activity. Thus, intrauterine children and neurological patients without cortical activity are not persons: "Such patients are more commonly described as being in a persistent vegetative state, biologically alive but considered by many to be former persons because they *appear to* lack any mental life" ([29], p. 39; our italics). But this is not enough for Farah and Heberlein: it would be necessary to specify which cortical areas are involved in personhood and what degree of functionality they should have. They recognize that this is impossible even for neuroscience and, *therefore*, personhood as such does not exist:

> If our analysis is correct, it suggests that personhood is a kind of illusion. Like visual illusions, it is the result of brain mechanisms that represent the world nonveridically under certain circumstances. Also like visual illusions, it is stubborn. ([29], p. 45)

Given this nihilist conclusion, the authors propose a double attitude: on the one hand, ethics must take a utilitarian stance and, instead of asking who is a person, consider the degree of intelligence or self-consciousness and, on the other hand, since in daily life we need to make a plethora of decisions with some moral implications, it is legitimate to behave with certain beings, such as human babies, as if they were really persons, because it is beneficial for their development: "Although the concept of personhood may be bad metaphysics and better suited to an earlier world, even today it serves us well" ([29], p. 46). It is worth noting that Martha Farah is one of the cofounders of the International Neuroethics Society, an institution that, through the Presidential Commission for the Study of Bioethical Issues, directly counsels the President of the USA.

It should be also mentioned that biology has irrefutably confirmed the existence of non-photosynthetic plants [32].

Unquestionably, the notion of person in current neuroscience is richer than the one presented here. We have just summarized two examples from very influential researchers: the interdisciplinary view of Northoff that assumes the cognitivist approach on the person and the nihilist account of Farah proposing merely practical attitudes. The idea that we want to communicate is as follows: neuroscience, due to its mainstream eliminativist stance, needs the simplest notion of person to analyze it in the laboratory. For that reason, the most adequate concept is that of Locke, who advocates for three simple features to be considered as person: intelligence, self-consciousness, and memory.

Albeit mainstream, this is not the only approach on the person in neuroscience. In the next section, we take the opposite point of view and ask how neuroscience should be to study a richer notion of person. As we will show, through open-mindedness and interdisciplinarity, it becomes possible to study personhood from a holistic point of view, which is more fruitful than eliminativist materialism.

The Concept of Person Needs an Enriched Neuroscience

Neuroscience, as any individual discipline, has several intrinsic limitations that undermine the study of human big questions. Technical issues, in our opinion, are not the most important. In previous sections, we have insisted on the absence of an ideal technique to measure human brain activity in normal conditions. We could conduct a mental experiment and assume that such technique exists, and we can exactly assess what is happening in our brains when we behave in certain way or think about something. This fictitious scenario may be compared with a stress test to evaluate cardiac activity or pulmonary or muscular function when we are under fatigue conditions. The equipment may indicate that our physiological response is abnormal, too high or too low according to physical effort, and this would be a crucial information to guarantee our wellbeing when we are under similar situations.

However, we would obtain no valuable information about our subjective experience during the test: in fact, it is assumed that subjective experience will not correspond with our physiology, approaching to dangerous situations where our wellbeing may be at stake. That is the purpose of a stress test. The "perfect machine" to assess brain activity in normal conditions would be the same: it would inform about how our brain responds in certain situations – moral or economic decision-making, witnessing or committing unfair actions, learning, habit acquisition, behavior in accordance to beliefs, and so on – but it would not provide any valuable information about our moral principles, memories, or beliefs. This is neuroscience's most important intrinsic limitation: having a restricted field of interest.

Hence, a proper understanding of the concept of person does not require a technically exquisite neuroscience, but a neuroscience that participates in a *comprehensive anthropology*. Just like we presented a concrete case to exemplify the eliminative stance of neuroscience, we will explain here the proposal by Thomas Fuchs, psychiatrist and philosopher in the University of Heidelberg, in order to demonstrate that neuroscience, even from materialist presuppositions, can provide valuable information to improve our understanding of personhood. Fuchs's main goal is to demolish "brain-centrism": we are not our brains because, for a start, the brain is not the only biological substrate of the mind. In his most recent book [33], he explains the ecology of the brain, that is, the environment where it is located and its relationship with the rest of the body and the person. First, he criticizes neurobiological reductionism and explains the person from a phenomenological perspective. To do so, he distinguishes – but also integrates – the notions of "living body" (*Körper*) and "lived body" (*Leib*); both, as a unity, configure human life. The former is the object of neuroscience, which must not eliminate the latter. Fuchs adheres to the *4e cognition* field, which advocates for an embodied, embedded, extended, and enactive understanding of the mind. The first term argues that the mind always appears in relation with a body, so both must be always studied as a whole [34]; the second stresses that the embodied mind is always in a concrete situation and context [35]; the third proposes that the mind goes beyond the limits of the body, that is, it is also in the cane used by a blind person to perceive the world [36]; and, finally, the enactive mind highlights the *Leib*, the fact that the mind is always within a living, dynamic, and complex being [37]. Note the radical distinction between these proposals and the assumption that the brain is the source from which the mind and all behaviors emanate. Going back to Fuchs, the fifth chapter of his book describes the brain as the organ of the person. It is focused on social and cultural influences in brain development since infancy: the human brain is physiologically premature – the newborn's brain is 25% the size of an adult's, whereas this figure is up to 50% in chimpanzees – so its development will go hand-in-hand with its ecology:

> The development of the embodied human mind does not only require interaction between brain, body, and environment, but essentially interaction with other humans. In the course of these biographically progressing interactions, the brain becomes a social, cultural, and biographically shaped organ. ([33], p. 175)

According to Fuchs, the person is the unity between the living body and the lived body: the former can be assessed from a third-person perspective, but the latter provides the person with his or her uniqueness:

> A person is a lived body (Leib) inasmuch as his or her subjective states, experiences, and actions are bound to the medium of the body. However, persons are also lived bodies for others, who directly perceive them "in the flesh" through their expression, attitudes, and acts—thus, not as a combination of pure physical body and hidden psyche, but as a unified entity. If someone greets me by extending a hand, this does not represent for me an inner, "mental act" involving a movement of the physical body as an outward symbol. Rather, this person is present for me by virtue of his greeting, in his offered hand. ([33], p. 74)

One essential element in this proposal is the role of the second-person perspective – intersubjectivity – in the configuration of the person. This is absent in neuroscience, but also in the most influential philosophical stances outlined above.

The relationship with others is not accidental, as mere receptors of moral acting; it is essential since conception – in the mother-baby relationship – and in the continuous development of the person. This can be minimally explained by neuroscience, because the brain is just a small part of it. Then, Fuchs assumes that the mainstream standpoint in neuroscience is similar to Farah and Heberlein's and argues as follows:

> The brain does not possess mental states or consciousness as such, for it does not live—it merely exists as the organ of an animate being or living person. Neither neuronal assemblies nor brains, but only human persons can feel, think, perceive, and act. It is erroneous to identify the brain with the human subject and to look inside for what makes up the person. What essentially characterizes a human person is being in relationships. The brain neither produces nor inherently contains such intentional and social relationships with the world. True, a human person's capacities and their realization as conscious acts of life are uniquely linked to brain functions. In this sense, the brain is a primary condition of possibility of personal existence in the world. However, a person is not a localizable part of the body, but is embodied and animate. We do not exist a second time inside ourselves. Human persons have brains, but they are not brains. ([33], p. 279)

Throughout the text, Fuchs mentions those brain areas associated with the recognition of the other as person. This is similar to the train of thought that followed Farah and Heberlein. However, whereas the neuroethicists concluded that personhood was an illusion, Fuchs demonstrates that those brain areas and processes serve the person to harmonically integrate with the rest of the body, the environment, and the other. This starts with primary intersubjectivity, through which prenatal and postnatal babies interact with their mothers in a natural, pre-reflective manner. Primary intersubjectivity allows the establishment of a shared space that involves bodies and environment. Later, at about 9 months of extrauterine life, secondary intersubjectivity is developed, and the child starts interacting simultaneously with his or her caregiver and concrete objects in the environment. This is the popular "shared attention," which is extremely important in the acquisition of all types of abilities, such as language [38]. In turn, together with tools, language is the environment of cultural development: "Therefore, humans, like no other creature, need their conspecifics in order to develop their dispositions into capacities" ([33], p. 205). Finally, we would like to note that Fuchs's definition of mind goes beyond cognitivism and the Lockean interpretation. For the German phenomenologist, the mind is "the overarching manifestation, the gestalt, and the ordered patterns of all relations that we have to our environment as animate beings, and as humans to our fellow humans" ([33], p. 207).

In conclusion, this kind of approaches allows neuroscience to provide valuable information on the big questions of the human being, such as personhood, freedom, morality, or spirituality. An integrative vision must assume the multiple dimensions of human beings, where the partial explanation of each dimension should contribute to improve our knowledge about the unity that they configure. This is the basis of a fruitful interdisciplinarity between the sciences and humanities.

Conclusion: Interdisciplinarity as the Cornerstone to Study the Concept of Person

Research on big questions must be guided by questions themselves, and not by the constraints of a given discipline. After posing the question and selecting the disciplines that can contribute to the answer, it should be clarified the own space of each discipline and the shared space between them. In the initial section of our text, we have briefly explained the basics of neurobiology and the main techniques to study the human brain, in order to delimit neuroscience's own space. The philosophical journey to explore the concept of person has served us well to realize the magnitude of the question and to assume that neuroscience is unable to give an answer by itself. This was exposed in the next section, where the neuroscientists' tendency to eliminate what remains inexplicable was exemplified in the case of free will and personhood. After these negative cases, we focused on the phenomeno-

logical approach by Thomas Fuchs as an example of constructive interaction between disciplines. This can be viewed as a growing spiral that, starting from the small restricted field of a discipline, increases its diameter through its interaction with others. Neuroscience, as a valuable source of knowledge about biology, may formulate hypotheses in dialogue with psychology, philosophy, or theology, in order to expand its field. The research on personhood by Farah and Heberlein is a good example of a decreasing spiral, where the constraints of certain field – neuroscience – reduces the diameter of the spiral and ends up in an extensionless point: personhood is an illusion.

The approach on big questions, therefore, does not mainly depend on the contribution of each discipline, but on the attitude of the researchers that carry the study out. They can expand or contract the spiral's diameter. In our opinion, educational systems should promote this attitude at all levels, from schools to doctorate degrees. Only thus, training humble open-minded students who are eager for dialogue and in honest search for the truth, we could try to answer the big questions of humanity.

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12 The Neuroethics of Beauty: Insights from Aldous Huxley's Theory of Knowledge

Luis Enrique Echarte Alonso

Beauty over Aesthetics

"Beauty is worse than wine, it intoxicates both the holder and beholder." These words, once attributed to Aldous Huxley, mock those who are *blinded* by beauty, as well as the recipients of excessive flattery. However, the phrase also hides a deeper lesson: beauty has *both* a subjective and an objective dimension. It is not just in the eye of the beholder; it is also in the holder, or so it was long believed – this is the posture of Plato, which lasts until scholastic philosophy with few exceptions. However, this common belief changed with modernity, when beauty was reduced to an aesthetic experience, namely, to its subjective dimension. It resulted, as Arthur Pontynen writes, in a drastic way, "Which approaches can be called aesthetic and which beautiful? It is helpful first to remember how the meaning of those terms. Aesthetics refers to sensation and emotional response whereas beauty refers to perfection in a purposeful world, and the splendor of wisdom. Aesthetics is non-cognitive, whereas the pursuit of beauty aspires to some significant degree of cognition, of understanding being, of ontology. Aesthetics refers to physics, to the realm of fact and feeling. Beauty refers to metaphysics, to the attempt to understand reality and life. The difference between aesthetics and beauty was once widely recognized, just as was the distinction between fact and truth. Today that distinction is blurred, and when blurred, a shallow aesthetics prevails" [1] (p. 326).

Of course, this does not mean that beauty is a simple matter of external and internal senses for all modern philosophers. For example, Immanuel Kant faces this interpretation, mainly sustained by British empiricists, arguing that beauty is a sort of judgment. He views it as "an experience submitted to rational considerations" [2]. However, in Kant's transcendental idealism, beauty has neither a representative nor an intentional function: it does not say anything to us about things as they are in and of themselves; neither does it lead us to reality. By contrast, philosophy of beauty, in the sense used in this chapter about Huxley's approach, is a philosophy about one of the transcendental properties of being – truth, goodness, and beauty, among others.

In light of these clarifications, I will delve into the role of beauty in moral decision-making, and, to carry this out, I will present some of Huxley's stimulating ideas about, first, the difference between knowledge and understanding; second, the problem of the increasing technification of our culture; and third, some tips for returning the

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arts to the place they deserve. These points positively contribute to the two subgroups of study that, according Adina Roskies, are included in neuroethics, namely, "the neuroscience of ethics" and "the ethics of neuroscience" [3]. For the first subgroup, I conclude that developing nonverbal education may help neuroscientists face the problems of methodologism and utilitarianism. With regard to the second subgroup, I argue that neuroscientists have a major responsibility in avoiding the centralization of power and subsequent danger of tyranny.

Three Transcendentals

Over the past centuries, much has changed concerning the idea of beauty – and not always for the better. It is no coincidence that one of the main challenges that Huxley faced in his work was retrieving the *perennial philosophy*, an interpretation of reality in which beauty has a central role.

I will start with a good example of classic wisdom that Huxley tries to recover. In *The Life of Pythagoras*, Neoplatonist philosopher Iamblichus Chalcidensis writes, "It is said that Pythagoras was the first to call himself a philosopher, not only introducing a new name, but also the first to teach the related matter thoroughly and usefully. He likened the entrance of men into the present life to the progression of a crowd to some public spectacle. There assemble men of all descriptions and views. One hastens to sell his wares for money and gain; another exhibits his bodily strength for renown; but the most liberal assemble to observe the landscape, the beautiful works of art, the specimens of valour, and the customary literary productions. So also in the present life men of manifold pursuits are assembles. Some are influenced by the desire of riches and luxury; others, by the love of power and dominion, or by insane ambition for glory. But the purest and most genuine character is that of the man who devotes himself to the contemplation of the most beautiful things, and he may properly be called a 'philosopher'" (Iamblichus, The Life of Pythagoras, 12). This rather long text is worth quoting in full because, in these words, we find the context and historical basis with which Huxley links science and philosophy. According to him, contemporary science and contemporary philosophy share the same roots that amount to beauty – the incommunicable secret, the objective subjectivity, of the *perennial philosophy*.

Beauty is the sacred and ultimate bridge among fields, among the seekers of knowledge – whether philosophers or scientists in the proper sense of those words. As Plato also writes, "Wonder is the feeling of the philosopher and philosophy begins in wonder" (*Thaetetus* 155d). Philosophical conversion is, then, a fortuitous event in which reality reveals itself as precious for a single gifted moment. That is often enough to go down the road of knowledge. But what does the adjective "beautiful" refer to? On this point, Huxley also follows the classics when he answers that the objective dimension of beauty speaks to a proportional relationship in reality. "Beauty arises when the parts of a whole are related to one another and to the totality in a manner which we apprehend as orderly and significant" [4] (p. 16). For him, the concept of beauty is impossible to understand without thinking about identity's inner purpose and the universe's outer purpose, i.e., harmony. Indeed, beauty is a sign of unity between the observer and an entity, which leads, in turn, to unity with the universe. This is possible, according to Huxley, because everything is called to such unity. However, this target is only possible if, first, reality maintains a particular order, the natural one, and second if the observer maintains a relationship of love with the observed.

Huxley points out here the intrinsic connection among three transcendentals: the first one, truth, refers to the intelligibility of reality and other aspects and dimensions that shape entities; the second transcendental, goodness, refers to one of these dimensions, i.e., teleology, which amounts to knowing that certain things are good or bad, to finding things' correct place in the universe; the third one, beauty, refers to the agentive dimension of knowledge, that is to say, its capacity to impel an observer to action that aims at protecting, serving, fostering, and, above all, seeking unity. Beauty's main commitment

involves being one with the beloved. In contemplating the beauty of a thing, the philosopher – this *old scientist* – is coherently inclined to look around at the surrounding reality. As Huxley notes, quoting Yung-chia Ta-shih, this idea is not exclusive to the West: "One Reality, all-comprehensive, contains within itself all realities" [5] (p. 8). Settling for less becomes impossible. Interdisciplinarity is, therefore, connatural for scientists, which explains their interest in a variety of methods and in the right way of reaching adjacent objects. For the same reason, scientists are concerned about professional ethics, in particular, as well as ecology, in general, which is the second transcendental. These concerns mobilize citizens – the third transcendental. It is therefore not surprising that, for Huxley, science is not a matter of simple curiosity or practical interest, but of demanding love. Facing reality implies the development of human sensitivity, that is to say, the capacity to be aware of other's needs and anticipate their demands.

Because it is not easy to implement such an interdisciplinary, moral, and committed attitude, scientists need scientists. However, according to Huxley, this necessity is not the main thing that unites them; rather, their shared condition as a part of a long chain of teleological natural dynamism brings them together. Quoting Huxley's words, "It is only when we have renounced our preoccupation with 'I,' 'me,' 'mine,' that we can truly possess the world in which we live. Everything, provided that we regard nothing as property. And not only is everything ours; it is also everybody else's." [5] (p. 108). Better yet, the exploration of reality always drives discovery of human beings' beauty. In this context, scientific community is not just a means but also an end: the place where humans can rest and delight in their most specific and noble task. However, things do not end there. Universal harmony leads observers to God: the first principle of natural order, the font of this complex web of purposes. "God is the final, deepest meaning of all that exists. God, then, is manifest in the relationship which makes things beautiful." [4] (p. 17). It is a long ascension from corporal unity to natural unity and then to divine unity.

To conclude, Huxley also agrees with the classics on knowledge's ultimate reward, namely, beauty. This is because philosophy does not just begin in wonder but also ends there. It is what makes philosophy a magnificent activity, an end in itself, the highest reward for any intellectual. For example, Aristotle observes this point when, talking about how Thales the philosopher became rich, he concludes, "thus showing how easy it is for philosophers to become wealthy if they so wish, but it is not this they are serious about" (*Politics* I, 11, 1259a). Using Huxley's words, the best scientists are those who deal with pure truth – not its fruits. "To the exponents of the *Perennial Philosophy*, the question whether Progress is inevitable or even real is not a matter of primary importance. For them, the important thing is that individual men and women should come to the unitive knowledge of the divine Ground, and what interests them in regard to the social environment is not its progressiveness or non-progressiveness (whatever those terms may mean), but the degree to which it helps or hinders individuals in the their advance towards man's final end" [5] (p. 80). Man's final end is not in the future; it is not even in the most limited view of tomorrow. Rather, for Huxley, it is in the eternal timeless present.

The Search for Understanding

Transcendentals do not have an equidistant position in Huxley's theory of knowledge. *Truth* is the main and general transcendental in which all aspects and dimensions of one's being are interrelated. On the other hand, goodness is only part of this explanation; it speaks to the teleological dimension of identity because not every being has a purpose. Beauty ultimately refers to an even more specific feature of these second kinds of beings; it is the driving force associated with the knowledge of said teleological dimension. When we observe the purpose of something, we are attracted or repelled by the particular place it takes in the universe, but not all teleological identities move us to action. In brief, transcendentals are like three *matrioskas*: they are found one inside another.

In the preceding conceptual framework, it is easy to understand Huxley's distinction between *knowledge* and *understanding*. According to him, "knowledge is acquired when we succeed in fitting a new experience into the system of concepts based upon our old experiences. Understanding comes when we liberate ourselves from the old and so make possible a direct, unmediated contact with the new, the mystery, moment by moment, of our existence" [4] (p. 193). *Knowledge* implies the acquisition of truths – including of purpose – but not those that stir observers to significant behaviors. Indeed, for Huxley, science or moral books do not trigger by themselves good or evil acts. It is because of beauty – a sort of direct experience – that intelligent beings take action.

Two mistakes must be avoided in interpreting Huxley's distinction. First of all, the metaphor of matrioskas is not entirely suitable because it leads us to believe that there is only one nesting doll inside another when, in Huxley's view, there may be several ones at the same level. For example, besides beauty, teleological identities have more dimensions and traits to contemplate. Secondly, these three transcendental ways of touching reality do not necessarily mean three different sorts of acts or even three different moments of the same rational act. For Huxley, as for Aristotle, beauty is in the very principle of any act of cognition. "For where there is perception, there is also both pain and pleasure, and where these, there is of necessity also epithumia" (*De Anima* 2. 413b 23–24). Similarly, Huxley writes: "The new is the given on every level of experience – given perceptions, given emotions and thoughts, given states of unstructured awareness, given relationships with things and persons. The old is our home-made system of ideas and word patterns. It is the stock of finished articles fabricated out of the given mystery by memory and analytical reasoning, by habit and the automatic associations of accepted notions. Knowledge is primarily a knowledge of these finished articles. Understanding is primarily direct awareness of the raw material. Knowledge is always in terms of concepts and can be passed on by means of words or other symbols. Understanding is not conceptual, and therefore cannot be passed on. It is an immediate experience, and immediate experience can only be talked about (very inadequately), never shared. Nobody can actually feel another's pain or grief, another's love or joy or hunger. And similarly nobody can experience another's understanding of a given event or situation" [4] (pp. 193–194). In this quote, we find a close connection between beauty and emotion. Any intelligence is emotional intelligence: purpose and drive are constitutive elements of any kind of real awareness and consciousness. What is the difference then between beauty and emotions? For Huxley, it has to do with the everlasting issue of appearances: a belief is *true* when it corresponds with reality, a moral value is *good* when it speaks to the real place of beings in the universe, and an emotion is beautiful when it compels an agent to build a better world. The opposite corresponds to false beliefs, bad values, and seductive emotions. Indeed, the opposite of beauty is not necessarily sadness and ugliness because some of these emotional perceptions may help us obtain a better understanding of our world and find more sympathy for its downtrodden creatures, which results in a better position from which to love – *the sublime experience* as philosophers universally referred to it. Huxley strongly believes that any deep enquiry into suffering is also a chance to discover the delicate and marvelous forces that sustain the world and being.

Huxley does not advocate for naïve realism. Beauty emerges from intuitions, but it often comes with a dose of what merely appears to be truth and goodness. "For the convinced believer, understanding or direct contact with reality is exceedingly difficult. Moreover, the mere fact of having a strong reverential feeling about some hallowed thing, person or proposition is no guarantee of the existence of the thing, the infallibility of the person or the truth of the proposition" [4] (p. 221). It is the task of reflection and dialogue, together with more contemplation, to discriminate between what is real and unreal. However, for Huxley, it is impossible to climb the stairs of transcendentals starting from a completely false impression. There must be some element of truth

in every experience. This vision is coherent with Huxley's concern for "free undistorted awareness." The challenge of reaching clearer understanding, especially among Westerners, corresponds to reopening the doors of perception, as Huxley quotes from William Blake, "were cleansed everything would appear to man as it is" [6] (p. 6).

This challenge does not involve the most human aspect of our intelligence but rather our sensory and visceral part, which we share with the rest of the animal kingdom. This is another way of saying that understanding is a capacity more deeply associated with children than with wise men. What is more, Huxley believes animals too have this capacity: those beings with sensitive souls, using Aristotle's terminology, who feed on matter as well as truth, purpose, and beauty from nature. Of course, we as humans go further with intuitions, reflections, and communications that are much stronger, more penetrating, and acute; animals do not have science, moral codes, or pieces of art. In the end, only human beings, thanks to rationality, can achieve a degree of relational unity with the universe that enables them to enhance and recreate it – for Huxley, this corresponds to the last *graces*.

A final critical issue is how these three dimensions of intelligence act upon each other. According to Huxley, such interaction is both a fact and also a risk because, if we are not careful, it leads us to think that one dimension can be deduced from another or, what is worse, be reduced to another. Both mistakes appear in the *naturalistic fallacy*: moral values cannot be deduced from scientific statements in the same way as *ought to be* cannot be reduced to *being*. Having said that, Huxley recognizes that adequate knowledge of matters is important for greater understanding: scientific knowledge can act as an inner lens – empty of content – that helps observers grasp the purpose and beauty of reality. Let me reiterate, however, that, for Huxley, a fly examined with a magnifying glass cannot be deduced from or reduced to the features of the magnifying glass. It should be acknowledged, however, that Huxley does not sufficiently explain how knowledge is capable of strengthening human intuition, which is a non-mediated act. But, on the other hand, he faces a problem that is still today insufficiently studied, that is, the similarities and differences between an elderly person's intuition and that of a child, which are both as mysterious as everyday phenomena.

Idealism and Alienation

What is normally an advantage can become a liability. Huxley warns against keeping a wealth of conceptualized knowledge and patterns of thinking, feeling, and behavior because they may stand between us and reality and hamper human intuition. On this basis, he distinguishes between *knowledge* and *pseudo-knowledge*; the former acquires its meaning from perceptions, while the latter is deduced and validated from other mental images or thoughts. For Huxley, this is not a minor matter: pseudo-knowledge prevents us from intuiting what is good and what is not and, most importantly, from hearing the voice of intuition that speaks to us about what to do and what not to do. In other words, too much pseudo-knowledge blocks understanding. The price to pay is high. "Meaningless pseudo-knowledge has at all times been one of the principal motivators of individual and collective action. And that is one of the reasons why the course of human history has been so tragic and at the same time so strangely grotesque. Action based upon meaningless pseudo-knowledge is always inappropriate, always beside the point, and consequently always results in the kind of mess mankind has always lived in – the kind of mess that makes the angels weep and the satirists laugh aloud" [4] (p. 196). It goes without saying that a lack of meaning is typical of aged and tightly corseted societies in which children, as well as the elderly, have left behind the noble and free impulses of the heart.

The disease of Western society, as Huxley diagnoses it, is the hypertrophy of pseudo-knowledge; its main symptoms are idealism, alienation, and separation from the physical body. With it, engines of beauty are lost and replaced by *puritan* and *voluntaristic* attitudes

that serve as a defense mechanism, as well as by solipsistic pleasure and vibrant promises for a better future pawned off by the entertainment industry. On the latter matter, Huxley claims, "[A]ny faith based upon hypothetical occurrences a long time hence must always, in the very nature of things, be hopelessly unrealistic. In practice, faith in the bigger and better future is one of the most potent enemies to present liberty; for rulers feel themselves justified in imposing the most monstrous tyrannies on their subjects for the sake of the wholly imaginary fruits which these tyrannies are expected (only an implicit faith in progress can say why) to bear some time, let us say, in the twenty-first or twenty-second century" [7] (pp. 26–27). Hope deflects attention from the present life – where reality is – to the future – where intuition is sterile. Again, like culture, *tomorrow* has a creative role in human life but only when the doors of perception are completely open. It is the present that teaches us our plans and the codes and methods to change them, which amount to moral enhancement.

The root causes of the West's idolatry of pseudo-knowledge can be traced to a forceful intellectual trend initiated three centuries ago, namely, the Cartesian split between natural sciences and spiritual sciences. It was then that mind and matter started to separate and, thus, to forget the intuitional basis of intelligence. From a Cartesian view, experimental methodologies are applied in *natural sciences*, while formal methodologies (dialogue and the logic of question and answer) are suitable for sciences of the spirit – today also called the humanities and social sciences. In both cases, objectivity is the final goal. Descartes identifies this term with the self's intuition – the only and ultimate way of knowledge. In other words, certainty, or perfect knowledge, is reduced to knowledge that can be communicated to everyone. That being so, the question of methodology became a central theme in the sciences and the humanities, which comes down to the search for the best way of knowing features of reality that are entirely communicable. The problem with this interpretation of objectivity is that it excludes noncommunicable knowledge from science, which is, as we have seen, the most important key for reaching understanding. Using Huxley's theory of knowledge as a framework, it is clearly wrong to think that only third-person knowledge corresponds to what is intrinsic to reality as it exists autonomously to any spectator. This mistake is not difficult to grasp once you comprehend the difference between knowledge and understanding, which is basically an intuitive act.

The legacy of the Cartesian split has increasingly affected the Western conceptual framework of reality and, most importantly, our sensibility for deeper truths: the delicate ability to capture purpose and beauty from nature, both of which are all the more mysterious, personal, and not directly transferable. Besides, as Huxley correctly claims, this problem is worsened by contemporary educators: "[They] have taken John Dewey's theories of 'learning through doing' and of 'education as life adjustment,' and have applied them in such a way that, in many American schools, there is now doing without learning, along with courses in adjustment to everything except the basic twentieth-century fact that we live in a world where ignorance of science and its methods is the surest, shortest road to national disaster" [4] (pp. 200–201). The Cartesian split and the primacy of objectivity have become an axiom, that is to say, a general premise, on which reasoning in the sciences and the humanities is based. But, using Huxley's criticism, the term *prejudge* is more accurate than *axiom* because it is more a cause of cultural ignorance and desensitization than of cultural progress.

Another turn of the screw is given in our modern way of thinking and feeling: less attention is given to non-applied knowledge, which is the same as saying that we suffer from a growing inability to value present reality, the here and now. Unfortunately, this was only to be expected: even the most exact and correct third-person knowledge makes no sense and is of no value if we throw out understanding. Thus, scholars have found it necessary to assign knowledge another value – an external one. Nowadays, any sort of search is mainly valuable in that it allows me to manipulate reality. Indeed, objectivity is being replaced by utility. By far, concern for practical knowledge – innovation as it is also called – far

outweighs concern for truth and, even more so, understanding, i.e., lived truth. In this regard, Huxley perfectly captured the independence of applied and non-applied knowledge: "There are in the lives of human beings very many situations in which only knowledge, conceptualized, accumulated and passed on by means of words, if of any practical use. For example, if I want to manufacture sulfuric acid or to keep accounts for a banker, I do not start at the beginnings of chemistry or economics; I start at what is now the end of these sciences. In other words, I go to a school where the relevant knowledge is taught, I read books in which the accumulations of past experience in these particular fields are set forth. I can learn the functions of an accountant or a chemical engineer on the basis of knowledge alone. For this particular purpose it is not necessary for me to have much understanding of concrete situations as they arise, moment by moment, from the depths of the given mystery of our existence. What is important for me as a professional man is that I should be familiar with all the conceptual knowledge in my field. Ours is an industrial civilization, in which no society can prosper unless it possesses an elite of highly trained scientists and a considerable army of engineers and technicians. The possession and wide dissemination of a great deal of correct, specialized knowledge has become a prime condition of national survival" [4] (pp. 199–200). However, though specialists are necessary and good, for Huxley, a society totally dominated by them is destined to slavery, boredom, and alienation – an ugly *brave new world*. In his most famous novel, he imagined a worst-case scenario in which everyone has been completely transformed into a specialist – a *hyper-specialists* is someone who knows all about their job and private life (i.e., is socially well-adapted and productive) but, at the same time, who does not understand anything at all.

The Neuroethics of Beauty

Dogmatic science has taken a Copernican twist that fosters all sorts of technified attitudes. However, nowadays another long-term effect of the Cartesian split, which is seemingly the opposite of utilitarianism, is found in the philosophical world. For many philosophers of the modern era, establishing a uniform methodology and adhering strictly to its terms are of utmost importance. Huxley coined this intellectual vice the *primacy of methodology, hereinafter referred to as methodologism*.

Then as now, there are academics, scientists among them, that are more concerned about the way than the destination. All that matters is playing by the rules and being consistent with the established framework of beliefs. The ultimate expression of this idealistic overview is Ludwig Wittgenstein's definition of philosophy as an *idol breaker*. It is at the service of science, with lowercase letters also. "The results of philosophy are the uncovering of one or another piece of plain nonsense and of bumps that the understanding has got by running its head up against the limits of language. These bumps make us see the value of the discovery […] The philosopher's treatment of a question is like the treatment of an illness" [8] (§119 and §133). Long gone are the days when philosophers were hunters of wonder and beauty. Now, the main focus is mind, arguments, and human logic – pseudo-knowledge, as Huxley would say. Nonetheless, he finds that both approaches – methodologism, present in universities, and utilitarianism, present in industry – share a disdain for ever-present nature. Dissatisfaction with what is real, a need for change, and a yearning for a better future lead to our current interpretation, valuation, and use of methods and tools. Conversely, methods and tools are taking us away from ourselves – alienating us – and, thus, truncating human beings' main sources of satisfaction. Both phenomena are cause and effect.

Neuroscience is probably the field in which the problems of methodologism and utilitarianism are most correlated. On the one hand, the study of the biological basis of consciousness (for biological, empirical should be understood) often results in a reductive perspective of consciousness in which *human experiences* are wholly identified with what we can learn from *laboratory experiments*. "Confronted by the data

of experience, men of science begin by leaving out of account all those aspects of the facts which do not lend themselves to measurement and to explanation in terms of antecedent causes rather than of purpose, intention and values. Pragmatically they are justified in acting in this odd and extremely arbitrary way; for by concentrating exclusively on the measurable aspects of such elements of experience as can be explained in terms of a causal system they have been able to achieve a great and ever increasing control over the energies of nature. But power is not the same thing as insight and, as a representation of reality, the scientific picture of the world is inadequate, for the simple reason that science does not even profess to deal with experience as a whole, but only with certain aspects of it in certain contexts" [7] (p. 28). Purpose and beauty are beyond the empirical approach's scope of direct analysis. Neuroscience establishes correlations between subjective impressions (such as intentions or feelings) and objective brain processes. For Huxley, that is no small thing: antecedent causes are part of what we are and, most importantly, of what we can do. However, without intuitional experience of purpose and beauty, the human material dimension and human projects become surreal and bizarre. When methodological reductionism ends in ontological reductionism – a sort of methodologism – then truth no longer matters, and power takes its place.

The preceding argument is extraordinarily important in the neuroscientific study of human decision-making, where three transcendentals come together to give full meaning to human behavior. From the measurable perspective, knowledge no longer describes reality. Therefore, truth ceases having an active role in decision-making. Intuitions about the truth or falsehood of any proposition come to form part of archaic superstitions, which are better left behind in order to survive. As many euphemistically claim, "truth statements only have functional value." Similarly, real purposes are removed or, if one prefers, reduced to complex antecedent causes. Among other consequences, what was known as human freedom ceases: one's own law reduced to physical law or at least to a series of specific actions in human biology – *autonomy*, as compatibilism's defenders call it. Intuitions about moral responsibility are, in this light, mere delusions of grandeur. "Human beings, it is more or less tacitly assumed, are nothing but bodies, animals, even machines; the only really real elements of reality are matter and energy in their measurable aspects; values are nothing but illusions that have somehow got themselves mixed up with our experience of the world; mental happenings are nothing but epiphenomena, produced by and entirely dependent upon physiology; spirituality is nothing but wish fulfillment and misdirected sex; and so on" [7] (p. 29). Finally, beauty is reduced to aesthetics, i.e., to purely subjective emotions and feelings – if anything, the only truth of our emotional life is its usefulness for survival. Therefore, there are no authentic feelings – true emotions – about the people that matter to us or about the war and violence that devastate our world. They only speak of the observer. There are no beautiful eyes or ugly, polluted cities. Hence, our motivation for action also comes from antecedent causes: we are not attracted by beauty but rather triggered by neural networks.

There is an important connection between the neuroscience of ethics and the ethics of neuroscience: this triple shift in perspective that neuroscience introduced – no matter if true or false – already influences people and organizations' decisions about truth, freedom, and love. However, it is not the only bridge. Many lines of exploration in neuroscience have to do with applied research, including therapeutic, as well as nontherapeutic, uses of biotechnology. However, without intuitions about nature, concepts such as *health* or *enhancement* become quite problematic because functional criteria are also subjective to circumstances and individuals. There are no guidelines for the use of technology; reality, human rights, and love are no longer baselines or reference points.

In short, a better-oriented neuroscience of decision-making would help us face many ethical challenges that will emerge in the very near future. Huxley hits the mark when he proposes that we should redefine the issue from mind/brain perspective to intuitional/processual perspective. The problem is not how consciousness emerges from the brain, how the brain produces consciousness, or how the brain and consciousness are gathered together but rather how intuitional and processual acts are related. Are intuitions and processes two sides of the same coin – dimensions, features, etc. – or, on the contrary, do they respond to different non-reducible entities or principles? Is the human brain more than an organ charged for operating processes or does it need to be connected to another thing to think? These are perennial questions.

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13

Radical Solutions to the Ontological and Epistemological Problems of Consciousness

Javier Andrés García Castro

Explorations into consciousness have a remote history, which probably begins with the first mystical adventures thousands of years ago in the context of magical and religious practices. However, interest in this philosophical and scientific study is relatively more recent. We can date it to the seventeenth century thanks to the work of the French philosopher René Descartes (1596–1650) and to the end of the nineteenth century with the foundation of the first laboratory of experimental psychology in Leipzig by the German psychologist Wilhelm Wundt (1832–1920), respectively.

Problems in the study of consciousness have been a constant that has generated deep discouragement to those who have tried to approach its ontological and epistemological roots. Suffice it to cite as examples the gnoseological pessimism of behaviorism of the first half of the twentieth century, the currents of eliminative materialism, and *mysterianism* or catastrophic predictions of some sectors of neuroscience, impotent before the task of explaining the emergence of subjectivity as a product of a synchronized discharge of various populations of neurons. These and other predictions, arising from a debate that has been described as "dead end" ([1], p55), place us before a bleak panorama in which many researchers conclude their work with an air of frank resignation [2–4].

Now, what are the problems of consciousness? We assume that it is not a single problem, but several, and all of them have deep roots, from both a neuroscientific and a philosophical perspective. These problems have been stated in ontological and epistemological terms. Briefly, ontology means the study of the reality of the world, that is, what is in it, while epistemology refers to the ability to obtain an objective knowledge of that reality. While from the philosophical point of view consciousness is ontologically objective and undeniable, from the epistemological point of view, it is a subjective phenomenon, difficult to address scientifically [5]. However, the supposed ontological objectivity of consciousness could be denied since we still do not have an objective and universal criterion to determine the self-conscious capacity of an organism, entity, or object in the known universe, beyond the famous and limited Turing test [6]. Modern versions of this test propose that, to find out if an organism or entity is "self-aware," it must be tested by another organism that we know for sure is self-aware [7]. Note, however, the *regressus ad infinitum* of this proposal. Therefore, except each one privately and subjectively, no one can be sure that the other is self-aware [8], and, accordingly, we do not currently have a valid and reliable criterion to prove

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the presence of self-awareness and/or phenomenal consciousness in other beings or devices.[1]

The objective of this chapter is to offer a recapitulation of the most groundbreaking solutions that have been proposed to try to solve the various problems posed by the study of consciousness. For this, the fundamental difficulties that this research field entails will be exposed, and each one of them will be answered from different theoretical approaches. Finally, a critical assessment of each of these contributions is proposed, and the possible lines of theoretical and empirical approach that are expected in the coming years are outlined.

The Problems of Consciousness

More than a few renowned philosophers have referred to the problem of consciousness in the singular [10, 11], when in reality the real problem of consciousness is that it is more than one problem, beginning with its conceptualization. Accordingly, there is currently no consensus on what could be the definition that best characterizes this elusive mental phenomenon [12]. In any case, this is not due to the lack of proposals, which have been numerous. However, none has been fully able to capture that which they aim to define; thus, the majority is inclined to delimit the concept by resorting to a mere list of obvious properties. Among them, its unity and continuity; its private, personal, and subjective nature; its coherence; and its ability to integrate multiple sensations and perceptions [13] should be highlighted. Indeed, and as William James [14] affirmed, private and subjective character, together with the sense of unity and continuity, are some of the problematic nuclear features of consciousness, though not the only ones.

The following section describes each of these problems. In this review, they have been reduced to three: the problem of reality, the problem of dualism, and the problem of the subjective quality of conscious experience (*qualia*).

[1] See however [9].

The Nature of Reality and Its Relation to Consciousness

The first difficulty that we must take into account when studying consciousness has to do with the correspondence that exists between the outside world or reality and the inner world of the subject or representation. Various philosophical currents have tried to address this issue, which remains immersed in deep debate. Thus, the problem of "double access" ([15], p291) raises the difficulty of verifying the fidelity of the "I-world" correspondence, precisely because we start from our own subjective representation of that reality and lack an external validity criterion that allows us to contrast the two. This and other evidences drawn from the research into perception, attention span, and certain hallucinatory phenomena [16, 17] question the possibility of a naive or radical realism. On the opposite extreme, *idealism* denies the existence of reality, which is reduced to a mere product of our thinking. Between the two, *constructivism* states that from information captured by the sensors, the cognitive system reconstructs a representation to some extent analogous to external reality.

In relation to consciousness, neuroscience postulates a materialistic directionality by stating that the brain creates consciousness [18]. This directionality is not new at all [19] but has been accentuated in recent decades. Faced with this point of view, authors who reduce all possible knowledge to subjectivity have not been lacking in the philosophical tradition [20] nor have those who consider that consciousness uses the brain as an instrument to self-manifest in the course of an evolutionary process [21]. A third way to resolve this directionality is that defended by Francisco Varela through the existence of a mutual overlap between mind, brain, and world, based on the concept of "embodied" mind, that is, one inextricably linked to a body [22].

To further complicate matters, the findings of quantum mechanics in the early twentieth century introduced notable difficulties in articulating the micro-phenomena of physics with macro-phenomena, extending their implications to the very study of consciousness. Among the propos-

als that have been made, Roger Penrose and Stuart Hameroff stand out. They suggested, through their *model of orchestrated objective reduction*, that consciousness could be explained from quantum computing processes [23]. For Penrose, consciousness, such as thought or creativity, is a non-computable mental phenomenon, since algorithmic computing contains a deterministic element that is incompatible with freedom and creativity. These authors, therefore, state in their hypothesis that consciousness arises as a result of quantum coherence in auspicious structures that make up the cytoskeleton of all cells of the human body: the microtubules. Microtubules are essential for a wide variety of biological functions that include cell displacement, mitosis, and maintenance of cellular form and functions. In addition, in neurons, they contribute to "maintain and regulate synaptic plasticity related to learning and other cognitive functions" ([24], p1872). The proteins that make up the microtubules are called tubulins and can adopt two possible configurations, α and β, which could be made equivalent to the binary computing system "0 and 1." The quantum computation developed in the microtubules in an isolated and superimposed way could be generalized to the whole brain, giving rise to a large-scale physical activity in accordance with the quantum nonlocality. This state of quantum overlap would suffer an objective reduction orchestrated by the molecular structures of the microtubule (tubulin) proteins to move to a conscious state thanks to a quantum gravitation mechanism, which would generate self-collapse by latent nonlocal variables [25]. All this would lead to the irreversible transition from a state of preconscious overlap to a reduced one that would coincide with the state of conscious experience in the phenomenal world of macrophysics (Fig. 13.1).

The efforts undertaken to try to explain consciousness from microphysics are abundant, and recent proposals have been made that attempt to overcome some of the intrinsic difficulties identified in the previous theory and other similar hypotheses [26].

Faced with the enigma of the ontological status of reality and its relation to the mind-body problem, Donald Hoffman has constructed a mathematical theory which he calls *conscious realism* [27]. For this author, consciousness is composed of three processes that he assumes as true in an axiomatic way by intuition: perception, decision, and action. From here, he defines the key concept of his hypothesis: the conscious agent. A conscious agent, when interacting with the world (W), has a conscious experience (X) that triggers a decision process (D), which consists of a deliberation on what courses of action to take. The possible course of action translates into an effective action (G) that transforms the world (W_i), which in turn will alter the subject's conscious experience (X_i). These processes are developed in spaces of probability, and the messages that are transmitted between the components (W, X, G) can be counted by a number N measuring the flow of information through stochastic channels that connect each node of the conscious agent (P, D, A) (Fig. 13.2).

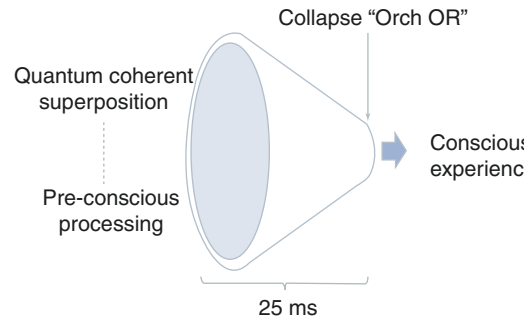

Fig. 13.1 An orchestrated objective reduction event that produces a conscious experience

However, the theory thus formulated would fall into dualism; on the one hand, we have the world (W) that is described in classical physics and, on the other, conscious (X, G), private, subjective, and ineffable experience. But, the hypothesis of conscious realism aims to be a monism in which consciousness is ontologically fundamental and thus overcomes the difficulties posed by the mind-body problem from materialism and its relation to reality. Therefore, Hoffman proposes replacing the world (W) with the dynamic interaction of conscious agents. Thus, the decisions and actions of a conscious agent would constitute the experiences of another conscious agent and

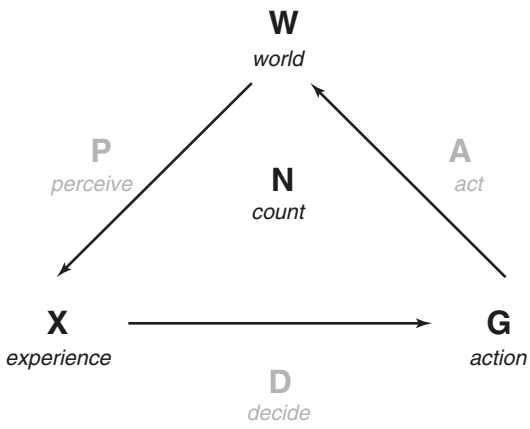

Fig. 13.2 Diagram of a conscious agent [27]. (Adapted with permission from the original author. Orignally published under a CC-BY license)

vice versa. The world, consciousness, would be composed only of conscious agents in reciprocal interaction.

To illustrate this idea, Hoffman uses the split-brain analogy, which consists of an interhemispheric communication difficulty secondary to a surgical intervention that cuts the corpus callosum. According to this author, this phenomenon reveals the existence of two conscious agents who interacted before generating the impression of a unified consciousness. In short, for Hoffman, the "subject-world" correspondence arises because consciousness creates brain activity and not vice versa, as neuroscience defends.

If consciousness, in addition to self-awareness, is also of the objects and entities of the outside world, it seems obvious that any research on consciousness must deal with the supposed objectivity of reality. Both physics and psychology have provided convincing evidence that, for the moment, we must not literally interpret the stimuli of external reality. In addition, studies on perception have shown that organisms have not been evolutionarily selected to perceive reality as it is but to optimally record those stimulus configurations that are most advantageous for their adaptation to the environment and survival [28]. A multidisciplinary approach, therefore, is necessary to continue moving forward on this issue.

The Problem of Dualism

Another of the great and traditional problems that have arisen to explain consciousness is dualism. Although it is a metaphysical interpretation already present in other cultures and eras, Descartes [29] establishes the modern distinction between res cogitans and res extensa, two independent but interacting substances, subject to different laws and principles and with different properties. The other possibility, monism, tries to explain and reduce all the phenomena that exist to matter, and therefore, subject to deterministic physicochemical laws. While dualism cannot satisfactorily explain the interaction between two substances of different nature, monism has not been able to complete successfully its reductionist project.

Cartesian dualism was harshly criticized by the philosopher Gilbert Ryle in the mid-twentieth century [30], leading to a current of animosity that would extend to the entire neuroscience research program. However, if monism cannot account for the main problem when studying consciousness – that is, the emergence of subjective experience from a physical process – many authors consider that the most reasonable alternative we have left is the dualism. So much so, neurosurgeon Wilder Penfield [31] goes on to state that dualism, perhaps, is the lesser evil of the existing solutions:

Taken either way, the nature of the mind presents the fundamental problem, perhaps the most difficult and most important of all problems. For myself, after a professional lifetime spent in trying to discover how the brain accounts for the mind, it comes as a surprise now to discover, during this final examination of the evidence, that the dualist hypothesis seems the more reasonable of the two possible explanations. (p123)

To resolve this controversy, numerous proposals have been formulated. Attempts to reconcile dualism with the contemporary scientific method have led to the interactionist dualism of John Eccles [32], who proposes interaction between the mind and the brain as two independent and autonomous substances. The soul (mind) would

act on the brain through a mechano-quantum field altering the probability of release of presynaptic vesicles. In turn, the body (the brain) would influence the mind through the process of exocytosis of presynaptic terminations on receptive fields of cortical neurons, which would be detected by the quantum field of probability producing a mental and conscious event.

For his part, Nicholas Humphrey [33], in his attempt to solve the mind-body problem, states that consciousness is actually an emergent property that arose as a result of the natural selection process and, therefore, with an adaptive function. This function is fundamentally social, although it later evolved by providing an internal model of itself. This process, presumably, would occur through the "internal monitoring of the body's external reactions to the outside world" (p19).

Another way to address the overlap between mind and brain is the so-called emergentism. For example, for Charlie D. Broad, phenomena can be explained mechanically or emergently. In the first case, observed phenomena can be explained from the complete knowledge of the properties of the components of that which is intended to be studied, while in the case of emergentism, this is not possible [34]. Although the classic example usually used to illustrate this idea is to obtain the liquid state of water from the covalent bonds of hydrogen and oxygen atoms, in a sense, it should be expected that the properties of these elements, when combined, produce that effect. This, however, does not occur in the case of the mind, since the same laws that govern the behavior of neurons do not have the status they should to justify the onset of mental states. Therefore, it is proposed that certain systems have associated new – emergent – properties, although the mechanisms that produce them are not explained.

From neuroscience, on the other hand, we work around the concept of neural correlate of consciousness (NCC), which currently accepts at least three different possibilities: (1) identity, a mental state is equal to a brain state; (2) causality, a brain state causes a mental state or vice versa; and (3) correlation, both mental and cerebral states are related. Each of these possibilities is supported by a concrete theoretical-conceptual framework. The first two start from physicalism, while the last one implies, in one way or another, some form of dualism (interactionist, substance, property, etc.) or even the possible existence of a psychophysical parallelism. However, we must bear in mind that "correlation," as a condition of possibility, does not imply causation but is only limited to indicating the co-occurrence of two or more events that could be related [35]. In addition, if the concept of NCC is far from being clarified, much less will be the explanatory implications on consciousness around that concept [36].

All these difficulties seem to demand a change of epistemological paradigm. The dominant scientific method currently tries to explain the phenomena from the principle of causality. Thus, consciousness would be the result of certain neuronal operations [37]. Neuroscience, in general terms, seems to be limited to the correlational study of mental and brain events, without examining in depth the theoretical implications of its findings. If we could formulate the problem as follows:

(a) $\Phi = \psi$
(b) $\Phi \rightarrow \psi$
(c) $\Phi \leftarrow \psi$

being Φ = physical events and ψ = mental events, we could establish a discussion about the directionality of causality or even the possible identity of both phenomena, an issue that remains open. When talking about correlation, we thought, in principle, "of a linear measure of the association between two variables" ([38], p890). Therefore, it does not seem legitimate, at the moment, to speak of causal directionality.

Faced with the sequential view that prevails in Western epistemology, the principle of synchronicity implies that there is a correspondence between two simultaneous states of two different phenomenal systems [39]. This connection is not of cause-effect but of homology of two events that concur in the same instant. These approaches would open the possibility to currents similar to psychophysical parallelism, according to which the physical and psychic processes are indepen-

dent, although they occur in a coordinated manner [40]. In this sense, synchronicity, understood as the "temporal coincidence of two or more causally unrelated events" ([41], p35), could be extrapolated to overcome the difficulties imposed by the concept of NCC. It would be interesting to investigate the necessary and sufficient physiological, spatial, and temporal conditions of this synchronicity. In fact, various investigations in cognitive neuroscience point to the need for a minimum time for the emergence of a correlation between neural and cognitive events [42, 43]. At the same time, neurophysiology has revealed the existence of synchronized discharges in certain neuronal groups with oscillations in the gamma band at 40 hertz when a moving object is perceived [44]. Whether these neuronal synchronies are causally decisive to generate awareness or not, or that they may have some link with the synchronicity between mental states and neuronal states, is something that has not yet been explored.

Following the line of what might be called neodualism, David Chalmers had already raised through his naturalistic dualism the possibility of investigating the mechanisms of interaction between subjective experience and the physical world [45]. Specifically, he proposed three psychophysical principles that could establish links between both dimensions: the *principle of structural coherence*, the *principle of organizational invariance*, and, finally, the *double aspect of information theory*. This last principle, of fundamental character, implies that certain types of information have a double structure in which there is a correspondence between the physical states and the phenomenal states. One of the corollaries of this principle is that wherever there is some kind of information, there could be some kind of equivalent consciousness, although this would be as rudimentary as that corresponding, for example, to a thermostat.

In a new proposal, Chalmers raises this relationship founded on the principles of quantum mechanics, based on the pioneering works of Eugene P. Wigner on the possibility that consciousness caused the wave function determined by the Schrödinger equation. According to its new model [46], in the universe, there would be a special property, called m-property, whose effect would be to respond with the collapse of the wave function every time it encounters an overlap state. Thus, for example, if a photon is in two overlapping positions (P1; P2), when it comes into contact with an m-particle, the photon would collapse into a defined state (i.e., M1-P1). The m-properties would be similar to the physical correlates of consciousness, which in turn should find neurobiological candidates that establish their relationship with brain mechanisms. One of them could be the phi (Φ) measure of the *integrated information theory* of Giulio Tononi [47], which provides a dimension of the amount of consciousness generated by a system. Here consciousness, as a phenomenal state, is taken as an intrinsic property of physical systems, being the result of the degree of integration of information into that system. Therefore, according to this model, consciousness is understood as a higher order function of physical systems, such as the brain, determined by its functional ability to exhibit the collapse of the wave function thanks to the existence of the m-property.

In sum, there are currently as many difficulties as arguments in favor of dualism as of monism. Adopting the attitude of denial as a solution, as proposed by eliminative materialism, is not convincing in the face of Cartesian evidence of self-consciousness. The inconsistencies of the concept of neural correlate to conform to the linear causality scheme imposed by the methodological framework of Western science should not be an obstacle to persist in neuroscientific research but rather an incentive to reform a possibly inappropriate epistemological paradigm which must be expanded and reformulated. Thus, the progressive conceptual enrichment derived from the convergence between apparently distant theories could be a crucial starting point to unlock an atavistic antinomy [48].

Qualia: The Subjective Quality of Conscious Experience

No less thorny than the other two is the problem of the subjective quality of conscious experience, what philosophers have called *qualia*. Indeed, the explanatory leap between the aseptic functioning

of neuronal computing and the intimate and private sensation of perceiving inwardly the quality of a color, a taste, or a pain is one of the greatest difficulties that physicalism has encountered in all its aspects [49]. From this perspective, the most thorough investigation of the neural intricacies of the perceptual pathways in the brain can tell us nothing about *qualia* [50]. Beyond the axon tracts, the exchange of neurotransmitters and the feedback and feedforward mechanisms, the mysterious sensation of *what it is to feel like* will always remain in the air; it seems to escape, for the moment, the methods of modern science.

At this point, many authors conclude in a logically impeccable way a reasoning that starts from questionable premises: consciousness does not exist; it is a mere epiphenomenon; it is not relevant [51–53]. However, at least since Descartes, the subjective experience of the world and of oneself is something clear and evident. Some contemporary authors have even hinted at the possibility that consciousness is a fundamental property of the universe, such as mass, electric charge, or space-time continuum in physics, thus approaching panpsychism [54].

A possible solution to this dilemma could be the intersubjective agreement reached through a neurophenomenological approach to the problem, a well-known philosophical tradition that has recently been revitalized in the field of cognitive neuroscience and the philosophy of the mind. Indeed, at the beginning of the twentieth century, the German philosopher Edmund Husserl founded the phenomenological movement in response to the epistemological limitations of positivism, materialism, and psychology [55]. Phenomenology questions the validity of everyday metaphysical and epistemological statements and tries to go to things themselves as presented to consciousness. By suspending the trial (*ἐποχή*), the belief that sustains the natural attitude about the objective existence of the world is in doubt. The goal is to obtain sufficient knowledge in itself, an absolute fact on which to build the immediate experience. This process is called *phenomenological reduction*.

More recently, the philosopher Thomas Nagel [56] has suggested the possibility of developing an objective phenomenology that allows a description of the subjective nature of the experiences in such a way that it is understandable to other beings lacking such experiences. Perhaps collecting the witness, neurophenomenology proposes a research program capable of articulating the relationships and mutual restrictions between the phenomenological experience and the findings of cognitive neuroscience [57]. Thus, one of the fundamental attitudes of the neurophenomenological method is that it does not persist in the "objective-subjective" opposition, but rather seeks to go further and cover the explanatory gap between the two from its fundamental correlation.

The working hypothesis of neurophenomenology would be to explore the structure of experience and its equivalents in cognitive neuroscience in order to "formulate relational principles and laws between the two to resolve apparent contradictions" (p343). The novelty of this proposal would be that the explanations of "first person" should be included as fundamental elements of validation of the neurobiological findings of "third person" and not as mere accidents in the course of experimentation. The aspiration to find a way to reconcile objective and subjective data in a unified epistemological framework is common in various authors and fields of knowledge and, therefore, is an ideal working field to search for convergent theoretical spaces. All this suggests a stereoscopic perspective in which both conscious experience and cognitive science must become active partners in a new way of understanding the relationships between the mind and the brain.

On the other hand, if we accept the existence of a phenomenal level of organization, within a stratification of increasing complexity between different ontological levels, new and original approaches are necessary to capture the essential properties of consciousness. According to Antti Revonsuo, the exploration of dream activity during sleep, particularly during the REM phase, which is when narrative dreams occur, could constitute an adequate model of the proposed phenomenal level of organization [58]. This is so because the dreaming brain doesn't need neither sensory input nor motor output to produce phenomenal consciousness. This could provide us

with the more accurate possibility of identifying the processes that are sufficient to produce subjective experience. In this line, new technologies such as virtual reality could offer us methodological opportunities to open unexpected fields in the study of consciousness. For example, Jeremy Bailenson's group has carried out interesting experiments in which, through a conscious change of perspective using virtual reality, they managed to increase the prosocial behavior of the participants [59]. In this sense, the development of technology can contribute to exploring aspects of consciousness hitherto inaccessible to experimentation.

Discussion and Conclusions

In this brief review of the different alternatives offered to problems in the study of consciousness, we have focused on three fundamental difficulties and some of the most radical solutions that have been proposed. However, other problems have not been considered here, although in one way or another they could be understood as precursors, related to or derived from those presented here (i.e., binding problem, free will, or self-consciousness, among others).

Faced with the problem of interaction between reality and consciousness, various authors have proposed solutions from the peculiar properties of particles at the subatomic level of quantum mechanics. Perhaps their biggest problem, as mentioned in various places [60], is that these theories try to explain a mystery through something even more mysterious, such as quantum mechanics, based on future advances that do not currently exist. In addition, they rely heavily on physical explanations, neglecting their link with the data that neuroscience research continues to generate [61]. However, a comprehensive explanation of consciousness will have to rely, eventually, on the ultimate foundations of the universe's matter or, at least, be in some way consistent with them.

The dualism-monism debate has been entrenched for centuries. It does not seem that new data or proposals generated from the same epistemological context can produce a satisfactory solution to this perennial problem. Although outlawed in the field of neuroscience, dualism remains in force due to the inadequacies of physicalism [62]. Therefore, a reformulation of the epistemological framework that allows us to overcome what has also been described as false debate is necessary. This would open new possibilities to other explanatory frameworks in which events do not follow a linear logic of causality but a spatiotemporal coincidence of transversality. The possible mechanisms of this synchronicity and the ontological nature of the two states, mental and physical, are issues that need even more radical proposals.

In relation to subjective experience, the acceptance of the privacy and subjectivity of conscious states does not imply that we cannot investigate the necessary and sufficient conditions to generate consciousness. It means that describing these conditions is not the same as producing those experiences [63]. Moreover, a phenomenological approach to the states of conscious experience may allow us, over time, to generate intersubjective communication codes that make possible the transfer of interspecies experiential qualities. Each level of organization requires levels of analysis, methods, and particular theoretical developments that should not become antagonistic and incompatible rivals but, rather, allies capable of building bridges that enable a global and articulated understanding for a complex problem.

On the other hand, emergentism has been repeatedly proposed as a panacea for the various problems of consciousness and, more specifically, to circumvent the explanatory gap. However, the difficulties of this formulation are notable. Among them and like most materialistic proposals, they merely expose the magical appearance of consciousness as an emergent property of the interaction between the different parts of a system, without really explaining how it arises or why [64].

In short, both the diversity of problems listed and the proliferation of theories to address them suggest that the science of consciousness is in a still immature stage of development [65]. The progressive integration between different epistemological frameworks, such as the case of neuroscience and cognitive psychology or the fusion between some metaphysical theories with the principles of quantum mechanics, indicates the

possible existence of a convergent movement that could result in new approaches that we do not contemplate today. At this time, creative and groundbreaking proposals that challenge conventional practices in both the philosophy of science and empirical research are more necessary than ever. The proliferation of new technological tools opens up unknown horizons for experimentation, whose data should enrich and improve the theoretical discussion. Such proposals should stimulate and generate exciting lines of research in the not too distant future, in order to overcome the difficulties posed by the different and numerous problems of consciousness.

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A Discussion of Anxiety Over the Last Millennium (1000 to 2000)

14

Michel Bourin and Monique Bourin

Introduction

Current forms of anxiety often seem to be related to our way of life. Is this reality or mythology? Did people really suffer from anxiety about the end of the world around the year 1000? Medieval texts are discreet about emotions, especially the earliest of them. It is true that the Christian religion imposes the notion of the Last Judgment and, therefore, of the end of the world, but it is difficult to rely on contemporary written testimonies to think that people sat around full of fear imagining the forthcoming apocalypse. These days, we think, in fact we know that the solar system will ultimately disappear, that the earth can be threatened by the fall of an asteroid or that we risk collective death following a nuclear accident; these threats are ever present within our current consciousness. In the face of these potential but real dangers, only a small proportion of humanity is afraid, often in a more intellectual than perceptual way.

Around the year 1000, the Christian church may have exploited this anxiety to make their parishioners fearful, motivating them to donate goods to the monasteries in exchange for redemption of their souls. However, finding evidence to support this hypothesis is difficult. If such anxiety generated by talk of the Last Judgment existed, it manifested itself well after the year 1000, toward the end of the eleventh century, when preparations for the First Crusade were being made. The minds of the people were dominated by forecasts of the apocalypse; no one doubted it. Some indulged in learned calculations; indeed, several periods had already been predicted as possible apocalyptic times. But the counting of the years since the incarnation of Christ was not uppermost in their minds: those who wrote the acts of sale and transfer of the goods, all members of the clergy, counted according to the years of the reign of the living king or according to a calendar of Roman origin. These figures had no doubt a very strong arithmetic and symbolic meaning for the most learned clerics, who were used to calculating the date of Easter, around which the entire Christian liturgical life was organized. Once again, there is very little material to testify to their fear of the approaching millennia. For the others, most of the population, the coming years were much less important than the cycle of the seasons. Daily life carried on as before.

According to the chronicles of the time, people could be the subject of collective panic, especially if they thought they were receiving heaven-sent signs from the cosmos. This

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happened to the troops of the Holy Roman Emperor, Otto I, during an eclipse in 968 [1].

The Death of Oneself

We should distinguish the anxiety created by thoughts of an end of the collective world against the anguish of our own individual death. In the year 1000, people knew that they were mortal. Each disease could have a fatal outcome, and death during pregnancy or childbirth was a common occurrence. At that time, life expectancy was low; death could strike at any age, so the priority was to entrust one's soul to God, to be ready always for the unexpected and possibly sudden passage to the other side. "She kills the young man or the adult in full force, he must make his pact with the Almighty well before he has time to become decrepit as an old man" as Emmanuel Leroy Ladurie once said [2].

In medieval times, did the constant threat of imminent death arouse permanent feelings of anxiety or, on the contrary, lead to its resolution? No doubt neither, but some sense of painful awareness, even within monastic circles, more than anything else tempered this notion. Imagine that we are, in the year 974, present in the large Lorraine monastery of Gorze. The abbot, Jean de Gorze, is dying, morally serene, but he suffers: "Troubled hearts, affecting our sick minds, no longer able to breathe the winds of reason …….. everyone feared only for himself." Hope, cultivated by these monks, namely, that a life of piety would lead to a sweet death without suffering had just vanished forever.

In current times, our death is more "programmed." We develop the feeling little by little, especially as we age, that we are getting closer to our final reckoning. This phenomenon of habituation does not remove the anxiety, quite the opposite in fact. It is not uncommon to hear an individual tell us that he is not afraid of death, so why talk about it?

Today, dying and death are mostly hidden from view. There is rarely anything comparable to the long procession of little people who visited Jean de Gorze as he lay dying, welcoming them from his bed "as much as the disease allowed him" [3]. Then came the time of Jean's "private" death, Jean gives leave to the crowd and is assisted in his final moments only by a small group of relatives. A very similar example is the ceremony held for William the Marshal a few centuries later. Should death be on display or hidden; is the difference so great? One might think that the sudden occurrence of death is more agonizing if it is not a regular presence in our lives. In fact, the panic that was evident in the monks attending Jean's dying emphasizes that one does not get used to the idea of his death nor of his suffering that preceded it.

Fear of Old Age

Today, old age often brings physical and intellectual decline, although we should be careful to note that the condition of an 80-year-old person of today is probably equivalent to that of a person of around 50 years of age during the medieval period. The fight against aging is a recurring theme in advertising, targeting women more than men. Beyond the age when we struggle not to grow old, there are far more surviving women than men [4].

The medieval image seems different: old age is valued, and it does not appear that women outnumber men in old age. (Fig. 14.1) Certainly, contemporary texts are full of references to widows, dynamic and liberated by the death of a husband who until then had hidden, according to

Fig. 14.1 Fear of aging

preserved documents, their real influence in society. But it is mainly the inequality of age at marriage that gives rise to this survival of the wife over the husband; they are widows, but not necessarily very old.

The noble old man existed too. There were even some who almost became centenarians. Evidence of impotence and senility in old age is not absent from contemporary documents; one can rightly imagine a population affected by hard work and physical effort, worn out early. But the dominant image is that of self-control and wisdom. The impetuous impulses of youth are finally tamed by reason. The old are the bearers of memory: they are witnesses of the customs, and their testimony, in a highly oral civilization, remains the foundation of this custom. In fact, the old man was an individual who was able to avoid the pitfalls of disease, especially infectious diseases such as tuberculosis or various forms of dysentery. Old age conferred on the individual a feeling of invulnerability and could be perceived by the youngest as a sign of divine election and therefore of wisdom. During the Middle Ages, old age is precious because it is rare; it is a biological triumph for those fortunate enough to make it that far.

Life expectancy in today's world has increased, and reaching old age has become the norm for most of us [5]. However, it does come with an increased risk of brain cognitive diseases (e.g., dementia). On the other hand, the likelihood of poverty in old age and a lack of descendants to ensure our well-being are much reduced. The death of a child is a major emotional event; we do not expect our children to die within our own lifetime leaving us to contemplate old age in the loneliest of circumstances [6]. Nevertheless, it is less likely to be the miserable and anxious existence than it was in times past.

Fear of Starvation, Sickness, or Deprivation

The fear of lacking the essentials of life, the fear of destitution for oneself living in solitude or for one's family, was undoubtedly strongly present in the medieval consciousness. Hunger is still prevalent today in third-world countries, but it is not generally a collective fear of Western societies.

Medieval communities lived in constant fear of a failed harvest [7]. Modern societies recognize it in a very different form which is specific to our agricultural industries; however, the effectiveness of the fight against fungal and other diseases has to some extent alleviated it. In extreme cases, a poor harvest results in scarcity of agricultural produce. The opening of trade after the eleventh century no doubt helped to reduce the number of occasions when hunger and starvation intruded into people's lives. Also, communal anger against hoarders, which manifested itself in revolts against unpopular regimes, reveals the latency of this anxiety of hunger, if not death by hunger.

But the risks to the individual of disease are undoubtedly pervasive, more so than famine [8]. The threat of somatic diseases or disabilities caused by accidents was, for the man of the year 1000 and until the eighteenth century, a major source of anxiety. The consequences could be disastrous as his resultant inactivity and therefore his nonproductivity could make it difficult or even impossible to feed himself and his family. This concern had probably been increasing because there existed in the Middle Ages forms of intra- or interfamily solidarity, as in most so-called "primitive" societies; however, this form of collective support seems to have diminished, partly because of migration and the increased chances for individual enterprise in an economy where innovation was becoming increasingly rapid and prevalent. Orphanages were created in the fifteenth century by Saint Vincent de Paul [9]. It can be assumed that before this period, intrafamily solidarity helped to relieve the loss of parents through death. In fact, there were many uncles who acted on behalf of their nephews and who may be presumed, in many instances, to have accommodated and raised them. From the twelfth and thirteenth centuries, the "linen of the poor" and the various "charities," fed by the collection of alms, were organized within the parish framework to distribute help and sustenance to

those in need; this had previously exclusively been a function of the larger ecclesiastical institutions. Of course, this was only a palliative and as such a case of only treating the symptoms and not the root causes of people's suffering; therefore, it cannot be linked exclusively to the sharing of goods as advocated by Christian doctrine, because it responds in the first place to the social need to find a solution to the impoverishment of some of its members. This led to the creation of various forms of insurance against risk which relieved the grip of individual responsibility within the family but made it more vulnerable in the end. It was necessary to wait for the very recent arrival of health insurance schemes where the risk is covered collectively by a company and no longer by family-generated solutions. It is difficult to make judgments about collective solidarity (social security) versus family solidarity, in terms of anxiety.

Superstitions

An inability to understand natural processes appears to be a defining characteristic of society in the year 1000 [10]; therefore, one might conclude that this failure to comprehend the forces of nature contributed to anxiety in general. As humans developed a greater understanding of the natural world around them, did their improved knowledge serve to alleviate anxiety? We have roughly understood the mechanisms of contagion; so, do we need to get rid of the hypochondriacal fear of the disease? During the Middle Ages, a whole system was developed to deal with and hopefully reduce anxiety. A major worry was salvation: the inculcation of Christian doctrine into people's lives was unrelenting; people firmly believed that they could only achieve salvation through the intercession of the saints and the virgin with her protective mantle. Also, the increasing weight of Christology in the Christian religion contributed to a greater understanding of this God made man [11]. These were all ramparts set against the prospect of the eternal torments to be endured by Christians who had committed evil acts in defiance of religious law.

Fig. 14.2 Fear of pride sin

As regards anxiety generated by the fear of incurable diseases, people put their trust in the miracles and the virtues of the relics. Rites of blessing, processions, and rogations were all ways to reassure oneself. Epidemics or cosmic phenomena were interpreted as signs sent by God to correct a society increasingly attached to their worldly possessions and values and to reassure it through penance for their sins [12] (Fig. 14.2).

Superstitions may be a convenient way to explain phenomena we do not understand and a guide to behavior which reduces risk. Has not the "disenchantment" of the world placed a heavy responsibility on the shoulders of humans and created new forms of anxiety about the future? It is interesting to compare the anxiety related to the lack of understanding of phenomena versus the anxiety generated by speculative visions of the future. Science has helped us to understand many things including death, but it has not responded adequately to either the interrogation of the future of humanity or the notion of the beyond.

Weaknesses of Historical Documentation

An analysis of available documents, in order to develop an accurate picture of the anxiety experienced by the man of the year 1000, is very difficult. Indeed, until the Fourth Lateran Council (1215) made twice-yearly confession compulsory, thus leading to introspection and the direction of conscience (cf. Saint Louis), it was not customary to ask these types of questions; therefore, it is difficult to know specifically how

Fig. 14.3 Fear of hell

evil manifested itself in daily life. In any case, especially before the 1300s, the pen was still very much in the hands of clerics; if the "psychology" sometimes is evident, it is that of the clerks and therefore of a very small part of the population; or at least the psychological notations have been filtered via their way of thinking. When evil and demons appear embedded in their writings, it is expressed in the vocabulary of confrontation between good and evil, which corresponds poorly to current concepts [13]. The sources do not facilitate our objectives because the historical record provides a poor basis for our analysis of anxiety in those times (Fig. 14.3).

Shared fears within a society can sometimes lead to paroxysmal events. For example, it is easy to explain large or violent displacements, like those of the crusades, pogroms of Jews, or revolts against despised rulers. The historical records often describe clashes between clans or between classes. A psychological review of these events would necessarily involve collaboration between historians and psychiatrists.

Analysis of specific narratives about anxiety becomes even more complicated. Among the very rare medieval autobiographies, a text can enable us to recover semiological aspects of acute anxiety. What follows is Guibert de Nogent's description of the anxiety his mother felt when she learned that her husband had been taken prisoner by an enemy lord and would never see him again:

> One particularly dark night, when, overcome by this atrocious anxiety, she huddled at the bottom of her bed, the Devil, accustomed to attacking souls filled with sadness, suddenly arrived in person as her enemy and went to bed on top of her while she was still awake. With his enormous weight, he crushed her so much that she was almost dead. While her breath was cut off by this crushing weight and she was totally deprived of the free use of her limbs, she was literally unable to utter the slightest sound. She, constrained as she was by her muteness, could only plead for divine help. [14]

This reactive anxiety that occurs during sleep evokes a panic attack or acute anxiety attack. The translation done by Labande is very faithful to the Latin text that is presented to us. It would certainly be easier sometimes to tell our patients that their shortness of breath (dyspnea) is caused by anxiety related to the weight of the devil on their chests.

It is also interesting to follow, throughout the speech, the obsessional neurosis of the narrator, evident in his writing. It does not seem possible to equate it, as Kantor did, with the notion Freud developed that religiosity was obsessive [15]. This poses the problem of the timelessness of psychoanalysis. Indeed, it is difficult to evaluate the role of religiosity in a cleric.

Conclusion: What Remains of Our Millennial Anxieties?

The approach of a new millennium does not seem to have changed current human behavior in the face of worry, either as death approaches or as we head toward an increasingly uncertain future. In this respect, the transition from the first to the second millennium probably did not have any major impact either. But in the longer term, what does the future hold for us? Has the loss of faith and the development of "positive" rationality worried or reassured human beings? Anxiety became the "ransom of freedom," according to Kierkegaard, when it became clear that man had some power over his destiny [16]. Alongside the religious response to the anxieties of the present, to overcome or at least save a soul, another trend has emerged: the escape of the present not to the hereafter, but in a historically feasible future. Did the man of the year 1000 have more certainty? Probably not, but culturally he had no choice.

The fact remains that it is difficult, when examining the writings of the religious clerics, to find much of consequence regarding the nature of anxiety in the year 1000. Autobiography is probably the only tool that fulfills the conditions needed to identify the characteristic symptoms of anxiety, but it is a very exceptional genre in the Middle Ages. So how can we define the different manifestations of anxiety as they are described in the modern classifications of the psychiatric affections such as the American classification (DSM 5) or the World Health Organization (ICD 11)? Did social phobias exist? Certainly, such phobias could have been generated by the frequent intrusion of strangers transiting through medieval villages. Could there have been post-traumatic stress or panic attacks? It is not impossible. What could have been the degree of introspection within the confessional? Despite these difficulties, serious research remains to be done in the light of the current psychiatric culture, specifically, to gain an appreciation of what historians have started using the methods of social sciences other than history, such as anthropology or sociology.

Strictly speaking, the scope of this discussion paper, as presented here, does not amount to a history of anxiety through the ages. It is commonly accepted by the media or by the general population that we now have more risk factors in our environment due to stress, urban violence, and the vagaries of employment. It seems that the environmental effects have changed, but we forget that our living conditions, for most of us, have improved: housing, sanitary conditions, and food, with some notable exceptions since many people in the world are still dying of hunger. It does not seem to us that the background of human anxiety has changed through the ages; it is likely that our basic fears are the same. When consulting their doctors, older patients may express their fear of death with the paradoxical phrase "Doctor, I am not afraid of death." This continues to provide the most stable support for our anxiety.

Another question arises as to the evolution of anxiety; did the most anxious of our ancestors survive or, on the contrary, was it those who could best control their anxiety? The great changes in our societies have probably reshaped our anxieties, but it seems to be more the expression of our anxiety and its paradigms than profound changes in our anxious nature [17].

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Part II

From Basic Neurosciences to Human Brain

Neurotransmitters and Hormones in Human Decision-Making

15

Luis Felipe Sarmiento Rivera and Amauri Gouveia

Introduction

Neurotransmitters, neuropeptides, and hormones in humans are directly related to behavior. It is suggested that the ultimate goal of these chemicals is to increase the probabilities of individuals to access to resources, to mating, and to get social status [1]. The relationship between neurochemicals from the brain and body and behavior has been commonly studied using pharmacology. It allowed to understand in a putative way how each neurotransmitter or hormone affects behavior [2]. Specifically, in this text, how different kinds of these neurochemicals are associated with different kinds of human's decision-making is studied.

Making a decision is to choose between different kinds of options or possibilities, in order to try to have the best outcome. In the living beings, this outcome is related with adaptation, in order to improve reproduction and life quality [3]. Precisely, in human beings, from the beginning of the day, people are making decisions. Some of them are simple decisions like what to wear and what to eat and more complex decisions like how to invest the money or how to save the marriage.

There have been classified different types of decision-making, due to the different approaches which study it. Usually, they are classified according to the kind of decision that person is making. In this manner, it can be found moral decision-making, economic decision-making, and social decision-making. It is probable that there are studies of other kinds of decision-making. However, they are inside of this categorization or they became outside of this manuscript.

Social Decision-Making

Social decisions are a kind of decision that are made each day, and, as it is suggested by their name, they are related to interactions and social relationship. Many of these decisions are related to empathy, social cognition, cooperation, altruism, hierarchy, mating, and relationship. New researches are approaching to the study of them using neuroscience and psychological and economical paradigms, like the game theory. It presents tasks as dilemmas. In them the decision-maker has to find a solution. The prisoner dilemma is one of the most famous examples of this kind of paradigms related to cooperation.

> Two persons are arrested for being suspects of thief by the police; they are put in different rooms and they are not allowed to communicate with each other. The crime has a punishment of 10 years of prison. The police do not have enough evidence to make a definitive verdict; but they can put them on prison for 2 years by a minor crime. If both

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> prisoners remain loyal to each other, not betraying their partner, they will pay 5 years in prison, while if both betray each other, they will pay 10 years in prison. Then, they have this alternatives, if both of them betray each other they will pay 10 years; if both remain loyal to each them, they will pay 5 years; but, if one betray, and the other remain loyal, the betrayer will pay 2 years while the other one will be paying 10 years. [4]

Will the person betray or not his/her partner? These kinds of task are mainly focused on the social decision-making studies.

Moral Decision-Making

Moral decision-making has a strong component associated to social relationship. For example, there is a woman who see a 20 dollar bill, just at the side of a man toward her. Should she tell the person, who probably lost the bill, and pick it up for him, or should she wait for the perfect timing to pick it up and save it for her [5, 6]? This decision has a strong moral feeling, and they have been studied specially through moral dilemmas.

An example of a classic moral dilemma is this:

> A runway trolley is out of control, it going directly to five people, which are working on the road. They do not see the trolley, so, they are going to die if you do not do anything. You are next to a big person in a bridge above the road. You can press a lever, which make the big person fall down. He will die, but you will save the five workers. It is morally allowed, to move the liver and sacrifice one life in order to save the five lives of the worker? [7]

Now, the decision-maker has a variant, he has the same situation but without the lever; this time he has to push the big person out of the bridge with his own hands. Is it morally allowed to push out the big person in order to save the life of the five workers [7]?

Although the consequence of this action is the same, the answer of the people is that they tend to prefer to make the decision, which is to save the five persons when there is no physical contact with the victim, while they are more reluctant to involve in physical contact – pushing the big person. The utilitarianism is going to judge if the action is acceptable or not, holding it in the outcome of the action. In this case, it is allowed to kill a person in order to save five, while deontology judges the acceptability of the actions based on what is done, for example, to kill is bad regardless the objective [7].

Economic Decision-Making

Some theories and models have been used to study how people make decisions in financial markets and business, like expected utility theory and prospect theory [3]. This manuscript is not the place to analyze how decisions are made, if it follow the expected utility rules or not, unless to understand this kind of decision and its relation with neurochemicals. Thus, economic decision-making is related to the options the decision-maker has in order to make the decision. It is also related to the possible choices that he has and the different kinds of outcomes, being some or all of them unknown. In this case this kind of decision is under risk and/or uncertainty because the decision-maker doesn't know the whole possible outcomes.

To measure decision-making under risk, there are different kinds of tests used. Most of them are choosing between different kinds of stimuli. The most famous task is the Iowa Gambling Task (IGT). In this task are taking into account emotional and instinctive responses in the options. Other tests to measure decision-making under risk are the Balloon Analog Risk Task (BART) and the Columbia Card Task (CCT). The IGT is a test which has four decks, A, B, C, and D. Two decks are disadvantageous or risky desk, because they give big rewards but at long term they result in loses. The other two decks are advantageous because they give wins in the long term [8].

Oxytocin

Oxytocin is a neuropeptide composed by nine amino acids. It is mainly released in the brain. However, it is also released in other parts of the body. Oxytocin acts as a neuromodulator of the central nervous system. Moreover, it has the abil-

ity to form nexus with other molecules in the whole body [10]. It is synthetized by the hypothalamus in the paraventricular and supraoptic nucleus – which synthetized the vasopressin too [9]. It is later liberated by the pituitary gland in the circulation [2].

Oxytocin has the ability to relate with other hormones. In the paraventricular nucleus, the corticotropin-realizing hormone (CRH) is liberated, involved in the stress response, which can be liberated at the same time than the oxytocin. Moreover, this nucleus has connections with the amygdala and the hippocampus. These connections relate oxytocin with emotions. Levels of this peptide tend to be very high in the blood and brain. Besides blood level having variation between individuals, they tend to be a stable measure through time for this neuropeptide [10].

Currently, there is a hypothesis claiming that oxytocin has evolved for more than 700 million years. At the beginning, it regulates cellular processes, homeostasis, and water balance. Later, during brain evolution, it acquired other new functions and became responsible of the development of the brain cortex in relation to social behaviors [11]. Nowadays, this hormone is strongly associated with social behaviors [12, 13, 15, 16].

Social Decision-Making

There is a relation between oxytocin, in human and animals, as is mentioned by Barazza [12], and parental behavior, social attachment, affiliation, and long-term monogamous relationship [14]. Moreover, it is reported that administration of intranasal oxytocin produces more social proximity – being more near to others – in rats [17]. In humans, it is found that there is an increase in oxytocin blood measure when there are observed positive social clues like trust or reciprocity [9]. Furthermore, it was found that oxytocin modulates social attention, social perception, social memory, emotion recognition, and communication [13, 15]. It also has to be taken into account that in humans the presence of a social environment that suits with the interaction is crucial in order to associate oxytocin with social proximity [18].

Vasopressin and Oxytocin in Social Behaviors

Vasopressin is closely linked to oxytocin. Partly it is because this molecules share the same receptors, they have similar structures, and they are produced in the same place [9]. Moreover, as with the oxytocin, vasopressin is linked with social bonding, and together, both molecules are related to anxiety [19] and defensive behaviors [11, 20]. Furthermore, there is an association in the increase of both neuropeptides when a person receives a massage [21].

Relationship Between Trust and Oxytocin

The oxytocin is frequently linked to trust and social behaviors. Even though there are plenty of researches supporting it, the mechanism of how it works is unknown [9]. Regarding to trust, it was found that during the trust game (TG), a task focused on the trust between the participants [22, 23], the people who portray more trust were the people with higher oxytocin levels measured in blood. Furthermore, when the experimenters administrate synthetic oxytocin, people exhibited higher levels of trust in the TG [10, 14].

Moreover, it was found that oxytocin administration increases generosity in the ultimatum game [10, 24]. On the other hand, there are studies with oxytocin which relates positive attributes and trust to commercial brands [25]. In the same way, a trust increment toward the government and political leaders was seen, especially in participants who show lower levels of trust [14]. However, it is unknown how this relationship works because it was discovered that learning under oxytocin influence affects the brain reward system and it is hypothesized that, more than a trust increment, it could be a learning difficulty [26].

Empathy and Prosocial Behavior

There are many studies linking oxytocin with prosocial behavior and empathy. Increasing levels of oxytocin have relation with the increas-

ing in donations to philanthropic social institutions [12]. There is evidence that oxytocin release is linked to empathy, while participants of a study are seeing a film in which a child has terminal cancer [27]. In relation with social behavior, it is observed that administration of intranasal oxytocin improves social interactions of the participants [27].

Moreover, some studies show that oxytocin administration helps to improve emotional recognition in autism [28]. Other studies relate it with social memory and increase in looking to the eyes of another person [10]. In rats, oxytocin administration during adolescence causes an increase in social gaming, while there was a decrease in anxiety and social preference [17]. *Furthermore, it was found that oxytocin increases generosity* [29] *and social interaction in rats* [30] *and other animals* [31].

In this way studies begin to link oxytocin, mostly the intranasal administration, in psychopathology treatments of autism, schizophrenia, and social anxiety [32]. Finally, functional Magnetic Resonance Imaging (fMRI) studies show that the ventral tegmental area (VTA) presents the highest activation in relation to oxytocin and social behavior [33].

Social Decision-Making

Oxytocin is related to moral decision-making. It was found that the receptor gene for the oxytocin – OXTR – is linked to utilitarian decisions vs deontological decisions in moral dilemmas, and even, a correlation in a variation of the gene associated with the utilitarian judgment and harm in moral dilemmas was found [34, 35]. In this manner, genetics would have a contribution in the way that people make moral decisions. (Fig. 15.1)**.**

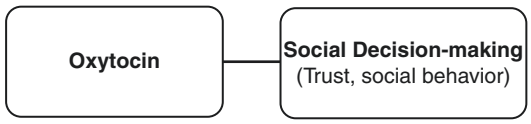

Fig. 15.1 Oxytocin is clearly related to social decision-making, especially with trust and reciprocity

Testosterone

Testosterone is a steroid hormone and the most common androgen. It is released through the hypothalamic-pituitary-gonadal axis; gonadotropin-releasing hormone is released from the hypothalamus into the hypothalamo-pituitary portal system; via this system, it is carried to the anterior pituitary gland; there, it induces liberation of gonadotropins by the anterior pituitary into the circulatory system, and posteriorly, the testosterone is secreted in the gonads [2]. It is not just linked to sexual and reproductive functions because it is also related with social behavior [36].

Absence or presence of prenatal testosterone has influence in the development of sexual dimorphism, and it has short-term and long-term repercussions [37]. Nevertheless, it only counts for a little part of the explication of sexual differences, which are not going to be discussed in this chapter. On the other side, this data could be used as an indicator for the understanding of why schizophrenia, stuttering, and autism are more prevalent in men. Besides, attention deficit hyperactivity disorder (ADHD) is 10 times more frequent in men than in women. These facts may also give an explanation of why anxiety disorder, affective disorders, and depression are more prevalent in women [2].

Testosterone and Social Decision-Making

Different studies have found relation between testosterone and humor, aggression, sexuality, and social decision-making [37]. In animals, it is linked to aggressive and nonadaptive behaviors, for example, testosterone increase in birds is associated with competitiveness in reproductive-related behaviors [38]; and, in rats, it has been seen having a relation with social dominance [52]. Despite this, in human beings, the testosterone has a weak and confusing relation with aggressivity [2, 37, 38], but it is linked to competitive behaviors [39].

A neuropsychological test has found that men tend to have higher punctuation in spatial skills compared to women. It has been suggested that there could be a link between testosterone and these skills. Furthermore, it has been observed that testosterone administration to women improves their performance in test that measures this skill. In the same way, social aggression is related to testosterone, and administration to women generates quicker responses to angry faces [37].

It was also found that higher prenatal testosterone levels in men were associated to what they decide to buy [41]; specifically, products related with determinate gender. They choose products more masculine and less colorful than other men exposed to lesser prenatal testosterone levels [40]. Persons which received testosterone administration compared with a placebo group have shown preference for brands which give them more social status than a similar product from another brand. Similar studies have suggested that there is cerebral differences in the sexual stimuli processing comparing two groups, one with higher testosterone levels and another with lower testosterone levels [37].

Reciprocity and Trust

Even though how it works is not understood, research suggests a link between reciprocity, trust, and testosterone [10, 42]. This link may be related to the fact that higher testosterone levels inhibit oxytocin release. It was found that dihydrotestosterone is associated with low levels of reciprocity. It may be the explanation of the higher trust levels shown by women comparing to men in the trust game [10]. Furthermore, it was also shown that trust decreases in women with testosterone administration in a test which portrays pictures of men faces. In the same way, there is a bigger quantity of rejects in the offers presented in the ultimatum game when the participant has higher levels of testosterone [42]. In men, testosterone administration diminishes the trust and also decreases the offer to the other participant in the ultimatum game. In the same way, the minimum offer received for the second participant gets higher, but, curiously, this effect was not seen in women [10].

Economic Decision-Making

It has been suggested that testosterone is related to economic decision-making [37], especially to decision-making under uncertainty. The test developed by Damasio [43], the Iowa Gambling Task (IGT), consists in a card game. There are four decks, two of them with some penalties to the higher rewards, while there is lower rewards and at the same time lower penalties, finishing in a sure winning. This test is a good measure of decision-making under uncertainty [44]. Men tend to choose more cards from the decks of lower reward in the short-term but higher reward in the long-term than women [45], and men with lower levels of testosterone are associated with a better performance of the test [46]. In young people, a higher risk-taking measure with the Balloon Analog Risk Task was found. It was associated with endogenous testosterone [47]. Nevertheless there are no other studies which find similar conclusions to decision-making under risk between men and women [48].

In more competitive situations, increased testosterone is associated with more risky decision-making compared with noncompetitive situations [49], even finding more financial risk [50, 51]. Nevertheless, testosterone rising, in competitive situation, is bonded to personal and social characteristic of the individual [53]. On the other hand, administration of testosterone in rats decreases their decision-making under uncertainty but also reduces their effort to reach a reward [54]. Moreover, there could be a link between propensity to take risk with the dopaminergic and reward system, because it was shown in rats that testosterone can joint to dopaminergic receptors D1 and D2 [37].

Moral Decision-Making

Testosterone affects the moral decisions and the moral judgment. People with higher testosterone tend to make more utilitarian decisions, especially if there is some aggression involved [55]. In another study, a relationship in women between testosterone administration and moral judgment was not found. However, taking into account the

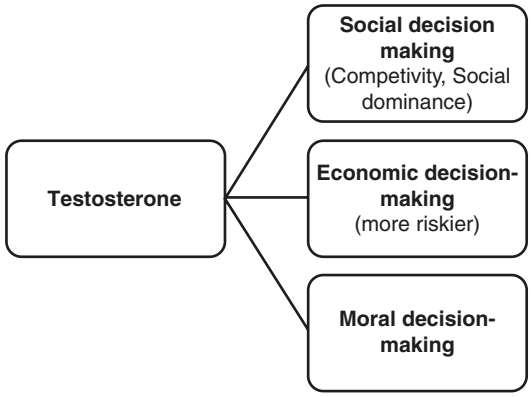

Fig. 15.2 Testosterone has relation with social decision-making, specifically in competitiveness and social dominance. It is also related to economic decision-making relating with higher risk-taking and with moral judgment

testosterone administration and a bigger difference in the digit ratio of the right hand's second to fourth – 2D:4D (the division of the second digit length and fourth digit length, which is related to prenatal testosterone exposure), it was found that they make more utilitarian decisions [56]. Then, prenatal testosterone is associated to utilitarian judgment. Even though the relation between testosterone and social behavior in adulthood is clear, it remains unknown how is it related to moral judgment made by people [57] (Fig. 15.2).

Arginine Vasopressin

Vasopressin and neuropeptides are synthetized in paraventricular and supraoptic nuclei from the hypothalamus. They are released in general circulation from the posterior pituitary [2]. Oxytocin and vasopressin brain circuits interact with gonadal sex hormones [58], and it has been found that in different kinds of mammals, this is important for the regulation of social behavior [59].

In rats a relation between V1a oxytocin receptor and social behaviors has been seen [60]. In reptiles, it is linked to the modulation of social behavior and parental care. However, in reptiles, the molecule is a homologue named vasotocin [61]. In zebra finches it has been related with affiliation behaviors [62]. In human beings, it has similar functions related to learning and social behaviors like social recognizing, parental care, affiliation, cooperation, and in psychiatric level with autism [58, 63–65] and anxiety [19].

Social Decision-Making

Cooperation

Arginine vasopressin (AVP) has been linked to cooperation. It has effects very similar to oxytocin, and it is thought that both of them are strongly related because they can share receptors. However, it is suggested that they have different neural circuits [66]. It has been found that intranasal administration of AVP increases task taking in looking for cooperation [64, 67]. Moreover, a higher cooperation in the prisoner dilemma in the second participant when it is administrated by intranasal oxytocin has been observed [27]. On the other side, differences between men and women has been found when there is intranasal administration of AVP, specifically in the lateral part of the insula and the right supramarginal gyrus [66]. In addition, using fMR studies, it was observed that, when the participants saw an emotional scene, charged emotionally plus the AVP administration, there was activation of the right amygdala, the ventromedial prefrontal cortex, and the inferior parietal lobule [59], structures previously linked to empathy.

Parental Care and Monogamy

It has been found that arginine vasopressin is linked with parental care [68, 69, 149] and monogamy. In studies with animals in relation to sexual fidelity, in the prairie vole, a monogamous specie, it was found that males with higher levels of vasopressin in the retrosplenial cortex V1aR receptors tend to have lesser partners outside of the original couple [70] (Fig. 15.3).

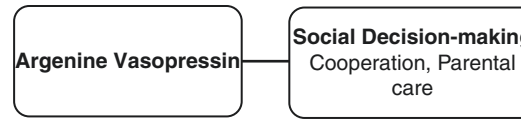

Fig. 15.3 Neuropeptide vasopressin is associated with social decision-making, specifically with cooperation and parental care

Serotonin

Serotonin is a monoamine known as 5-HT (5-hydroxytryptamin), derived from the amino acid tryptophan [71]. This neurotransmitter is specially located in the raphe nuclei, in the brain stem, and it is associated to cognitive and emotional processes, besides impulsivity, temporal discounting (the loss of value of a bigger reward delayed in time versus a shorter but immediate reward), and cooperation [73], all of them important for decision-making. Furthermore, serotonin is related to modulation of decision-making under risk [74–77].

Serotonin plays an important role in the most advanced functions related to behavior. In the most frontal part of the brain, the medial prefrontal cortex (mPFC) is associated with attention, direct behaviors toward a goal, planning, and cognitive control. This brain area has projections from subcortical structures as the amygdala, nucleus accumbens (NAc), and habenula. Another part of the prefrontal cortex, the orbitofrontal cortex receives projections from the anterior cingulate cortex (ACC) forming a circuit linked to emotional valences. These brain areas are known for the presence of serotonin in them. Serotoninergic neurons are originated in raphe nuclei, and these nuclei project to almost all subcortical areas including the NAc, amygdala, habenula, and mPFC. These serotoninergic neurons together with dopaminergic neurons are in charge of the prefrontal cortex modulation [78].

In psychiatry, serotonin has been related to psychopathologies [79, 80], such as anxiety and depression [81]. It has also been suggested that it is associated with the kinds of food that people consume, particularly, choosing which will benefit in the long term [82, 83]. In the same manner, serotonin plays a crucial role in inhibitory learning and behaviors related to aversive predictions, known as Pavlovian aversive predictions [81, 83, 84]. Moreover, drugs that inhibit serotonin reuptake tend to affect social behaviors like affiliation, social interaction, playing behaviors, reproductive behaviors, and parental care of the next generation [79].

Social Decision-Making

The role of serotonin in affective and social decision-making is highly complex; a relation with the valuation of aversive outcomes is suggested [81]. It is also related to mood, appetite, and sleep, and it is important for learning and cognition [73]. Furthermore, there are relations between serotonin and psychopathologies which affect socially to people [79, 80] as anxiety and depression [81].

Serotonin is an important modulator of social decision-making, especially in cooperation. In animals, it has been observed that the rise of tryptophan increases cooperation and affiliation. In humans, serotonin reuptake inhibitors augment cooperation in the prisoner dilemma [13]. In the same manner, tryptophan depletion reduces cooperation in the prisoner dilemma [77, 85]. Alteration of the serotonin will affect the results in the ultimatum game, making more rejection to offers perceived as injustice. In addition, it is suggested that it is very important to identify psychological characteristics of other people [9].

Impulsivity and Decision-Making Under Risk

The neurotransmitter serotonin has been linked with risk in decision-making [74, 86], loss aversion [87], and cognitive flexibility, focusing in reversal learning and temporal discounting [78], with self-control and impulsivity [88–91]. Decreasing the amino acid precursor of serotonin and tryptophan, there is an increased impulsivity [73] and aggression. Likewise, there are some studies linking serotonin with patience [92, 93].

Serotonin elimination in rodents using neurotoxins produces a high rate of immediate decisions that seek immediate rewards [13], equally to tryptophan elimination [75]. In humans, it was found that discount rate increases with tryptophan elimination, and it equally affects risky decision-making. Nevertheless, in humans, it increases loss aversion [14].

Specific serotonin receptors as 5-HT1B and 5-HT_2 are associated with decision-making. The

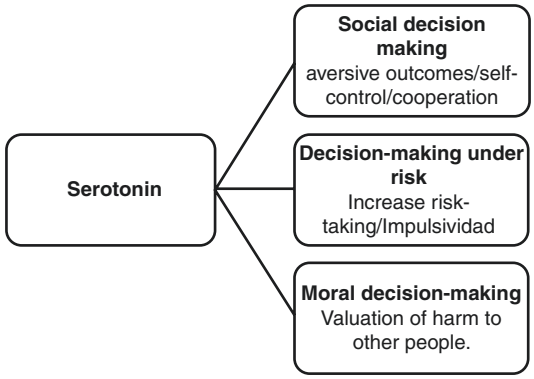

Fig. 15.4 The neurotransmitter serotonin is related to social decision-making with self-control, impulsivity, and valuation of aversive consequences. In economic decision-making, it is associated with higher decision-making and impulsivity. In moral decision-making, it is related to valuation of harm to another people

5-HT1B receptor relates with risk in decision-making [94]. Moreover, 5-HT_2 receptor relates with risk but also with motor impulsivity. Moreover, it is suggested that this receptor antagonist can be used pharmacologically to change behaviors, which affects impulsivity in decision-making [95].

Moral Decision-Making

Serotonin can also be very important in the moral judgment, specifically in valuation of harm, justice perception, and harm aversion [96, 97]. It has been associated with valuation of harm to other people in moral dilemma [97]. Increasing serotonin can make people more cooperative, while decreasing it can make it less cooperative. Furthermore, studies with patients taking citalopram – a serotonin reuptake inhibitor – have shown a significant increase in more altruistic behaviors [96, 98]. Finally, tryptophan elimination causes people to become more punitive to what they consider an injustice [14]. (Fig. 15.4).

Stress Hormones

Physical or psychological stress produces the same physiological response. It helps the animal to respond to the stressor stimuli. This response is accompanied by the rising of cytokines and adrenal hormones. The stress response is mediated by the activation of the adrenal cortex-anterior pituitary system. It releases the adrenocorticotropic hormone (ACTH) from the anterior pituitary. It facilitates the release of glucocorticoids from the adrenal cortex, which is a physiological parameter of stress [2].

Stress is a physical, psychological, and emotional response to a stimulus that is perceived as a threat. It is critical for survival because it allows the individual to adapt to new situations. However, long periods of stress or chronic stress can be nonadaptive for the individual. This has been related to decrease in the neurogenesis and neuroplasticity. In a psychiatric level, it may lead to psychopathologies such as anxiety and stress [99, 100].

Neuroimaging studies and behavioral studies suggest that stress produces a higher response in subcortical regions which are thought as related to instinctive and emotional behavior. Likewise, the activation of the frontal region, linked to executive control and deliberate thought, decreases. Then, stress will be related to the interaction between behaviors that are more emotional and more deliberate thinking producing more instinctive and emotional reactions [101]. Moreover, it has been suggested that it has a modulator role in the steroid hormone, testosterone [102].

Related to behavior, it has been found that rising of glucocorticoids affects negatively learning [100] and visual attention [10]. Furthermore, it has been observed that this rising is critical for different kinds of decision-making because it can impede the evaluation of all probabilities to make the right decision [99]. However, the function of these hormones has a U-shape response because low levels of stress can improve cognition, while higher levels can impair it [10].

Economic Decision-Making

Stress hormones are affected in decision-making under risk. Testosterone and glucocorticoids are related to risk-taking in decision-making [49, 102, 103]. In studies where cortisol was increased

pharmacologically, it was found that men tend to score lower than women do in the Iowa Gambling Task, but both are affected by stress. In the Balloon Analog Risk Task (BART) test, men took more risk, while women take lesser risk [104]. Other studies have corroborated these results finding that acute stress increases risk-taking in men, but they do not find the same effect in women [104, 150]. Additionally, in adolescence, a period of high-risk decision-making [99], it has been observed that under stress, young ones tend to make more risker decisions compared to a placebo group and adults [107].

Moreover, stress hormones are linked to strategic decision-making. Using the beauty contest game, a game in which a participant has to develop strategy in different steps in order to win, it was observed that people under stress were affected in their strategic reasoning compared with people without stress [108]. Furthermore, in studies in militaries, it was found that those who were in the condition of higher stress were more likely to fail in their decisions comparing with the other condition of low stress [109].

Moral and Social Decision-Making

Many social and moral decisions are made in stress condition. Higher levels of stress make more utilitarian [10] and altruistic [104] decision. Furthermore, it has been observed in social decision-making that participants under stress trust lesser than participants without stress [105]. In the hawk-dove game, a social decision-making game, to pick the hawk was associated with low cortisol level; this selection is related to social dominance behaviors, and higher cortisol levels would be associated to inhibitory behaviors as defense behavior [106]. (Fig. 15.5).

Women Cycle Hormones

Men and women have the same hormones. However**,** they differ in their proportions and functions. The biggest difference is that male gonadal hormones are stable while female

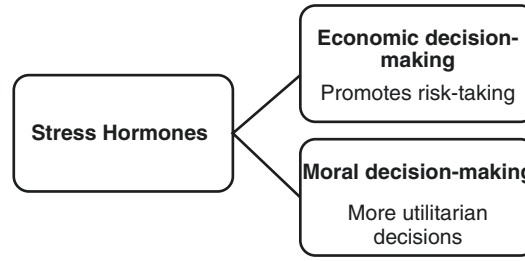

Fig. 15.5 Stress hormones are related to economic decision-making, increasing risk-taking and moral decision-making, focusing in the common well-being

gonadal hormones are cyclic. The hormones fluctuate each 28 days, more or less, and specifically the estrogen and progesterone are in charge of the cycle [2].

The menstrual cycle is divided in five phases: menstrual phase, follicular phase, ovulatory phase, luteal phase, and premenstrual phase. The length of the cycle varies between individuals, in which follicular phase is the most variable while ovulation, luteal phase, and premenstrual phase are more stable. Estrogen reaches its peak in the ovulatory phase, and progesterone in luteal phase [110]. Besides, in menopause hormonal changes that affects emotional processing are produced [115]. It takes to hypothesize that hormones play a key role in the emotional aspects of decision-making.

Social Decision-Making

Women cycle hormones are associated with emotional changing that has effect in social decision-making. In this manner, it was found that women show less trust in premenstrual and menstrual phases [10]. Another study found that it is more likely that women dressed red clothes, associated with courtship, during their most fertile period than any other part of the cycle. Moreover, estradiol-progesterone ratio was a predictor of the probabilities of wearing red clothes [116].

There are hypotheses which relate women hormonal cycle with mating. It has been found that during the follicular phase, women tend to look for men with more dominant appearance, while in the luteal phase they tend to observe

men with lesser dominant appearance [10]. However, this link is not clear because there are studies with contradictory results. When women are in their fertility period, it was found that there was no change related to the preference of physical characteristics of men. Nevertheless, there was a difference in choosing between long-term relationship partner and short-term relationship partner [117]. Other studies have not found a relation between preferences for male characteristics and hormonal cycle [118, 119]. However, it was observed that the qualification of the male body given by women is higher during the fertile phase [119].

There is a relation between women with higher testosterone levels and physical attractiveness of female faces but not male faces. In the same manner, they prefer attractive faces than non-attractive faces [120]. However, this relation was not found in another study which found a positive relation between preferences for male faces and higher level of testosterone measure in saliva. This result was affected when women were under some kind of hormonal contraceptive method [121]. Moreover, there were not found differences during menstrual cycle phases in preferences for men physical attractiveness, based on beard or boddy hair distribution [122]. These results suggest that there is some kind of link between women hormonal cycle and physical attractive preferences, but it remains unknown how it is affected.

On the other hand, it is suggested that female cycle hormones are related to social status and competitiveness. Specifically, testosterone is linked to a higher risk-taking of looking for a higher social status [123]. Furthermore, a link between competitiveness, the menstrual cycle, and hormonal contraceptive method was observed, but the role of the estrogen and progesterone in it is unknown [110].

Economic Decision-Making

Women cycle hormones are associated with decision-making under risk. It was observed that women tend to choose more risky option during ovulation. It was also found that they have lesser loss aversion in that phase than men [111]. Besides, it has been seen that women in luteal phase have less control and are more impulsive when they are buying, evenly correlating with remorse because they pay more than they can spend and remorse for buying in the next day [112]. In the same way, in biddings, it was found that in the fertile phase women tend to take higher risk. This behavior is explained evolutionary in higher chances of conceiving and higher quality and variety of the offspring [113].

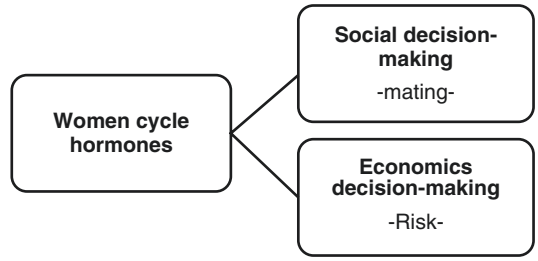

Fig. 15.6 Hormones involved in women menstrual cycle link to social decision-making in behaviors as mating, and with economic decision-making. They relate with decision-making under risk

Finally, there is a relation between risk, learning, and dopaminergic system [87, 114]. Estradiol and progesterone modulate dopaminergic tone, specifically in dopamine receptor DRD2. During menstrual phase, women present different levels of these hormones. Even some studies show that in follicular phase there is an improvement in the sensitivity to reward, compared to luteal phase. In the same manner, learning will be affected for negative feedbacks [124] (Fig. 15.6).

Dopamine

This neurotransmitter is a monoamine synthetized for the tyrosine amino acid. The tyrosine becomes L-dopa, and then, it became dopamine. It is mainly located in the dopaminergic mesolimbic system, related to reward [2]. As well as the prefrontal cortex, development finishes during adolescence, specially their connections with the ventral striatum [125]. In addition, it is strongly associated to motivation and seeking behaviors [126].

Dopamine is related to seeking behavior and learning [127]. In animal models, it has been observed, specifically in cleaner fish – *Labroides dimidiatus* – that blocking dopamine receptors inhibits motivationally the fish to approach to a reward [128]. Moreover, blocking dopamine receptors in mouse affects motivation in decision-making, specifically in decision which requires higher effort to reach the reward [129].

In human beings, it is linked with learning [130] and motivation [131–133]. In studies in Parkinson disease, it has been observed that dopamine is the neurotransmitter involved in learning difficulties presented by aging [2, 134]. Moreover, in schizophrenia, a disease characterized by dopaminergic system impairing [135], patients present a decrease in their motivation, which is also needed for decision-making that requires some kind of effort [136].

On the other side, as it was mentioned previously, neuropeptides oxytocin and vasopressin are very important in decisions and social behavior. Studies in rodents have found that there is an interaction in social behavior between oxytocin, vasopressin, and dopamine. This interaction is given in brain areas involved with learning and reward, as the striatum, where there are receptors for dopamine as well as oxytocin and vasopressin. Thus, there is a hypothesis which suggests that oxytocin is related to trust, vasopressin is related to social bonding, and dopamine became a reinforcer and motivator to social encounters [137].

Moral Decision-Making

Parkinson disease patients that usually portray alterations in nonmoral decision-making have also shown alteration in moral decision-making, suggesting that dopamine is involved with it [138]. Together with serotonin, they interact to make a valuation of the harm to make a moral decision [97]. Moreover, a difference in the dopaminergic system associated with moral judgment between men and women was found. It was found in women that they make the judgment less emotional and more rational [139].

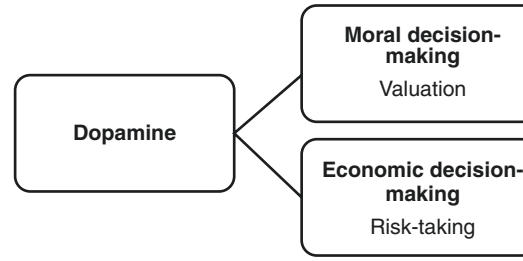

Fig. 15.7 The neurotransmitter dopamine is associated with moral decision-making, in the valuation of harm to other people, and in economic decision-making related to risk-taking

Economic Decision-Making

Dopamine is associated with risk-taking [114, 140] and reinforcement [141]. In rats, it was found that it is important for the valuation of the outcomes in decisions [142], and in human beings, it is very important for the valuation of the decision [143]. Dopamine is associated with decision-making under risk and behavior involving risk-taking. It is mentioned that D2/D3 receptors are related to the modulation of the weight of the probabilities of a reward [140]. On the other hand, dopamine has been linked to temporal discounting; it implies that affectation of the NAc in rats makes them prefer smaller reward but immediately bigger rewards which take more time to be earned [144] (Fig. 15.7).

Norepinephrine

It is a catecholamine of the monoamine group. It is synthetized from the amino acid tyrosine that became later dopamine. Then, another enzyme transforms dopamine in norepinephrine [2]. Noradrenergic neurons are located in the locus coeruleus. Despite being relatively low in number, they project to almost the whole cortex, cerebellum, and spinal medulla. Posteriorly, other enzymes synthetized the norepinephrine and became adrenalin [71].

Norepinephrine is involved with reward. As well as dopamine, it is located in the circuits of the frontal cortex related to reward [3]. Moreover, it is associated with the flight or fight response

[72], and it is linked to major depression. However, this association may be wrong, because this discovery of blood norepinephrine in depression could be related more to daily smoking than the psychopathology [145].

Moral and Social Decision-Making

It is suggested that the more basic emotions, as hunger and fear, are related with social attitudes and moral judgment. In this manner, norepinephrine is strongly related to social and moral decision-making [72]. Studies have found that when propranolol, a noradrenergic antagonist receptor, is administered, participants said it was unacceptable to take measures that harm other people in a moral dilemma [146]. Moreover, it was found that propranolol affects emotions diminishing the response of the amygdala when the participants see emotional pictures or emotional facial expression [72].

Economic Decision-Making

Norepinephrine, as serotonin, is linked with loss aversion. It was found that people with lower levels of norepinephrine transporters in thalamic pathways feel more strongly the losses than wins [147]. Furthermore, it was observed that after propranolol administration the sensibility to possible losses in a bet task decreases [87]. Finally, norepinephrine is intimately related to dopamine in impulsivity [3] and with multicomponent behaviors, which are to make different action in a specific order to reach a goal [148] (Fig. 15.8).

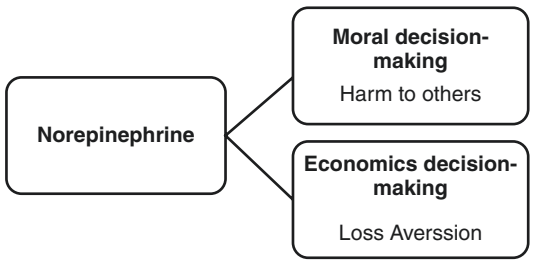

Fig. 15.8 Norepinephrine is associated with moral decision-making, when there is harm to other person, while in economic decision-making link to loss aversion

Discussion

The present model aims to describe the relationship between each one of this neurotransmitters and hormones with the different kinds of decision-making: social decision-making, moral decision-making, and economic decision-making. Manipulations of these neurotransmitters or hormones affect or alter in some ways these kinds of decision-making. It became necessary, for the different kinds of decisions, the interaction between these different chemicals to produce the outcome of the decision. However, it is necessary to consider that there is simply no mere game of neurochemicals to make the decision. All these substances will interact with individual characteristics, experiences, and their current situation, which will eventually lead to some kind of decision (Figs. 15.9, 15.10, and 15.11).

Related to social decision-making, it is observed that the interaction between vasopressin, oxytocin, and serotonin is very important. This whole interaction leads to a cooperation. Linked to moral decision-making, there is a relationship between stress hormones, serotonin, dopamine,

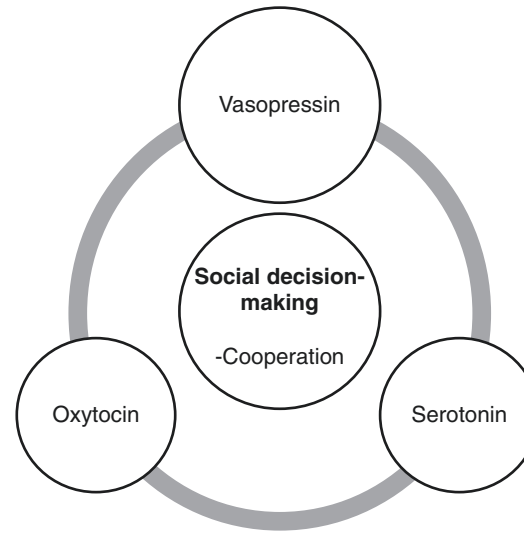

Fig. 15.9 In social decision-making, specifically in behavior related to cooperation, there is an interaction between neurotransmitter serotonin and neuropeptides, like vasopressin and oxytocin

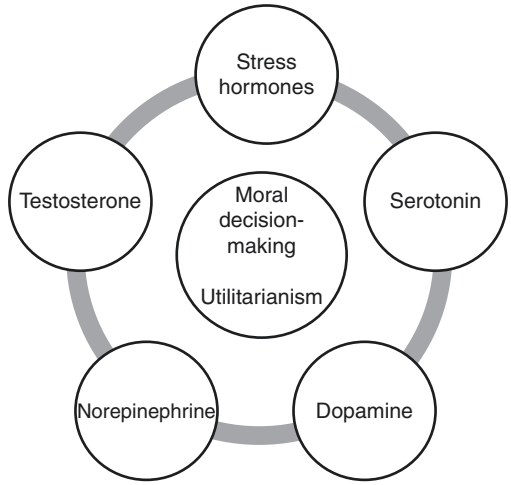

Fig. 15.10 In moral decision-making, there is an interaction between stress hormones, dopamine, norepinephrine, serotonin, and testosterone in utilitarian judgment

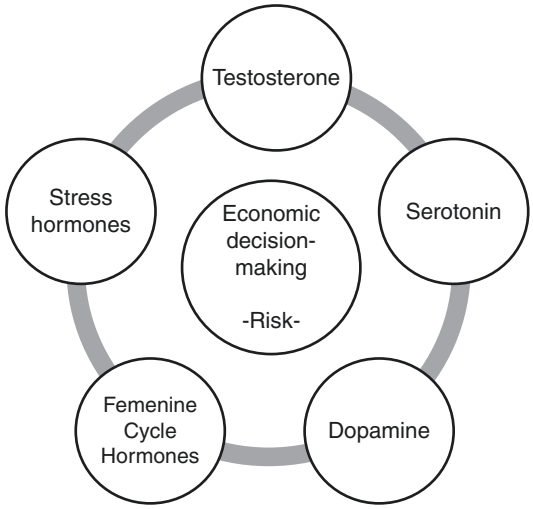

Fig. 15.11 In economic decision-making, there is a relationship between stress hormones, testosterone, serotonin, dopamine, and feminine cycle hormones, specifically in risk-taking

norepinephrine, oxytocin, and testosterone associated to decision that benefits the common well-being, as the utilitarian decisions. In economic decision-making, there is a relationship between risk-taking and hormones and neurotransmitters like dopamine, serotonin, testosterone, stress hormone, and feminine cycle hormones.

There are different ways to study some of this brain and body chemicals. However, it is necessary to mention that there are many limitations for the understanding of them in the human being. Blood measure, medical drug administration, or neuroimaging methods still have huge limitations which do not allow yet a full comprehension of how the relation and how these neurotransmitters, neuropeptides, and hormones in human decision-making is affected.

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Glial Cells in the Schizophrenia Puzzle: Angiotensin II Role

16

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Introduction

Schizophrenia is a severe mental disorder with 1% worldwide prevalence, which means that it affects the life quality and longevity of more than 21 million people worldwide according to the *World Health Organization* [1]. This pathology is characterized by profound disruption of thoughts, language, perception, and the sense of self, which often includes psychotic experiences, such as hallucinations and delusions. Together, they are called positive symptoms and were initially considered as the main psychological signs of this pathology. However, schizophrenia presents other psychological features such as social behavioral deficits, lack of motivation, and anhedonia – grouped as the negative symptoms – and cognitive dysfunction [1]. Historically, the classical neuron-centric view that has long-dominated neuroscience and the pharmacological research, fundamentally by using antipsychotic drugs, constituted the largest part of the characterization of schizophrenia etiopathology. In this sense, the bibliography is full of reports pointing out to neurotransmission misbalance – essentially dopaminergic or glutamatergic – as the main factor in the development of this pathology. Over the last years, the dopaminergic-centrist theories have given place to a more complex interpretation as schizophrenia becomes a multifactorial puzzle where glial cells are one of the new targets of interest. Glial cells (oligodendrocytes, astrocytes, and microglial cells) are essential pieces in brain microenvironment function as they play crucial roles in metabolic and ion homeostasis control, synaptic establishment and function, modulation of several neurotransmission systems, as well as neuroprotection, tissue repair, and inflammation [2, 3]. Regarding these glial functions, it is not surprising that alterations in their functionality and integrity could be related to psychiatric disorder development. In this sense, it has been reported, in human and animal research, that glial cells are involved in several mental diseases including Parkinson's disease, major depressive disorder, addiction, and schizophrenia [2]. The intricate patterns involved in both etiopathology and symptomatology make schizophrenia an unreadable enigma for the moment. Indeed, more than 100 years after its first description by Kraepelin and other psychiatrists, there is not a theory that explains all the features observed throughout its development and the consequent symptoms. Genetic, neurodevelopmental, and environmental theories can be mentioned among the multiplicity of the hypothesis that attempts to

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clarify the complex scenario of this pathology. Each one of them could represent different components, of the underlying cascade, that trigger the neurotransmitter imbalance and the subsequent schizophrenic symptom expression. These series of concatenated pathological events would occur throughout life, producing deficits in dendritic spine formation, due to an interplay between genetic factors and obstetric complication, along with an excessive apoptosis and synaptic pruning during adolescence, leading to brain disconnection and the subsequent psychotic symptoms [4]. Moreover, the different stages of schizophrenia development are transversally crossed by neuroinflammation. Some of the first insights of a possible association between the schizophrenic syndrome and neuroinflammation come from a century ago, when an increase of schizophrenia diagnosis rates was reported after an influenza epidemic that had happened in 1918 [5]. Although with some inconsistencies, more recent studies are in line with this asseveration, where schizophrenia development seems to be related with influenza as well as other maternal infection like herpes simplex virus type 2, *Toxoplasma gondii*, and nonspecific bacterial infections [6]. Taking into account the heterogeneity of etiological infections, the increased risk to suffer schizophrenia probably involve alterations related to immune system activation and not due to specific pathogenic pathways of each microorganism. To this respect, increased proinflammatory cytokine levels during pregnancy have been related to higher risk to suffer schizophrenia [6]. On the other hand, high genetic contribution to schizophrenia development was observed in studies made in twin with a heritability up to 80% [7]. Located on the short arm of chromosome 6, major histocompatibility complex (MHC) was consistently related to this high contribution to schizophrenia susceptibility [8–11]. In this region, at least 250 genes that encode human leukocyte antigens and many other immune and nonimmune genes are present. Variations on many genes encoded in this genome's region could induce an unsuitable immune reaction leading to exacerbate response and the consequent neuroinflammation throughout life. Together with the genetic vulnerability, the early neuroinflammatory insults abovementioned could imprint long-life marks over microglia cells, displaying an increased immune reactivity throughout the life of schizophrenic patients [12]. Both processes could explain the high levels of inflammatory markers, such as proinflammatory cytokines and C-reactive protein, that have been described in the blood and cerebrospinal fluid of schizophrenia patients [13, 14]. This immune hyperactivity during brain development could lead to less dendritic spine formation and to an enhanced mesencephalic progenitor differentiation into dopaminergic neurons, promoting some features of schizophrenia, like disruption of cortical synaptic connectivity and hyperdopaminergia [12, 15].

The central brain angiotensin II (Ang II) effects are mediated mainly by the two G protein-coupled receptors, the Ang II type 1 receptor (AT_1-R) and Ang II type 2 receptor (AT_2-R). AT_1-R is present in astrocytes, microglia, and brain endothelial cells pointing out its crucial role in neuroinflammatory responses [16, 17]. To this last respect, Ang II, via AT_1-R, is one of the most important inflammation and oxidative stress inducers by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex activation [18]. Nowadays, a large body of evidence supports the existence of a local renin-angiotensin system (RAS) with all of its components synthesized in the central nervous system (CNS). The presence and synthesis of angiotensinogen have been described in neurons and astrocytes. Whereas angiotensinogen production in neurons is restricted to some brain regions, its astrocytes' synthesis is the most important and widespread source [9–12]. Moreover, it has been reported an intra- and extracellular location of renin, while the Ang II converter enzyme (ACE) has been found extracellularly, as soluble and membrane-bound forms. Additionally, Karamyan [19] described alternative central pathways for Ang II synthesis that involved elastase, proteinase 3, cathepsin G, and tonin activity. Further evidence supports intraneuronal generation and activity of Ang II, as well, in brain microvessels [17, 20, 21].

In the present chapter, we attempt to summarize the role of the glial cells in the schizophrenia unmasking the AT_1-R involvement in the complex glial scenario.

Glial Cells in Schizophrenia

Microglia

Microglia, resident macrophages of the brain, participates in the progressive loss of synaptic connection during normal neurodevelopment after birth. This physiological process called synaptic pruning could lead, beyond a critical threshold, to cortical disconnection and psychotic symptoms. Moreover, a similar pathological state could be triggered in normal brains after an excessive microglia activation with an excessive synaptic pruning [15]. Furthermore, it has been hypothesized that an inadequate immune activation to a predominant type 2 response could induce an excess of kynurenic acid (KYNA) formation, an endogenous N-methyl-d-aspartate (NMDA) antagonist released mainly by astrocytes, known to lead to a glutamatergic hypofunction, an important hallmark of schizophrenia [12]. Moreover, it has been observed that about 10% of patients with an initial schizophrenia diagnosis have NMDA receptor antibodies [22]. In addition, a meta-analysis performed by Miller et al. shows that some cytokines, like interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor growth factor β (TGF-β), could be markers for acute exacerbations, since their levels were high during psychotic episodes and normal after antipsychotic treatment [23].

Anti-inflammatory effects of antipsychotic drugs are another important finding that relates immune imbalance with schizophrenia etiopathology. In this sense, a meta-analysis performed by Tourjman shows that antipsychotic treatment reduces the plasma levels of pro-inflammatory cytokines IL-1β and interferon-γ and increases soluble interleukin-2 receptor [24]. Further evidence, which supports an inflammatory response, came from the benefits of nonsteroidal anti-inflammatory drugs (NSAIDs) in the treatment of schizophrenia. In this case, the addition of NSAIDs to antipsychotic treatment generated an improvement, although modest, in symptom control [25, 26].

Under physiological conditions, Ang II and AT_1-R are expressed mainly in neurons and astrocytes; meanwhile, in nonactivated microglia, they are present at really low levels. However, under a pathological state, such as neuroinflammation, stroke, or multiple sclerosis, Ang II and AT_1-R increase their expression in activated microglia promoting an inflammatory feedback. Bhat [27] has reported that lipopolysaccharide (LPS)-induced gliosis is associated with RAS overactivation evidenced by increased Ang II level and AT_1-R expression in both astroglial and microglial cells. Moreover, in the same study, they showed that AT_1-R blockade blunted the neuroinflammation via suppression of glial activation and imbalance in inflammatory cytokines in both cell types. To this last respect, a large body of evidence reported that Ang II, via AT_1-R, plays a key role in oxidative stress and inflammation, promoting NADPH oxidase activation. Moreover, a cross talk between AT_1-R and the microglial toll-like receptor 4 contributes to Ang II pro-inflammatory signal inducing ROS production, NF- κB, and pro-inflammatory cytokine release, including IL-1β, IL-6, and TNF-α, and leads the microglial cell activation [28, 29]. In microglial cells, the NADPH oxidase activity is the main regulator of the shift between M1/pro-inflammatory and M2/immunoregulatory microglial phenotypes, where high oxidative levels promote the pro-inflammatory and inhibit the immunoregulatory phenotype [30]. In this sense, it is known that an M1/M2 phenotype balance is necessary to prevent brain damage, so it is not a surprise that the Ang II overactivation could lead to a chronic neuroinflammatory state. In this sense, the AT_1-R involvement in microglial neuroinflammation has been extensively studied in models of Parkinson's disease and hypertension [18, 29, 31–33]. To this last respect, both M1 and M2 markers have been found upregulated in an Ang II-induced mouse model of hypertension, whereas the microglial depletion reduced the neuroinflammation and the blood pressure, as

well as the levels of peripheral hypertensive hormones in a mice model of hypertension [34, 35]. In the same way, in an animal model of parkinsonism with MPTP, it has been shown that the Ang II/AT_1-R activation of the microglia is involved in the dopaminergic degeneration. This neurotoxin induces an increase in the RhoA/Rho-kinase pathway, which plays a critical role in the inflammatory and oxidative effects of Ang II that is inhibited by AT_1-R blockade or depletion [36]. Moreover, Rho-kinase activation upregulated AT_1-R expression in microglial cells leading to a feedforward mechanism. AT_1-R regulates the microglial response through two main pathways, Rho-kinase and NADPH oxidase, thus controlling superoxide generation, microglial motility and phagocytosis, and the release of inflammatory cytokines [29].

Astrocytes

Astrocytes are the neurovascular unit's center of integration mediating the vascular response to couple the neuronal activity, through the activation of their metabotropic glutamatergic receptors, Ca^{+2} oscillations, and release of gliotransmitters and vasoactive substances. In this way, astrocytes control the blood flow in response to the neuronal function (functional hyperemia), and the metabolites exchange through the blood-brain barrier. Furthermore, astrocytes exert a critical contribution to neurotransmission systems since they are responsible for the metabolism, recycling, and/or degradation of glutamate and GABA. Classical examples of these astrocytes' functions are glutamine-glutamate or KYNA circle. On the other hand, astroglia is involved in the immune response and tissue repair after damage [3, 37–39]. Taking all together, since astrocytes have a critical role in maintaining neuronal function, it is not surprising that alterations in their functions have been associated with several psychiatric disorders. However, regarding schizophrenia, the astrocytic contribution is not clear [37, 40]. Initial studies have reported signs of gliosis in a regional-dependent manner and usually closely related to the illness' history and severity [41, 42]. In this sense, Arnold [43] reported gliosis only in a schizophrenia subgroup of patients with a high prevalence of severe cognitive impairment and functional disability. However, later studies that focused on glial fibrillary acidic protein (GFAP) mRNA or protein analyses found no changes [44–47] or decreased expression [48–52]. In another study, augmented astrogliosis –described for schizophrenia – was reported to be concomitant with increased neuroinflammatory marker expression, and the authors suggested that astrocyte reactivity could be due to external factors (i.e., differences in psychiatric etiopathology, illnesses coexistence, or treatments received) [44]. In line with this hypothesis, it has been shown an increase in GFAP immunoreactivity after chronic antipsychotic treatment [53]. However, in animal models (rats), the treatment with haloperidol – typical antipsychotic – has no effects over GFAP expression [51]. Focusing the attention over the astroglial enzymes, products, or gliotransmitters, the reports over astrocytes' role in schizophrenia become more inconsistent. Among the main astrocyte's enzymes, the glutamine synthetase (GS) is a critical component of glutamine-glutamate cycling expressed mainly in astrocytes. This enzyme catalyzes the ATP-dependent condensation of ammonia and glutamate to form glutamine, playing a fundamental role in the glutamate neurotransmission and homeostasis, as well as neurotoxicity prevention by clearing of ammonia [54]. In the available bibliography, there is no agreement about GS involvement in this pathology, considering that the expression of this enzyme has been reported to be increased, decreased, or with no changes in schizophrenic patients [51, 53, 55–57]. Similar results were found for glutaminase, another glutamine-glutamate cycling enzyme, responsible for glutamine-glutamate transformation [55, 57]. However, it has been suggested that these changes could be due to antipsychotic treatment. In this sense, in astrocyte cultures, risperidone increased GS as well as the glutamate uptake and

glutathione content; meanwhile, haloperidol increased reactive oxygen species (ROS) but did not show any effect over these astrocyte functions [58]. Moreover, it has been shown an increase in glutamine synthetase-like protein in patients with schizophrenia, which becomes even higher after the treatment with olanzapine [59]. Other critical players in the glutamate-glutamine circle are the excitatory amino acid transporters (EAATs), expressed primarily on astrocytes. The evidence suggests that abnormalities in these glutamate transporter localization and function could underlie alterations in the kinetics of perisynaptic glutamate buffering, clearance, and cycling, contributing to the glutamatergic dysfunction described in schizophrenia [60, 61]. Regarding this last respect, it has been reported a decreased expression of these transporters in critical areas implicated in schizophrenia physiopathology [61]. Interestingly, McCullumsmith [60] showed an increased expression of these glutamate transporters in neurons and suggested that this could be a way to balance out the loss of astroglial reuptake capacity. Moreover, KYNA was found to be increased in critical regions of the CNS in schizophrenic patients, an effect that contributes to the reduction of the glutamatergic neurotransmission [62]. Studies performed in rats showed that the treatment with antipsychotics, like haloperidol, clozapine, and raclopride, caused a reduction in KYNA levels in the caudate putamen, hippocampus, and frontal cortex [63]. Another astroglial marker is the S100B, a neurotrophic factor released from several cell types, but within the CNS, it is released by astrocytes, and it is used as an astrocyte integrity indicator. Through its paracrine and autocrine role, in low concentration, S100B regulates proliferation and differentiation of neurons and glia and modulates dopamine and glutamatergic synaptic function. On the contrary, the over-release of this factor has been related to neuronal dysfunction and apoptosis due to increased expression of inducible nitric oxide synthase (iNOS) or proinflammatory cytokines [64, 65]. Regarding schizophrenia patients, several studies have revealed increased S100B levels in the peripheral blood and cerebrospinal fluid (CSF) of patients [65–70]. This increase in S100B has been associated to a more severe negative psychopathology and cognitive deficit, supporting the key role of astroglia in the schizophrenia etiopathology [65, 68, 69, 71]. Moreover, it has been reported higher levels of this factor at the cellular level in the early stages or acute paranoid schizophrenia that could be associated with astro- and oligodendroglial activation. These evidences suggest that glial activation and structural damage lead to a neurodegenerative-like process in schizophrenia [65, 72, 73]. Interestingly, an increased inflammatory markers' expression has been linked to higher levels of S100B in CSF of schizophrenic patients, suggesting that inflammatory processes could lead or exacerbate the glial dysfunction in these patients [66]. Furthermore, different reports showed a decrease in the S100B levels in CSF of patients after antipsychotic treatment. However, this effect appears to be selective for patients with predominantly positive symptoms, since the patients with negative symptomatology showed high levels of S100B even after antipsychotic treatment [65, 69, 72, 74]. Indeed, Qi et al. [67] reported that S100B was significantly higher in patients with refractory schizophrenia which were treated with both clozapine and typical antipsychotics, without significant difference between these two treatment groups. In animal research (monkeys), it has been reported lower S100B expression after chronic haloperidol or olanzapine treatment [75].

Succeeding the description of Ang II activity within the CNS, the first evidence of brain locally produced components was observed as the co-localization of angiotensinogen and the main astrocytic marker – GFAP – whereas the local synthesis of the precursor by astrocytes was confirmed short after [76–78]. Furthermore, it was simultaneously evidenced that Ang II binds to receptors in glial cells with similar binding properties and different functionality when compared to angiotensin actions over neurons [79]. These receptors were later identi-

fied as the AT_1-R subtype, promoting PLC activation and inositol-phosphate hydrolysis [80, 81]. Moreover, it was observed that Ang II, through AT_1-R, increased intracellular Ca^{+2} levels as an initial peak (via IP3) followed by a sustained plateau (for Ca^{+2} influx from extracellular sources) [82, 83]. Afterward, several cellular effects through different signaling pathways have been described for these receptors in cultured astrocytes including CREB phosphorylation, JACK2/STAT3 and MAPK/ERK activation, and inducible early gene transcription [84–87]. Overall, nowadays it is widely accepted the constitutive presence and expression of AT_1-R in astrocytes through which Ang II autoregulates its activity by controlling the synthesis of all RAS components [88, 89]. However, under certain pathological conditions that involve neuroinflammation, AT_1-R overactivity in astrocytes has been found to be detrimental. In this sense, AT_1-R activation stimulates ROS production and IL-6 synthesis and release in cultured glial cells via the NF-κB signaling pathway [87, 89], whereas Ang II promotes human astrocyte senescence by superoxide production, after membrane translocation of NADPH oxidase's subunits. Interestingly, these effects were blunted by AT_1-R blockade and the antioxidant tempol [90]. Moreover, in vitro studies showed that the inflammatory condition stimulated by LPS involves AT1-R activation for the upcome of astrogliosis, Ang II synthesis, and AT_1-R upregulation, concomitant with NF-κB nuclear translocation, ROS production, and TNF-α release [27]. In the same direction, experimental autoimmune encephalomyelitis in rodents implies AT_1-R overexpression in glial cells, triggering the upregulation and activation of transforming growth factor-β (TGF-β) and sustaining the inflammatory condition. Specifically, the AT_1-R blockade decreased thrombospondin-1 (TSP-1) secretion from astrocytes, which later promotes TGF-β activation, and improved clinical scores in rodents [91]. Furthermore, AT_1-R antagonists effectively blocked TNF-α release by astrocytes under hypoxic conditions [92]. Interestingly, the immunomodulatory actions of dopamine in neurodegenerative conditions involve the misbalance of RAS component production by astrocytes [93]. This interaction has also been observed after amphetamine exposure, where the psychostimulant-induced astrocyte reactivity involves AT_1-R activation, concomitant with vascular-network rearrangement and apoptosis in cortical areas [94]. This way, accumulating evidence supports the synergic activity of AT_1-R, pro-inflammatory mediators, and ROS in astrocytes contributing to the upcome of neuroinflammatory scenario [95, 96].

Oligodendrocytes

Oligodendrocytes are the glial cells responsible for the myelination processes**.** The appearance of myelinating oligodendrocytes facilitated the conduction of the nervous impulse, making the synaptic transmission faster and more efficient and leading to vertebrate CNS increase in complexity. In this sense, it is known that myelination processes have a critical role in cognitive functions, such as attention, learning, and memory. In humans, the myelin structure formation begins postnatally, and it is completed in young adulthood, around the time of the first psychotic episode expression and schizophrenia detection [97, 98]. Initial researches reported changes in gray and white matters and in the size of the ventricles. These findings gave a new meaning to oligodendrocytes and the myelination process in the schizophrenia etiopathology. Furthermore, several studies showed an atypical myelination in patients; the association observed in healthy controls between age, education, and the myelin water fraction in the frontal lobe's white matter is not found in schizophrenic subjects [99]. Indeed, a decreased myelin fraction was observed in schizophrenic patients on their first episode, suggesting that

these myelin changes precede the pathology occurrence and the possible pharmacological treatments [99]. Moreover, it has been reported that white matter density alterations are related to illness' severity, where the low density of the corpus callosum and anterior commissure suggests an aberrant interhemispheric connectivity of anterior cortical and subcortical brain regions and reflecting decreased hemispheric specialization in schizophrenia [100]. All together, these results support the reduced lateralization observed in schizophrenic patients [101, 102]. On the other hand, these myelin structural alterations are companied by oligodendrocyte functional alterations, integrity loss, and/or decreased population [103]. To this last respect, it has been reported a reduction of oligodendrocyte density in the hippocampus, frontal cortex, and anterior cingulate cortex [99, 104–108]. In the same way, decreased oligodendrocyte- and myelin-related genes have been found reduced in schizophrenic patients [108–113]. One of the main genetic risk factors in schizophrenia are the disrupted-in-schizophrenia 1 (DISC1) genes, expressed in oligodendrocytes regulating negatively their differentiation and maturation. *Bernstein* et al. [114] have shown augmented oligodendrocyte-positive expression for DISC1 genes in patients with paranoid schizophrenia. Furthermore, the use of knockout mouse models, missing oligodendrocyte-, or the myelin-related genes linked to schizophrenia recreates the demyelination observed in the human disease in animal research [113]. Interestingly, it has been shown that subchronic olanzapine improved oligodendrocyte- and myelin-related gene expression in rats [115]. In the same way, in cellular culture and in vivo, it has been reported that antipsychotics, such as haloperidol, olanzapine, and quetiapine, promote the differentiation of oligodendrocytes through transcription factors 1 and 2 (Olig1 and Olig2), without having any effect over their proliferation [97, 116, 117]. Other studies described an oligodendrocyte development stage-dependent effect for haloperidol. In this sense, in a proliferation phase, haloperidol promotes the cellular spread but inhibits their differentiation in the maturation process [118]. It seems that there is a special susceptibility period during oligodendrocyte development, mainly in gestational and perinatal phases. In this sense, it has been reported that inflammatory processes during early gestational stages produce a decreased number of oligodendrocytes and myelination alterations in the offspring's adult brain [119, 120]. These results are supported reciprocally with prenatal or gestational inflammation, since, as mentioned above, schizophrenia has been strongly related to maternal inflammatory insults.

Only a few studies have been focused on Ang II involvement over oligodendroglial functions. However, the confirmation of AT_1-R expression on oligodendrocytes suggests undescribed physiological roles of Ang II over these cells [88]. Several lines of evidences suggest an indirect Ang II involvement in re-myelinization through its action over astrocytes or microglial cells. In this sense, angiotensinogen, the precursor of Ang II, has been suggested as a potential biomarker of progression of multiple sclerosis, an illness that results in myelin sheath damage [121]. Moreover, clearance and recycling of lipid debris are necessary processes for an adequate myelination and depend on a suitable lipid transport. The main apolipoprotein (Apo) involved in this lipid transport in the brain is Apo E, which is produced by astrocyte. Since Ang II, through its AT_1-R, modulates astroglial function, this peptide could alter Apo E synthesis and indirectly myelin transport and recycling [122, 123] (Fig. 16.1).

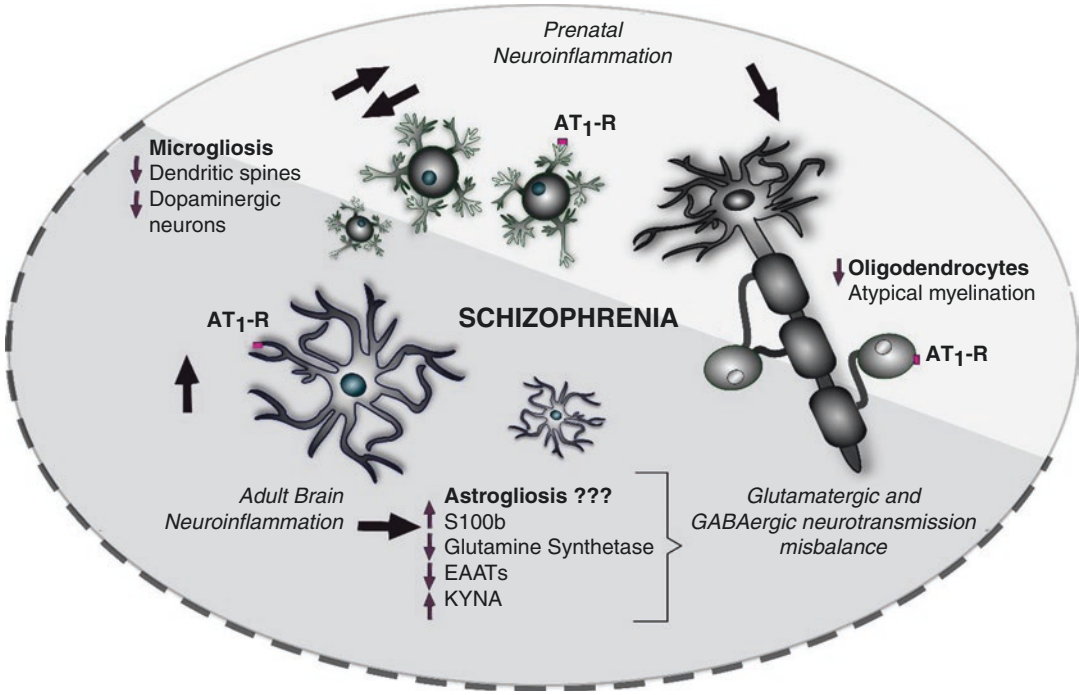

Fig. 16.1 The available evidences underpinning the glial system as a key player in the etiopathology of schizophrenia. The AT_1-R broad role over neurotransmission systems and glial function encourages their consideration in the development of this pathology and allows to postulate them as a new target for treatment. Indeed, it is important to be conscious that the actual knowledge is the tip of the iceberg in this multifactorial pathology and more studies become necessary to fill out the blank in the schizophrenia puzzle

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17 Somatostatin and Neurotensin Systems in Schizophrenia

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Introduction

Recent researches have focused on the possible therapeutic use of neuropeptides in the development of psychiatric and neurological disorders [1]. These molecules can behave as neuromodulators being responsible for a series of long-lasting events that modify the rapid actions of classic neurotransmitters. Although, many of these substances complete all the criteria to be considered neurotransmitters, since they are molecules synthesized and distributed in the CNS, which are stored in vesicles, to be released from the nerve terminals by a suitable stimulus [2]. Nevertheless, many peptides may be not considered true neurotransmitters because there is a limited acknowledgment about the mechanisms involved in the end of neurotransmission [2]. Neuropeptides are attractive therapeutic targets for mood disorders because they may participate in the biochemical alterations of these diseases [3].

Vasopressin and oxytocin play a pivotal role in behavioral regulation and in memory processes [4]. For this reason, it is known that vasopressin would be involved in the pathophysiology of major depression and the vasopressin receptor antagonists may be potential agents for the depression treatment. In this respect, oxytocin is also considered as an endogenous antidepressant or anxiolytic hormone [5].

Opioids are well-known as pain stimuli modulators, but they are linked to human being's behavior, particularly emotion and pleasure; then beta-endorphin, a natural opioid, seems to be involved in the rare behavior of autistic children. New therapeutic agents are synthesized to activate (in depression) or deactivate (in schizophrenia) the orexin system [6, 7].

These are a few examples of neuropeptide involvement in mental pathologies. However, our interest consisted of a description of neurotensin and somatostatin involvement in schizophrenia.

Neurotensin

Neurotensin is a tridecapeptide that was isolated from bovine hypothalamus extracts by Carraway and Leeman [8]. Intravenous administration of these extracts to exposed areas, i.e., ears, produced significant vasodilation. Since this peptide was found in the brain and behaved as a vasodilator, it was called neurotensin. Then a sequence of neurotensin amino acids (Glp-Leu-Tyr-Glu-Asn--Lys-Pro-Arg-Pro-Tyr-Ile-Le-OH) was found and used to obtain a synthetic peptide to develop antisera for radioimmunoassay (RIA); these assays made it possible to know both neurotensin distribution in the body as biological effects. In this

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sense, the neurotensin distribution in the rat brain was performed using specific antisera against different targets of the neurotensin sequence and followed by the reverse-phase liquid chromatography (HPLC) purification. In this assay, high amounts of neurotensin-like immunoreactivity (NTi) were found in the hypothalamus, septum, core of the terminal stria bed, and core of the central amygdaloid; moderate amounts in the ventral tegmental area (VTA), the thalamus, the locus coeruleus, and the dorsal roof of the spinal cord; and small amounts in the cerebellum and cerebral cortex [9], where the coexistence of NTi and tyrosine hydroxylase (a catecholamine marker) was limited to the nerve endings. These findings led many researchers to study the relationship of neurotensin, the dopaminergic system, and schizophrenia [10].

Like neuropeptides, neurotensin is synthesized as a precursor molecule that contains neurotensin and neuromedin N sequences found in the C-terminal domain, flanked by three sequences of Lys-Arg. These basic sequences are consensus sites that are recognized by pro-protein convertase enzymes [11]. In the brain, the precursor molecule cleavage originates both neurotensin and neuromedin N, which are released after neuronal depolarization [12, 13]. Neuromedin N is rapidly hydrolyzed by aminopeptidases, while neurotensin is degraded by metalloendopeptidases that produce the cleavage of the neurotensin molecule in the C-terminal domain [14].

Neurotensin can behave either as a neuromodulator, primarily of the dopaminergic neurotransmission, or as a neurotransmitter binding to specific recognition sites, which were studied using radioactive ligand binding techniques, such as [^{125}I]-NT. Mouse membranes obtained intestinally and cerebrally generally contain two different types of receptors: a high-affinity site, called NTS1 (Kd = 0.1–0.3 nM), sensitive to Na + and GTP ions which decreases the affinity of neurotensin for its receptor and a low-affinity site, NTS2 (Kd = 3–5 nM), much less sensitive to Na^+ and insensitive to GTP [15]. At the CNS level, there is a parallelism between neurotensin regional distribution and its receptor site localization [16]. The first molecular identification was achieved by Tanaka and colleagues, who cloned the first neurotensin receptor (NTS1) and demonstrated that it belongs to the G-protein-coupled receptor family [17]. The neurotensin interaction with the NTS1 receptor produces three different mechanisms: a transient increase in cGMP concentrations, a decrease in cAMP concentrations, and an increase in 1,4,5 inositol triphosphate and calcium concentrations [18, 19]. At variance, neurotensin stimulates phosphatidylinositol hydrolysis, without producing any changes in cAMP and cGMP levels in H29 cell line. Besides, neurotensin binds to the NTS1 receptor, producing MAPK (mitogen-activated protein kinase) activation and krox-24 gene induction, both mechanisms related to cell growth in this cell line [20–22].

In addition to NTS1 and NTS2 receptors, another receptor, NTS3, is localized intracellularly [23]. This receptor is involved in the regulation of glucose transporter vesicle biosynthesis and its translocation and in the clearance of circulating neurotensin. It has also been implicated in the development of certain types of cancer, where this peptide may act as a growth factor [24, 25].

After exocytosis, free neurotensin in the synaptic cleft is internalized by a presynaptic process that depends on the sodium ions' presence and the appropriate temperature [26]. The peptide molecules internalized are led from the periphery to the perinuclear region. NTS1 is not recycled to form part of the plasma membrane but is degraded within the cell [27]. The NTS1 receptor region related to internalization corresponds to the intracytoplasmic C-terminal domain [28]. Both enzyme degradation and receptor internalization are processes that could be considered as two mechanisms that put an end to the neurotensin actions [29].

G-protein-coupled receptors can form dimers [30] that are important for receptor function, including agonist affinity, efficiency, and G-protein specificity. NT triggered multiple responses because of independent signaling cascades. It is also shown that some regions involved in G-protein coupling are also involved in the control of the receptor regulation triggered by agonist stimulation. Heterodimerization modifies

ligand binding or coupling properties and trafficking characteristics of the receptors [31]. Molecular interactions between neurotensin receptors have been studied including NTS1/NTS2 heterodimerization. This situation affects the intracellular distribution and trafficking of NTS1 and could represent an alternative mechanism in the regulation of neurotensin responses mediated by NTS1 and NTS2 receptors [32].

After, neurotensin receptor activation, multiple signaling pathways occurred, leading to neurotensin effects. Neurotensin mainly activates the ERK signaling, which in turn activates downstream transcription factors, leading to cell proliferation [33]. Besides, it has been reported that RAS is activated and the pathways of phosphatidylinositol 3-kinase and mitogen-activated protein (MAP) kinase are stimulated, as well as MAP kinase ERK1/2 enhancement [34].

Several findings indicate that neurotensin binding to the NTS1 receptor produces diverse effects such as the stimulation of phosphoinositide hydrolysis and the inositol phosphate production, the calcium mobilization, and the increase in GMPc [35, 36]. Neurotensin also increases tyrosine hydroxylase activity and induces this gene expression through nitric oxide and protein kinase pathways [36]. Furthermore, neurotensin is implicated in cognitive disorders due to aging, because the interaction to its receptors in the hippocampal formation decreases the signaling pathways with age [37]. Neurotensin produces several central effects such as analgesia, hypothermia, the hormone release reinforcement, the reduction of locomotor activity, and the potentiation of CNS depressant agent actions. Neurotensin is found in the stomach, duodenum, jejunum, and ileum but not in the rat liver and lungs [12]. At the peripheral level, neurotensin causes vasodilatation, the stimulation of uterine contraction, the duodenum relaxation, increases in vascular permeability, and decreases in gastric secretion [27, 38].

Neurotensin is associated with dopaminergic transmission and considered to have potential antipsychotic activity. Therefore, several lines of research were devoted to the development of neurotensin analogs which were synthesized to easily cross the blood-brain barrier and provide it with resistance to degradation by prolonging the half-life [39, 40].

Somatostatin

Somatostatin is a 14-amino acid peptide that prevents the release of growth hormone (GH) from the pituitary [41]. But, two other peptides are part of the somatostatin system: the SS28, a 28 amino acid peptide, and SS28 (1–12), a 12 amino acid peptide formed by the cleavage of the SS28 in the N-terminal domain. All these peptides show a specific laminar distribution in the cerebral cortex; SS28 is found in the cell body and SS28 (1–12) in the nerve terminals of the hypothalamus and amygdala neurons [42]. For example, somatostatin-like immunoreactivity (SLI) is detected by a specific radioimmunoassay in rat striatum homogenates. The SS28 immunoreactivity is found in dorsal root ganglion cells and the superficial laminae of the rat spinal cord, while the dorsal root ganglion cells exhibit the presence of SS14 immunoreactivity [43].

Somatostatin regulates multiple biological processes via five genetically distinct, G-protein-coupled receptors. All of them are G-protein-coupled, seven-transmembrane receptors, which interact with a wide range of downstream signaling targets. Several pharmacological studies and sequence analyses have allowed to divide them into two groups, one formed by sst2, sst3, and sst5 and the second by sst1 and sst4. In mice and rats, the sst2 receptor can be found as two splice variants, sst2a and sst2b, which differ by length and C-terminal amino acid composition [44]. The mRNA expression patterns of the somatostatin receptors are useful to disclose the somatostatin receptor subtype distribution: Sst1 subtype receptor mRNA is widely distributed throughout the CNS. It is in the presynaptic membrane and can modulate somatostatin release. Sst2 subtype receptor mRNA is expressed at high levels in cells in the cerebral cortex, medial habenula, dentate gyrus, and cerebellum. Sst2a and sst2b subtype receptors can mediate regional and the cell-specific postsynaptic responses. Sst3 subtype

receptor mRNA pattern is found in the cerebellar Purkinje and granular cell layers, the deeper cortical layers, the hippocampal formation, and the myelencephalon nuclei. It is also detected in the sensory neurons of the dorsal root ganglia and in the rat trigeminal ganglia. The mRNA pattern of the sst4 receptor subtype is found in the formation of the hippocampus and predominantly located in the postsynaptic membranes. The sst5 receptor subtype RNAm pattern is both found in the hypothalamus as the preoptic area. Low expression levels were detected in the hippocampus, the cerebral cortex, the amygdala, and the striatum [44, 45].

Several studies were conducted to clarify somatostatin neural actions. Somatostatin induces a Ca^{2+} current inhibition in cultured cells of chicken in neurons of ciliary ganglion. Opioid peptides, applied before somatostatin, prevent the somatostatin inhibitory effects on the Ca^{2+} currents, but when applied after somatostatin, they change a voltage-dependent somatostatin-mediated calcium current by a voltage-independent opioid-mediated modulation [46].

The high voltage-activated somatostatin current is mediated by a G-protein from the Gi/Go subclass. Somatostatin produces the inhibition of the spontaneous firing in the noradrenergic neurons of rat locus coeruleus, leading to the opening of an inwardly rectifying K channel current in a somatostatin concentration manner [47]. In humans and rodents, the sst1 and sst5 receptors mediate the inhibition of insulin secretion, and the sst2 receptor seems to mediate the inhibition of glucagon secretion in these species [48]. Somatostatin also produces central effects that have involved it in diseases such as epilepsy, depression, Huntington's chorea, and Alzheimer's disease [49].

Schizophrenia and the Neuropeptides: Neurotensin and Somatostatin

Schizophrenia is a severe psychiatric disorder that affects approximately 1% of the world's population, regardless of race, gender, or social status. It appears in later adolescence and adulthood, and it is likely to persist after an initial outbreak throughout the patient's life. A variety of symptoms have been described in schizophrenia such as hallucinations and delusions (positive symptoms), apathy, flattening mood, poverty of oral discourse, and social withdrawal (negative symptoms) [50].

Following the neurodevelopmental theory, two sets of genes would be involved in schizophrenia: genes that would be responsible for the connectivity of the new specifically human circuitry and genes that would be associated with high emotionality in the interpretation of the signs. In schizophrenia, there is a transmission of a combination of genes that variably predispose symptoms related to language-thought disorders and schizotypal features. Besides, the obstetric complications at birth and the environmental conditions mainly during a child's emotional development can cause alterations coincident with a model of late neurodevelopmental damage (excessive increase in neural pruning), the run-up to adolescence. Finally, there is a possibility that in subjects without a decisive alteration in neurodevelopment, a lesion in some circuits or a neurochemical dysfunction results in a drug-related disorder transitory or permanent of the neural connectivity. All of this leads to a clinical phenotype that is a continuum of symptoms ranging from isolated psychotic symptoms to chronic schizophrenia [51].

Although the exact cause that predisposes to the disease is not completely clear, it is known that the interacting neurotransmission systems are altered. In accordance with the glutamatergic hypothesis, NMDA receptors, specifically in cortico-encephalic projections, could be hypoactive in schizophrenia, resulting in mesolimbic dopaminergic hyperactivity with positive symptomatology. Besides, the hypofunction of the NMDA receptor in the cortico-troncoencephalic projections would lead to hypoactivity in the mesocortical dopaminergic pathway, thus explaining the appearance of negative, affective, and cognitive symptoms in schizophrenia [52].

Neurotensin

To study the basics of schizophrenia, researchers use extremely helpful animal models; although they are not superimposable, they enable the study of the biochemical aspects of the disease. Using Black's animal model consisting of postnatal administration of a nitric oxide synthase inhibitor, it has been reported an alteration in behavioral tests and permanent dysfunction of neurotensin system that influence Na^+, K^+-ATPase response to neurotensin [53, 54].

It should be noted that neurotensin and somatostatin are implicated in schizophrenia since both peptides can regulate neurotransmitter systems related to schizophrenia. Neurotensin is also involved in the antipsychotic agents' mechanism of action [55, 56]. Dopamine and neurotensin are colocalized in some mesencephalic neurons, which project to the prefrontal cortex, a brain area devoid of dopamine and intrinsic neurotensin, but whose fibers are stained by tyrosine hydroxylase, a catecholamine marker, in the deep layers of the prefrontal cortex. These fibers also exhibit the presence of NTi (neurotensin-like immunoreactivity) determined by radioimmunoassay [57, 58]. Many lines of evidence demonstrated that neurotensin particularly exerts a direct regulation of midbrain dopamine neuron activity. It is known that neurotensin can increase dopamine turnover in the terminal fields of the mesolimbic-mesocortical pathways [10]. At nanomolar concentrations, neurotensin decreases the affinity of dopamine agonist binding to subcortical limbic sites. It might be due to intramembrane interaction between neurotensin and dopamine receptors. Moreover, using electrophysiological techniques applied to a culture of dopaminergic neurons, it has been enabled to prove a bidirectional interaction between the D2 receptor and NTS1 [59]. Neurotensin acting through NTS1 [56] receptors can reduce the function of D2 autoreceptors due to PKC and a calcium-dependent mechanism [60]. Likewise, prior activation of D2 receptors also reduces the ability of NTS1 to induce intracellular calcium mobilization [61]. The functional interaction between dopamine and neurotensin is also carried out by the formation of heteromers. It has been reported that expression of the dopamine D2 long (D2L) receptor and neurotensin receptor subtype NTS1 leads to physical interaction and the formation of heteromers in transfected human embryonic kidney cells and at the striatum cells [62–64]. In testing dopamine receptor agonists, partial agonists, and antagonists, it has been possible to establish different ligand-binding profiles and heteromerization based on functional selectivity; thus in the presence of neurotensin, the agonist FAU 326 displayed a 34-fold decrease of binding affinity in cells co-expressing D2L and NTS1 [62].

The intracerebroventricular administration of neurotensin to rats produces a neuroleptic-like effect, and neurotensin blocks dopamine hyperactivity due to psychostimulants when it is directly microinjected into the nucleus accumbens. However, neurotensin fails to induce catalepsy in rats and produces a decrease in stereotyped inhalation caused by dopamine stimulant drugs, leading to the hypothesis that neurotensin may behave pharmacologically like an atypical antipsychotic agent [65].

Neurotensin regulates serotonin release through the involvement of the high-affinity neurotensin receptor (NTS1), not associated with serotonin terminals, in the rat frontal cortex, striatum, and hippocampus slices [66]. Neurotensin has been located on the nerve terminals of the raphe nuclei. In the dorsal raphe nucleus, neurotensin induces an increase in the firing rate of a subpopulation of serotonin neurons while in the nucleus ventral part, the neurotensin induces excitation, which is selectively blocked by the non-peptide neurotensin receptor antagonist SR 48692 [67]. The activation of serotonergic neurons by an experimental acute sound stress enhances the stress activation of the median raphe nucleus; this effect is attenuated by endogenous neurotensin and unmasked by NT antagonist SR48692 [68].

Neurotensin is a mediator of the antipsychotic effects due to the dopaminergic neurotransmission modulation [69]. Prepulse inhibition is a robust operational measure of sensorimotor gating that is deficient in schizophrenics. Thus, using neurotensin null mice has shown that PPI is not reversed by haloperidol or olanzapine, indi-

cating that the elevation of neurotensin by antipsychotic agents is significantly involved in their action [70].

It is worth noting that striatal induction of neurotensin by both haloperidol and olanzapine confirms the results of previous single-gene studies that reported enhanced neurotensin mRNA and peptide release in response to typical and atypical antipsychotics [71]. Na^+, K^+-ATPase is inhibited by neurotensin through the NTS1 receptor interaction. Acute administration of haloperidol prevents the enzyme inhibition by neurotensin in cortical and striatal membranes due to a desensitization phenomenon. Differently, acute clozapine administration stimulates cortical enzyme activity by neurotensin. However, the acute administration of antipsychotics does not alter Na^+, K^+-ATPase basal activity suggesting that antipsychotic administration only modifies the neurotensin neurotransmission [72, 73]. Neurotensin antagonizes the prepulse inhibition (PPI) disruption produced by dizocilpine, an effect that is prevented by the neurotensin antagonist, 142948A. These facts demonstrate that the neurotensin interaction with NTS1 receptors presents antipsychotic-like properties [74]. The use of immunofluorescence histochemistry technique allows demonstrating the coexistence between glutamic acid decarboxylase (GAD) and tyrosine hydroxylase as well as the presence of neurotensin and galanin in cell bodies in the arcuate nucleus [75].

The ability of neurotensin to increase GABA levels in the prefrontal cortex is also blocked by pre-treatment with NTS1 antagonist SR 48692. This finding is consistent with the observation that NTS1 is localized in GABAergic interneurons in the prefrontal cortex, particularly in parvalbumin-containing interneurons [76]. The methylazoxymethanol acetate (MAM)-treated rats induce a reduction in the expression of parvalbumin (PV)-containing interneurons. Hence, a decrease in intrinsic GABAergic signaling may be the cause of the prefrontal cortex hypo-functionality of the hippocampus in schizophrenia [77].

Since neurotensin establishes relationships with other altered neurotransmitter systems in schizophrenia, it was thought that neurotensin receptor agonists could represent a novel class of antipsychotic agents [78]. Although neurotensin analogs have been shown to be effective in schizophrenia models, clinical studies showed no significant results [79]. Other research reports that neurokinin receptor antagonists acting on the neuropeptide system may be effective in the treatment of schizophrenia [80]. These contradictory findings have led current lines of research to focus mainly on the anorectic effects, directing the study towards the involvement of neurotensin in diseases such as obesity and diabetes [81].

Somatostatin

Somatostatin has been related to the dopamine system. It is known that dopamine antagonists inhibit somatostatin release and downregulate to somatostatin receptors [82]. In addition, it has been described that D2 and SSTR5 receptors form heterooligomers that reduce the formation of cAMP. Heterooligomerization is understood to be a ligand process initiated either by somatostatin or dopamine [83]. Somatostatin-like immunoreactivity (SLI) is reduced in hippocampus samples of patients with negative symptoms of schizophrenia [84]. Similarly, somatostatin-like immunoreactivity level is decreased in cognitively impaired schizophrenic patients, as in many other disorders with cognitive impairment [85].

Somatostatin-like immunoreactivity (SLI) is reduced in schizophrenics, but they are increased after haloperidol treatment, although this increase is not correlated with a psychopathological improvement [86]. Plasmatic somatostatin is increased in schizophrenic patients predominantly affected with positive symptoms, specially delusion and hallucination [87]. Although a reduction in cortical somatostatin levels has been reported, this reduction might not be due to gender differences in sex, age, or time between death and autopsy [88]. However, another research has found no significant differences in the mean initial somatostatin levels between schizophrenic, nonschizophrenic, and surgical

patients [89]. In this sense, it should be noted that the number of patients participating in the study as well as characteristic symptomatology of the pathology could lead to conflicting results.

It is known that MK-801 induces schizophrenia-like symptomatology. Therefore MK-801 administration produces the reduction of somatostatin mRNA levels in the prefrontal cortex, posterior cingulate-retrosplenial cortices, and hippocampus. After clozapine but not haloperidol administration, the somatostatin mRNA levels have been restored in these areas [90].

Furthermore, schizophrenia cognitive deficits are associated with a dysfunction of the dorsolateral prefrontal cortex (DLPFC) resulting, at least in part, from abnormalities in gamma-aminobutyric acid (GABA) neurotransmission. Accordingly, alterations in the inhibitory circuitry of the DLPFC in schizophrenia include reduced expression of the mRNA for somatostatin (SST), a neuropeptide present in a subpopulation of GABA neurons [91]. Recent findings indicate that altered DLPFC circuitry in patients reflects changes in gene expression that encodes to presynaptic and postsynaptic components of GABA neurotransmission [92]. In relation to somatostatin receptors, a significant reduction in sst2 receptors in layers 5–6 of the DLPFC pyramidal neurons in schizophrenia has been described [93].

In schizophrenia, functional abnormalities of the hippocampus are not ruled out. Neuroimaging studies have shown that the hippocampus is hyperactive in schizophrenia apparently due to abnormalities in the GABAergic neurotransmission, a reduced number of interneurons, and a marked decrease in the expression of both genes and interneuron proteins positive for somatostatin and parvalbumin [94]. Subsequently, such hyperactivity could be corrected by a transplantation of stem cell-derived interneurons in mice undergoing a schizophrenia model [95]. Moreover, a decrease in the somatostatin immunoreactivity of the amygdala neurons has been reported in schizophrenic and bipolar disorder patients, which has been interpreted as a decrease in the expression of somatostatin and an alteration of fear and anxiety responses [96].

The interneurons connecting the prefrontal cortex with the hippocampus have been extensively studied. Through optogenetic studies that stimulate or inhibit the somatostatin-positive interneurons, it has been observed that they are involved in the cognitive deficits of schizophrenia [97, 98].

Alherz et al. (2017) have reported three basic aspects of the relations between somatostatin and schizophrenia. Firstly, they refer to the changes of somatostatin or somatostatin RNA in different brains using schizophrenia models. Secondly, they analyze the changes in the GABAergic interneurons that contain somatostatin and sst2 receptors and its decrease in a model related to the glutamatergic hypothesis. The association with gamma-band oscillation (GBO) abnormalities which are involved in the schizophrenia cognitive deficits was also analyzed. The generation of GBO is also dependent on the inhibitory action of GABAergic interneurons as well as the activity of NMDARs [99].

Conclusion

This review has attempted to describe the basic and clinical aspects of neuropeptides, neurotensin and somatostatin, and its ability to interact with the main neurotransmission systems altered in schizophrenia, such as the dopaminergic, GABAergic, and glutamatergic systems, and their potential participation as therapeutic agents in mental disorders.

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18 Mechanisms of Action of Anxiolytics

Michel Bourin

Introduction

Anxiety and stress disorders are a major public health issue. However, their pathophysiology is still unclear. Given the complexity of the different types of anxiety and that the anxious behavior is regulated by a network of brain regions, addressing the mechanism of action of antianxiety medication is an important challenge. While there are similarities between symptomatological generalized anxiety, panic disorder, and social anxiety, biological and genetic determinants seem to be of a different nature [1].

Regulation abnormalities of neurobiological substrates are widespread and various including serotonin, GABA, glutamate, and also the autonomic nervous system, the hypothalamic-pituitary axis, and various neuropeptides such as cholecystokinin, substance P, galanin, etc.

Studies exploring the pharmacological properties of the different subunits have led to a better knowledge of the interaction between benzodiazepine-sensitive GABAA receptor subtypes and more or less specific ligands.

Furthermore, although the medical imaging furthers our understanding of the neurobiology of anxiety, animal models, achieved by utilizing transgenic animals in which specific receptors or subunits are mutated or knocked out, proved to be determining in explaining the mechanisms of action of anxiolytics.

The Amygdala and the Brain Circuit of Fear

The amygdala is the cornerstone of neuroanatomical models of fear and anxiety [2]. Several studies have in fact demonstrated the importance of the amygdala in the manifestation of anxiety disorders in humans. The volume of the amygdala (left) is reduced in children suffering from anxiety disorders [3], and this region is more activated when presenting photos of spiders to individuals with a specific phobia or during the presentation of photos of contexts or unhealthy objects to patients suffering from obsessive-compulsive disorder [4]. Increased amygdala activity has also been observed during the viewing of war photos by veterans with post-traumatic stress disorder [5].

A work in particular has made it possible to clarify the role of the complex amygdala (the amygdala has more than a dozen structurally and functionally different nuclei) in the conditioning of fear [6] (Fig. 18.1).

In these experiments, rats are exposed to a visual (light) or auditory signal (short tone) which is immediately followed by an electric shock from the metal floor. The animal quickly

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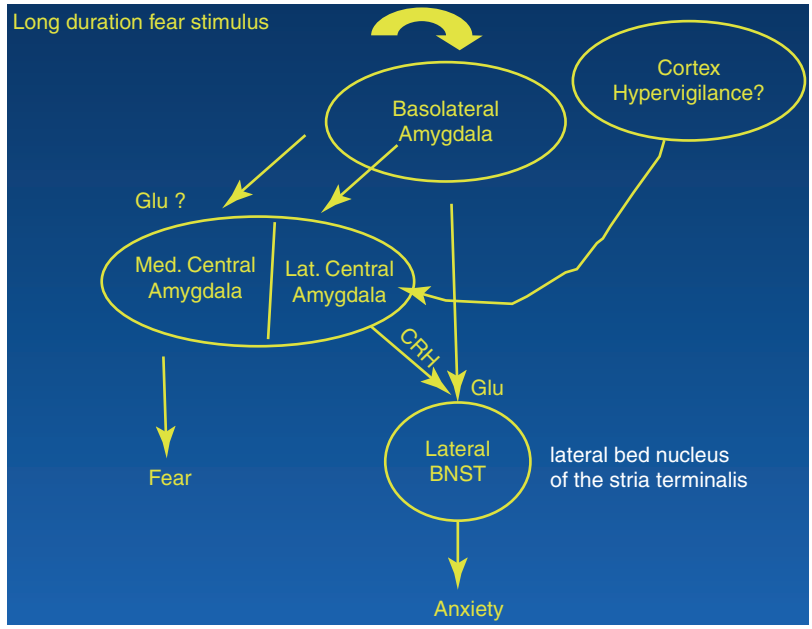

Fig. 18.1 Biological differences between fear and anxiety

associates the neutral stimulus (light or sound) with the aversive stimulus (shock) and manifests a deep tonic rigidity – freezing – when it perceives light or sound. This apprehension can last several seconds or even a few seconds.

In addition, if the electrical discharge is replaced by a sound stimulus, there is an exaggerated motor reaction (startle) in the animal. Thanks to this relatively simple procedure, the researchers mapped the neural circuits responsible for this conditioning. These circuits, too complex to be detailed here, mainly involve not only the amygdala but also the thalamus (a gateway to sensory information and an important integrating center of brain activity), the hypothalamus (a regulatory center of the release of several hormones), the central gray substance (a structure of the brainstem and freezing center), the hippocampus (a structure responsible for memory formation), and two cortical regions, the prefrontal cortex and the anterior cingulate cortex (or cingulum), both involved in the control of emotions and social behavior. In vivo observations of human brain activity have also shown the activation of these brain regions during the processing of fear-conniving stimuli. The prefrontal cortex plays an important role in inhibiting the activity of subcortical regions and inappropriate behavioral responses [7].

GABAergic System and Anxiety

The gamma-amino acid butyric acid (GABA) neurochemical system has been strongly implicated in their pathogenesis and treatment by numerous preclinical and clinical studies, the most recent of which have been highlighted and critically reviewed [8]. Changes in cortical GABA appear related to normal personality styles and responses to stress. While there is accumulating animal and human neuroimaging evidence of cortical and subcortical GABA deficits across a number of anxiety conditions, a clear pattern of findings in specific brain regions for a given disorder is yet to emerge. Neuropsychiatric conditions with anxiety as a clinical feature may have GABA deficits as an underlying feature. Different classes of anxiolytic therapies support GABA function, and this may be an area in which newer GABA neuroimaging techniques could soon offer more personalized therapy. Novel GABAergic pharmacotherapies in development offer potential improvements over current therapies in

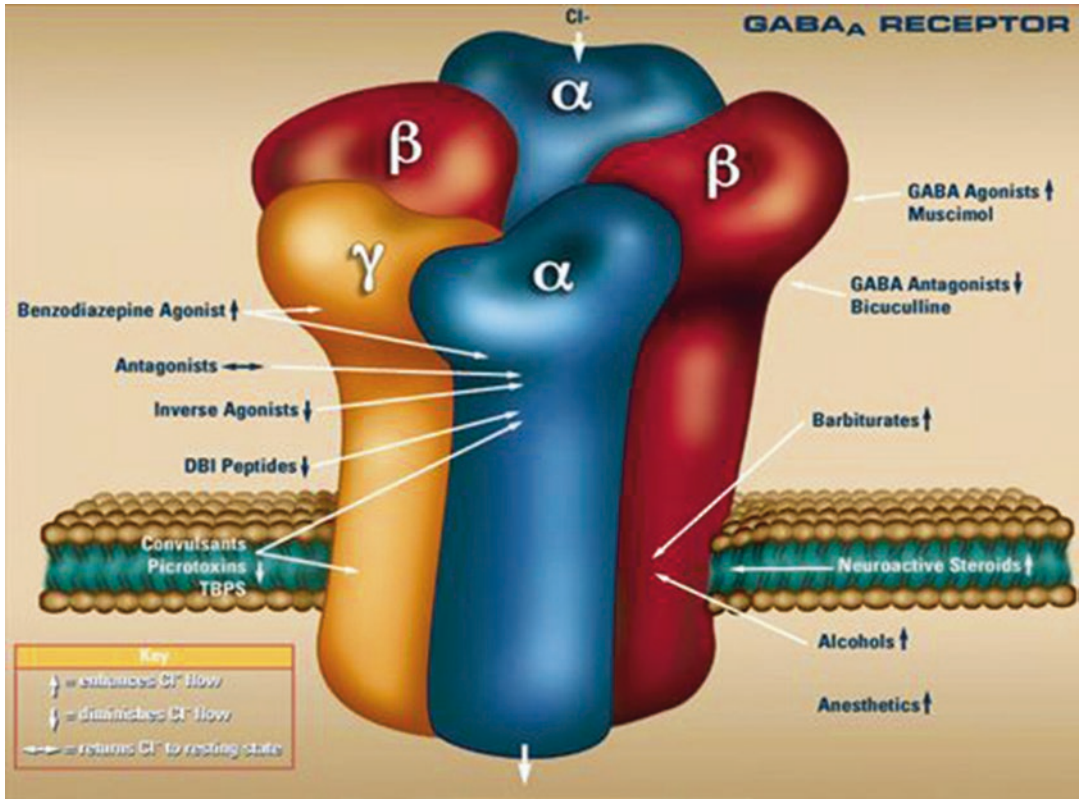

Fig. 18.2 Schematic GABAA receptor

reducing sedative and physiologic dependency effects while offering rapid anxiolysis [9].

By their allosteric modulation, GABAA receptors serve as control elements through which the level of anxiety, alertness, muscle tension, and epileptogenic threshold can be regulated. GABAA receptors with benzodiazepine (BZD) functional sites exist in the rat brain when γ2 subunits are expressed [10].

However, these receptors are not able to participate in the opening of the chlorine channel. The presence of multiple GABAA receptors in the brain may contribute to synaptic plasticity, GABAergic neuron response differences, and variation in drug activity profile. Thus, much research continues to be undertaken to find new agonists specific for BZD receptor subtypes.

Evidence is that GABAA receptor function is disrupted in anxiety and that patients respond poorly to BZDs. Furthermore, a genetic inactivation of 5-HT1A receptors in knockout mice for this receptor results in the downregulation of GABAA receptor alpha subunits, a decrease in GABA binding, and resistance of these mice to the anxiolytic activity of GABA receptors. (Fig. 18.2). These changes have been brought closer to an abnormal expression of the alpha subunit in the amygdala and hippocampus.

The inverse agonists of the BZD receptor are anxiogenic. Thus, FG 7142 administered to healthy volunteers induces anxiety accompanied by vegetative symptoms including cardiovascular; this action would be via the BZD receptor because the symptoms are antagonized by the BZDs. Studies using low doses of BZDs have shown increased attention in healthy volunteers, suggesting that in this case BZDs such as lorazepam or alprazolam [11] may have partial agonist properties (Fig. 18.3).

Flumazenil, which has an affinity for the BZD receptor comparable to more affective BZDs, does not alter GABAergic transmission and induces panic attacks in patients. One of the

hypotheses of the patient which had abnormal sensitivity: in this case, flumazenil would not be an antagonist but an inverse agonist. This modification of sensitivity of BZDs could also be involved in withdrawal and tolerance phenomena [12].

Although inverse agonist-induced activity and BZD-induced anxiolysis appear to be a direct consequence of GABAergic transmission, it would be simplistic to say that anxiety disorders result from primary alteration of the GABAA/channel chlorine complex. Indeed, in humans, the GABA mimetic substances do not show frank anxiolytic activity (tiagabine, gabapentin) (Fig. 18.4) [13]. Thus, the role of GABA in anxiety would be rather indirect. There are indeed

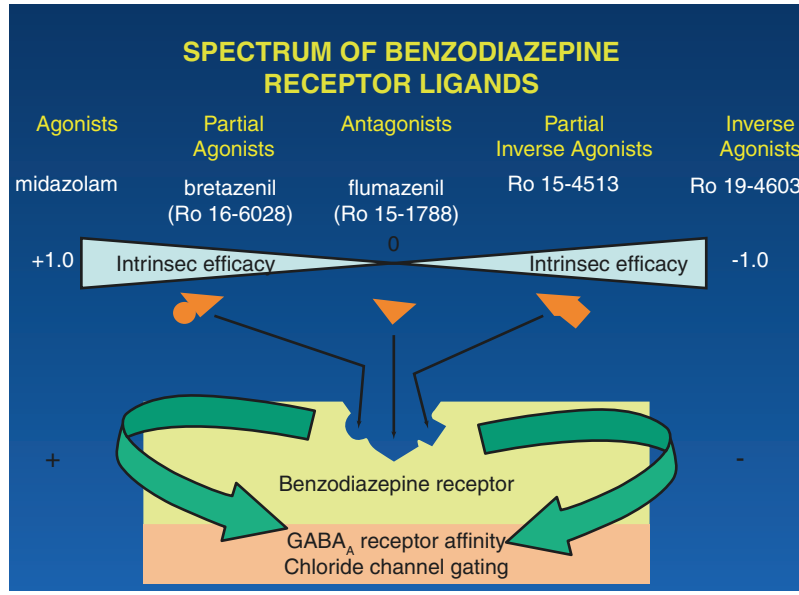

Fig. 18.3 Spectrum of benzodiazepine receptor ligands

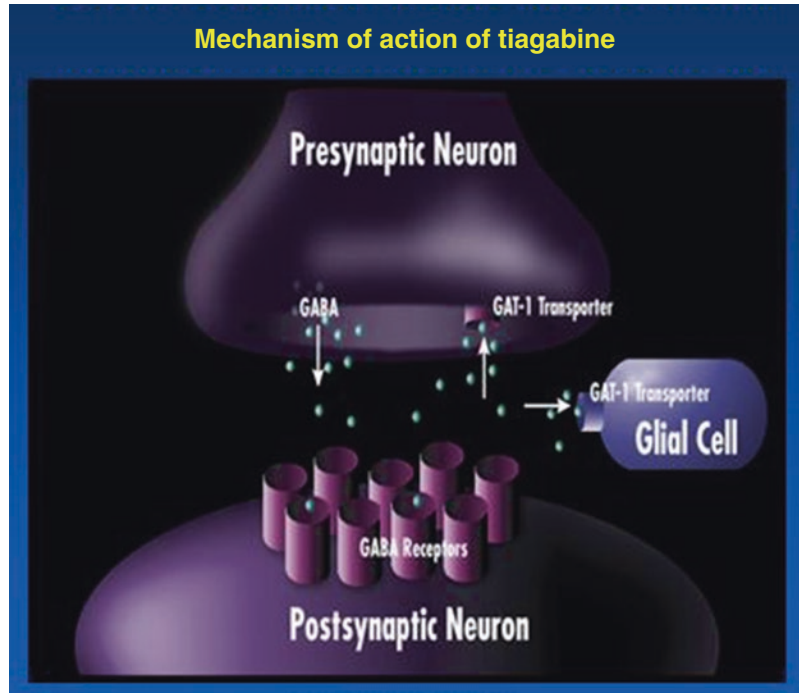

Fig. 18.4 Mechanism of action of tiagabine

interactions with the noradrenergic system at the locus coeruleus and with the serotonergic system at raphe, which could explain the activity of some antidepressants in the treatment of generalized anxiety. On the other hand, the anxiety-reducing activity and the decrease in serotonin turnover induced by BZDs were maintained over repeated doses in an animal model of anxiety [14]. Anxiolytic effects of BZDs were neutralized by intravenous serotonin.

Role of the Serotoninergic System

According to several animal studies, an increase in 5-HT transmission would be likely to lead to anxiety, whereas a reduction would attenuate it [15].

Considering the involvement of 5 HT1A, 5 HT2, and 5 HT3 receptor subtypes in the pathophysiology of anxiety, they should be considered as a key target for anxiolytics. m-Chlorophenyl piperazine (mCPP), which is direct-acting at 5 HT1 and 5-HT2 receptors, induced anxiety in humans. This occurs both in healthy patients and panickers [16]. Therapeutic investigations, using indirect serotonergic agonists such as 5-hydroxytryptophan (5-HTP), clomipramine, and fluvoxamine that act as serotonin reuptake inhibitors, revealed biphasic responses resulting in improvement following the initial symptom exacerbation. Serotonin increase leads to anxiety.

5-HT1A Receptors

The 5-HT1A receptor is involved in anxiety disorders. Indeed, within the azapirones family (buspirone, gepirone, ipsapirone, tandospirone, and zalospirone) that acts as 5-HT1a partial agonists are anxiolytics. Buspirone is the only substance to be marketed for the treatment of generalized anxiety disorder. However, buspirone has a long onset of action and appears not to be sufficiently effective in anxiety disorders, including panic disorder [17]. Other 5HT receptor agonists, such as ipsapirone, gepirone, tandospirone, and flesinoxan, exhibit anxiolytic activity; however, to exert this therapeutic effect, these substances require higher daily levels and could have significant adverse effects. Quetiapine, an antipsychotic, exhibits anxiolytic activity on the Vogel test (considered as an anxiety test in rats), due to its partial agonistic 5HT1A [18]. The 5HT receptor agonists have been suggested to reduce anxiety as an agonist at 5-HT1a on the somatodendritic autoreceptors, antagonist activity at postsynaptic receptors, or by a combination of both mechanisms.

The involvement of 5-HT1A receptor in modulation of anxiety behaviors is supported by other studies using 5-HT1A knockout mice that showed an increase in anxious behavior in several animal models [19, 20]. The effects of selective 5-HT1A receptors ligands have been extensively studied in a variety of animal behaviors. 5-HT1A partial agonists (buspirone, ipsapirone, gepirone) and full 5-HT1A agonists have been shown to induce an anxiolytic-like effect. Furthermore, anxiolytic-like effects of antagonists of 5-HT1A receptors were reported in the light/dark test (L/D) and the elevated plus maze (EPM). However, negative results have also been reported in the light/dark test and the Vogel conflict test (where drinking behavior is punished by electric shocks). These conflicting results have been linked to either a pre- or postsynaptic stimulation, to a different anatomical location of these receptors, or to the animal model used.

5-HT2A Receptors

Preliminary research studies involving the 5-HT2 receptors in anxiety have used nonselective 5-HT2A/2C antagonists (most studies used ritanserin or ketanserin). Studies showed that the administration of ketanserin, trazodone, pirenperone, and spiperone reduced the aversion induced at the periaqueductal gray [21] and, thus, is in accordance with clinical data. However, many of these molecules have an affinity for dopaminergic, alpha-adrenergic, histaminergic, or 5-HT1 receptors; moreover, there are few studies on these neurotransmitters' interaction**.** This antagonistic effect alone is not sufficient to explain their anxiolytic properties.

A plethora of studies using ketanserin or ritanserin showed results, ranging from an anxiolytic effect to anxiety-provoking or no effect. The emergence of 5HT2A receptor antagonists could elucidate the role of this receptor subtype in anxiety [22].

A few studies have closely examined the effects of the 5-HT2A receptor antagonists, probably due to the lack of available selective ligands and strong interest in blocking the 5-HT2A receptors. Serazepine is among the various compounds with antagonistic properties for the 5-HT2A receptors evaluated in clinical studies and proved to be effective in generalized anxiety [23]; deramciclane is also studied in several clinical trials [24]. Cyamemazine, an atypical antipsychotic, is widely used as an anxiolytic and has been found to have a 5HT2A agonist action [25].

5-HT2B Receptors

The demonstration of the involvement of the 5-HT2B receptor in anxiety has been hampered by the lack of detection of this receptor in the rat brain and low detection in mouse and human brains [26]. However, the identification of the expression of the 5-HT2B receptor in the rat CNS, especially in areas involved in anxiety (amygdala, hippocampus, and hypothalamus), is driving a renewed interest in the exploration of its effects in animal models. Recent findings suggest that 5-HT2B receptor can mediate anticonflict activity in those tests. SB 206553 also increased the percentage of time spent on the open arms and the total number of entries into the arms in the EPM in the rat; however, it has no effect in two experimental models in mice. The discovery of anxiolytic effect of BW 723C86, a potent 5-HT2B receptor agonist, in EPM in the rat [26], has led to assess the involvement of this receptor subtype in animals' models of anxiety. However, clinical trials cannot be carried out on humans.

In fact, compounds such as norfenfluramine which is the common metabolite of fenfluramine and benfluorex induced valvulopathies in humans [27].

5-HT$_{2C}$ Receptors

Clinical studies have shown that mCPP could induce anxiety symptoms in healthy volunteers. mCPP increases also panic attacks in patients with panic disorder and obsessive symptoms in patients with obsessive-compulsive disorder. These early studies suggest an involvement of this receptor subtype in anxiety control [28].

However, it must be emphasized that mCPP exhibits an affinity for several receptor subtypes including 5-HT1B/1D/2A/2B, and moreover, it can act as 5-HT2B agonist or 5-HT2B antagonist. 5-HT2C receptor blockade may prevent some of the anxiety-inducing effects of mCPP in the rat. The nonspecific 5-HT receptor antagonists, mianserin, cyproheptadine, and metergoline, have been shown to block the anxiogenic effects of mCPP in the rat. Since the stimulation of 5-HT2C receptors induces an anxiogenic response, it was expected that blocking these receptors may induce anxiolytic effects. Several 5-HT1C receptor antagonists (old classification of this receptor subtype) such as mianserin, 1-naphthylpipérazine, ICI 169369, pizotifène, and LY 53857 increased the time spent in active social interactions in the rat (predictive anxiolytic test). Regulation of anxiety involves 5-HT 2C receptor. Indeed, 5-HT2c receptor knockout mice exhibit an apparent reduced anxiety revealed by open-field test and "zero maze" [29]. The involvement of the 5-HT2C receptor in anxiety disorders is also supported by its central distribution. Several intracerebral administrations of 5-HT2C agonists or antagonists support their role in anxiety depending on the explored structure [30].

Because of the conflicting results obtained with the 5-HT2 receptor ligands (anxiolytic, anxiogenic, or no effect) whether in spontaneous models or conditioned models, there is confusion over the precise role of 5-HT in anxiety disorders (increased or decreased neurotransmission) and a lack of clinical implications and declining research of the possible role of this receptor in anxiety. However, the extensive therapeutic use of compounds which act by modulating serotonergic neurotransmission, specifically selective serotonin reuptake inhibitors (SSRIs), in the

treatment of anxiety disorders has increased the therapeutic importance of this neurotransmission system. It was suggested that the clinically active anxiolytics act through 5-HT2 receptors. In an instance, antidepressants exhibit affinity for 5-HT2 receptors and share the ability to regulate the binding ligands of the 5-HT2 receptors. Long-lasting administration of SSRIs and IMAOs results in 5-HT2 receptor desensitization. Some of the effects resulting in long-term administration of SSRIs and IMAOs and the behavior related to anxiety may involve desensitization mechanism. Recent studies indicate that discriminant effects of IRSSs may involve one of the components of the 5-HT 2A/2C, suggesting that there could play a significant role relating to some of the effects of this class of molecules in the CNS [31]. Several antipsychotics also exhibit affinity for the 5-HT2 receptor and act as 5-HT2 antagonist or 5-HT2 inverse agonist which may contribute to their anxiolytic effects [32]. It appears that the use of 5-HT2C antagonists in the treatment of anxiety is fairly obvious [33].

5-HT3 Receptors

Identification of 5-HT3 receptor was soon followed by the synthesis of 5-HT3 receptor antagonists (ondansetron, zacopride, zatosetron) and their evaluation in several animal models of anxiety [34]. The most convincing evidence (anxiolytic-like properties) of 5-HT3 receptor antagonists has been discovered in the dark/light test in mice. Several 5-HT3 receptor antagonists (especially ondansetron) have been investigated clinically as an effective therapeutic tool for anxiety disorders. However, the results have been disappointing; this led to a decrease in the interest regarding investigations into the pharmacology of these receptors. Nevertheless, it has been suggested that compounds acting as 5-HT3 receptor antagonist and exhibiting significant affinity for a variety of other receptors or using an alternative mechanism (such as reuptake inhibitors) may be beneficial in anxiety and offering a new line of research. Two subtypes of 5-HT3 receptor were identified, 5-HT3A and 5HT3B [35]. Anxiolytic-like behavior was investigated in 5-HT3A knockout mice. The dark/light test has revealed decreased anxiolytic-like behavior, and this correlated with the results obtained with 5-HT3 receptor antagonists [36]. In the dark/light test, 5-HT3A knockout mice have spent more time in the light compartment compared to control animals. It was also noted an increase in the total number of entries into the arms in the EPM in the mice. These findings suggest that the 5-HT3A receptor may be involved in anxiety disorders. Progress in the field of chemistry development of specific ligands should be made with a view to verifying this hypothesis. More recently, it has been shown that 5HT3 antagonism could help manage the fear and emotions in humans [37].

5-HT2 Receptors and the GABAergic System

The 5-HT2A receptors were found on GABAergic neurons in several brain regions [38]. Furthermore, it has been shown that GABAergic interneurons of the cortex express 5HT2A receptors. In the piriform cortex, the 5-HT2A receptors are present on GABAergic interneurons localized in layers II and III while the 5-HT2C receptors are located on the pyramidal cells.

Anatomical studies have revealed the expression of mRNA 5-HT2A and 5-HT2C subtype receptors in interneurons of CA1 region in the hippocampus. Serotonin appears to increase GABAergic synaptic transmission in the CA1 region through the activation of presynaptic 5-HT2 receptors. The choroid plexus expresses both the GABA receptors and large numbers of 5-HT2C receptors. Distribution of GABAA receptors and 5-HT2C receptors in the CNS indicates that these receptors may coexist in other areas of the CNS such as the telencephalon, diencephalon, midbrain, pons, medulla, and cerebellum. Serotonin appears to modulate GABAergic function in the ventral tegmental area (VTA). In VTA, 5-HT2A receptors are present on non-dopaminergic dendrites and presumably on GABAergic neurons. Recent data indicate that activation of 5-HT2C depolarizes non-dopaminergic

neurons, presumably GABAergic cells in the AVT which, in turn, inhibit the release and subsequent release of transmitters from dopaminergic neurons [39].

Glutamatergic System and Anxiety

Glutamate, the primary excitatory neurotransmitter in the central nervous system (CNS), exerts neuromodulator actions via the activation of metabotropic glutamate (mGlu) receptors. There are eight known mGlu receptor subtypes (mGlu1–8), which are widely expressed throughout the brain and are divided into three groups (I–III), based on signaling pathways and pharmacological profiles. Group III mGlu receptors (mGlu4/6/7/8) are primarily, although not exclusively, localized on presynaptic terminals, where they act as both auto- and hetero-receptors, inhibiting the release of neurotransmitter. Until recently, our understanding of the role of individual group III mGlu receptor subtypes was hindered by a lack of subtype-selective pharmacological tools. Recent advances in the development of both orthosteric and allosteric group III-targeting compounds, however, have prompted detailed investigations into the possible functional role of these receptors within the CNS and revealed their involvement in a number of pathological conditions, such as epilepsy, anxiety, and Parkinson's disease. The heterogeneous expression of group III mGlu receptor subtypes throughout the brain, as well as their distinct distribution at glutamatergic and GABAergic synapses, makes them ideal targets for therapeutic intervention [40].

A growing body of evidence suggests the involvement of the hippocampal glutamatergic system in anxiety disorders [41] (Fig. 18.5). Local injections of products that improve the release of glutamate in the ventral hippocampus (i.e., a cocktail of GABAB and mGlu2/3 receptor antagonists) exert a strong anxiolytic effect in rats exposed to prenatal restraint stress (PRS). In addition, chronic treatment with conventional antidepressants enhances glutamate release in the ventral hippocampus and corrects the

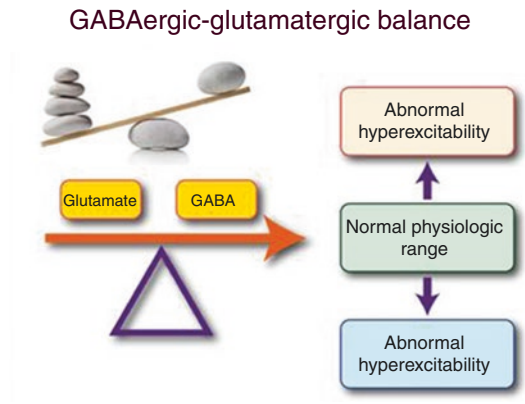

Fig. 18.5 GABAergic-glutamatergic balance

PRS-induced anxiety/depressive phenotype. These results reinforce glutamatergic theory in stress-related mood disorders and suggest that ventral hippocampal deficiency and its influence on the striatal circuit are key elements of research into the mechanisms of action of anxiolytics [42].

New promising agents are under development, including modulators of the N-methyl-D-aspartate (NMDA) receptor, 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propanoic acid (AMPA), and active substances on metabotropic glutamatergic receptors (mGlu) [43]. This type of development was carried out about 20 years ago but without success because of teratogenesis problems. We hope that these new derivatives will reach the clinic [44].

Adenosine and Anxiety

Adenosine is a nucleoside formed when adenine is attached to a ribose nucleus, released by neurons and glial cells. It plays an important role in biochemical processes, such as energy transfer – such as adenosine triphosphate (ATP) and adenosine diphosphate (ADP) – as well as in signal transduction like cyclic adenosine monophosphate, cAMP. It also acts as a hormonal neurotransmitter [45].

The process of stabilization/elimination of synapses is a key process of brain development. Although stabilization/removal of active/inactive synapses is well established, the mechanisms

involved are still poorly understood. Adenosine, a degradation product of ATP, positively or negatively controls the synaptic release of neurotransmitters via the activation of A1 and A2 receptors, respectively [46]. Although the role of these receptors is relatively well-known in adults, their function in the developing brain remains little explored [47].

The A2A receptor appears to control the stability of nascent synapses. This led us to propose that postsynaptic A2A receptors act during development as detectors of presynaptic activity. Release of ATP or adenosine of presynaptic and/or glial origin would activate postsynaptic A2A receptors. The signaling pathways thus activated by A2A receptors would stabilize the newly formed synapses. A lack of activation of A2As receptors would cause the disruption of the synapse after a critical period. In other words, a transient postsynaptic A2A receptor function would signal to the postsynaptic element that the presynaptic element is active and that the synapse must be maintained. A2A receptors appear to have an important role in the pathophysiology of anxiety disorders [48, 49]. In fact, it is an indirect evidence that suggests that adenosine and its receptors play a role in anxiety. Caffeine is a nonselective receptor antagonist (A1 and A2A) to adenosine which proves to be anxiogenic. Indeed, in high doses it can lead to panic attacks [50]; but also, at low doses it is anxiolytic. Attempts have been made to synthesize A2A receptor agonists that would have anxiolytic properties, hitherto unsuccessful [51]. It is difficult to unravel the role of adenosine in mood disorders and anxiety disorders.

Neuropeptides and Anxiety

Neuropeptides are an important and broad class of molecular messengers carrying information between neurons in the brain. There are more than 100 different neuropeptides in the mammalian brain, and most of them are made and released from the hypothalamus. Some hypothalamic neuropeptides are released directly into the bloodstream and exert their peripheral effects just like hormones. Among these hypothalamic neurons, magnocellular neurons with oxytocin and vasopressin have been shown to be a model system, as they reveal major aspects of many neuronal functions, including the release mechanisms of the neuropeptides themselves. The classical interneuronal communication system is carried out via neurotransmitters, molecules packaged in small vesicles released mainly at the level of the neuronal synapse. In contrast, neuropeptides, contained in other vesicles, are released via mechanisms different from those of conventional neurotransmitters, from many membrane sites including nerve endings, cell bodies, and dendrites of neurons. The neuropeptide release mechanisms at the level of dendrites can be quite different from those at axonal endings, and, for vasopressin and oxytocin, this differential regulation allows peripheral effects of the released neuropeptide to occur independently of its central effects.

In addition to the classical neurotransmitters, neuropeptides represent an important class of modulators for affective behaviors and associated disorders, such as anxiety disorders. Many neuropeptides are abundantly expressed in brain regions involved in emotional processing and anxiety behaviors.

Cholecystokinin

Cholecystokinin (CCK) is the most abundant neuropeptide in the mammalian cerebral cortex and limbic system [52]. CCK is evident in significant quantities in regions known to be important in the mediation of panic disorder, including the cerebral cortex, striatum, amygdala, hippocampus, and brainstem nuclei where it is believed to function as a neurotransmitter or neuromodulator. Specific CCK recognition sites, presently divided into CCK-A and CCK-B subtypes, have been characterized, and drugs selectively active on these receptor subtypes have been synthesized [53].

Interest in the role of CCK in anxiety and panic stems led to microiontophoretic experiments, which showed that benzodiazepines selec-

tively attenuated the excitatory action of CCK8s on hippocampal pyramidal neurons in rats [54].

One of the best ways to study panic disorders is to cause panic attacks, in the laboratory and under controlled conditions, in healthy volunteers or patients with this disorder. To do this, many pharmacological agents have already been used, the most frequently studied being sodium lactate. A panic model has been developed, using cholecystokinin tetrapeptide (CCK4) as an inducing agent [55]. Studies show that CCK4 offers a safe and safe way to induce anxiogenic reactions in human's healthy volunteers and most patients with panic disorders. These patients describe the symptoms induced by CCK4 as very similar or identical to the symptoms of their spontaneous panic attacks. Moreover, in these patients, the panicogenic threshold is lower than that of healthy volunteers, and at equal dose the panic rate is much higher than in healthy subjects. A dose-response relationship has also been demonstrated for CCK4. The effects of CCK4 are reliable and reproducible in terms of symptom onset time, duration, and intensity. Ineffective drugs for the treatment of panic attacks do not seem to block the symptoms of CCK4, whereas those commonly used in this indication prevent the onset of seizures. CCK2 receptor antagonists were developed unsuccessfully, probably because they poorly crossed the blood-brain barrier [56].

It is still unknown whether CCK plays a role in PD alone or whether it is involved in the pathogenesis of other anxiety syndromes. PD patients and normal control subjects display marked differences in their sensitivities to the panicogenic effects of CCK4 [57]. Although the effects of CCK4 in patients suffering from other psychiatric disorders indicate a nonspecific effect of CCK4, it is possible that lower doses of CCK4 will differentiate PD patients from those with other pathological states. Additional provocation studies could help to determine whether CCK4 shows selectivity of action in PD and whether it could be used as a diagnostic tool.

CCK-B agonists could be used to map out neurotransmitter networks leading to the phenomena of anxiety and panic attacks. Strategies of pharmacological dissection of the panicogenic action of these agonists could be used in humans with agonists/antagonists of postulated target systems.

The hypothesis of CCK-glutamate interactions as mechanisms for CCK4-induced panic asserts that CCK4 activates a glutamatergic pathway which mediates panic symptoms. If selective glutamate receptor subtype antagonists blocked CCK4-induced behavioral actions, then the anatomical site(s) mediating glutamate/CCK4 interactions could be analyzed through site-specific microinjections.

Interactions between CCK and GABA may also contribute to the etiology of anxiety disorders [58]. These neurotransmitters are co-localized in several structures in the brain which control emotion and cognitive processing. GABA has been shown to inhibit the release of CCK from the rat cerebral cortex [59] while CCK8 increases K-evoked of GABA release from several brain areas in vitro [60]. Brain site-directed injections of CCK- and GABA-selective drugs and application of recent developments in molecular biology could give new insights into the role of interactions between CCK and GABA in the regulation of fear and anxiety (Fig. 18.6).

The main attention will be paid to the study of serotonin – GABA, CCK, nitric oxide (NO) pathways. The drugs both increasing and decreasing the activity of these neurotransmitter systems are going to be used. The experiments measure the changes in the activity of NO synthesis due to anxiogenic and anxiolytic pharmacological manipulations that are also to be planned [61].

Corticotropin-Releasing Hormone

It is a neuropeptide that plays an important role in stress-related responses. Corticotropin-releasing hormone (CRF) injected into the rat brain produces many signs and symptoms observed in patients with anxiety signs [62]. CRF1 receptor antagonists have been shown to be active in rodent models of anxiety, but clinical studies in humans have been disappointing [63].

CRF and CRF-related peptides are not only responsible for activation of the hypothalamic-

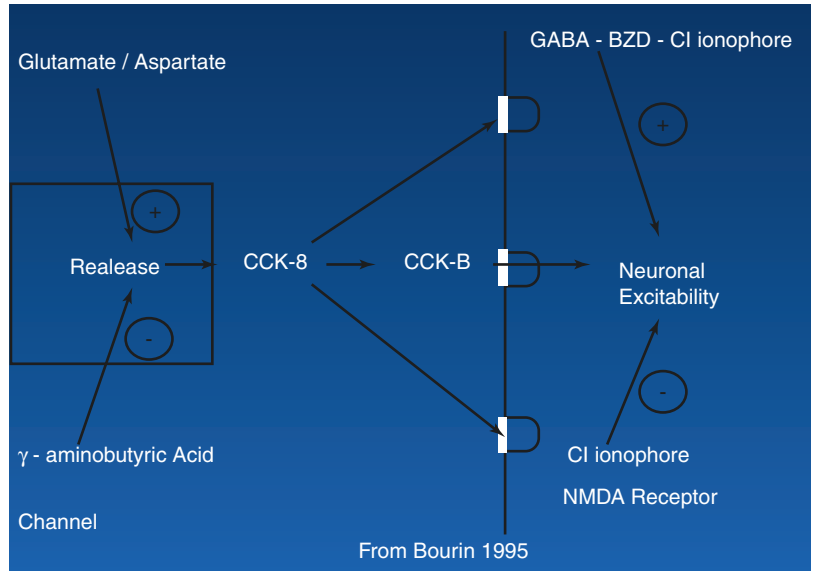

Fig. 18.6 The role of CCK in anxiety

pituitary-adrenal axis and activation of the sympathetic system in the brainstem in response to stress. They also have a powerful effect on the basal telencephalon that mediates behavioral responses to stress. CRF produces behavioral activation, facilitates behavioral responses to stress, and decreases appetite. CRF receptor antagonists very effectively produce a reversal of CRF suppressor or enhancer behavioral effects [64].

These effects appear to be mediated by the action of key elements within a telencephalic macrostructure called the "extended amygdala," comprising the central nucleus of the amygdala and the nucleus of the terminal striae. Furthermore, neuropeptides related to CRF have been identified, "urocortins" (urocortin 1, urocortin 2, and urocortin 3), and have been described as having high affinity for CRF receptors: CRF1 and CRF2 [63]. Urocortins are located mainly at the subcortical level. Urocortin 1 produces certain CRF-like effects and even reduces the intake of food in private animals or is fed at will more efficiently than CRF. Urocortins 2 and 3 do not possess the activating and anxiogenic effects of CRF in the rat but retain powerful anorexic effects. In transgenic mouse models, mice that overexpress CRF show anxiogenic responses compared to the wild strain, and mice that do not express the CRF1 receptor possess an anxiolytic behavioral profile compared to the wild-type strain [65]. The results obtained so far lead to the hypothesis that CRF1 receptors could participate in the mediation of CRF-like effects of neuropeptides on behavioral responses to stress whereas CRF2 receptors could participate in the mediation of the induced anorectic effect by neuropeptides of the CRF type. The specific brain targets responsible for the stress-like effect of CRF most likely involve CRF1 receptors in the extended amygdala; as for the anorectic effect, it would rather be the CRF2 receptors in some specific subregions of the hypothalamus. Recent studies also suggest that CRF in the extended amygdala has a major role as a mediator of the stress-like effect of withdrawal from most abused drugs such as alcohol, benzodiazepines, cocaine, opiates, and marijuana [66–68].

These effects may be important in prolonged abstinence and vulnerability to relapse. These data therefore suggest that CRF and neuropeptides related to CRF in the brain mediate the behavioral response to different types of stress. Dysfunction in such a fundamental brain activator system may be the key to a variety of pathophysiological conditions that lead to abnormal responses to stress, such as in anxiety disorders, affective disorders, substance abuse, and anorexia.

Substance P and Neurokinins

There are three main neurokinin receptors, NK1, NK2, and NK3, which are the preferential receptors for three tachykinins: substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), respectively [69]. However, SP acts on these three neurokinin receptors according to different degrees of affinity: mainly by binding to the NK1 receptor, then NK2, and finally NK3 [70]. Tachykinins and their receptors are widely distributed at the level of the central nervous system and peripheral nervous system [71]. Tachykinins bind to their receptors preferentially but not exclusively. Neurokinin receptors belong to the family of G-protein-coupled receptors.

NK1 is widely expressed at the central and peripheral levels and is present in neurons, vascular endothelial cells, muscles, and different types of immune cells. Internalization of the NK1 receptor appears to be a reliable and quantifiable indicator of this receptor's activity [72] and could potentially be a good indicator of MS activity.

The NK2 receptor is detected peripherally in smooth muscle cells, certain neuronal cell types and immunocytes, the middle layers of the dorsal horn (lamina II to IV) [73], and the specific nuclei of the brain.

The NK3 receptor is abundantly expressed in the CNS, and, although NKB does not appear to be peripherally expressed, the NK3 receptor is detected in some peripheral tissues [74]. Specifically, the NK3 receptor has been found in the cerebral cortex, the nucleus of the solitary tract, and the dorsal horn of the spinal cord [75].

Alterations in the SP-NK1 system have also been observed in human anxiety disorders, yet little is known about the relation between this system and individual differences in personality traits associated with anxiety propensity and approach-avoidance behavior, including trait anxiety, neuroticism, and extraversion [76].

Some animal studies tend to show anxiolytic properties of substance P antagonists [77]. Antagonists of the substance P (SP) preferring neurokinin 1 receptor (NK1R) represent a promising novel class of drugs for the treatment of stress-related disorders such as depression and anxiety disorders [78]. Some animal studies tend to show anxiolytic properties of substance P antagonists [79]. The development of nonpeptide receptor antagonists revealed species differences in neurokinin-receptor pharmacology, and the cloning of human neurokinin receptors has led to the development of compounds with optimized affinity for the human target receptor, but to date there is no anxiolytic drug issued from this research.

Galanin

Galanin (GAL) is a peptide consisting of a 30-amino acid chain produced in a number of regions in the brain. These regions include the amygdala (a region of the brain involved in the treatment of emotions) and specific regions of the hypothalamus. The galanin gene and its encoded protein may be involved in the regulation of food and alcohol consumption, but other genes could also be involved, and the DNA sequences examined here may not be the only regulatory factors of the gene.

Galanin and its three receptor subtypes (Gal R1–3) are highly expressed in the dorsal raphe nucleus (DRN), a region of the brain that contains a large population of serotonergic neurons [80]. Galanin is co-expressed with serotonin in approximately 40% of the DRN neurons, and galanin and GALR2 expression are elevated by antidepressants like the SSRI fluoxetine suggesting an interaction between serotonin and galanin [81]. Administered directly into the central nucleus of the amygdala, galanin blocks the anxiogenic effects of yohimbine. In addition, mice with a deficiency of galanin 1 receptors exhibit anxious behavior. This behavioral response depends on the balance between norepinephrine, neuropeptide Y, and galanin [82]. The anxiolytic effect induced by activation of Gal2 receptors may depend on the serotonergic tone. Finally, the role of galanin in panic-related behaviors remains uncertain.

Oxytocin

Recently, brain imaging has shown that oxytocin affects the functioning of the amygdala (emotional center of the brain) as well as parts of the prefrontal cortex (in charge of controlling emotions and behaviors). During this experiment, volunteers were subjected to images associated with fear as well as the possibility of being subjected to electric shocks. After nasal absorption of oxytocin, researchers were able to observe a change in amygdala activity accompanied by a decrease in the feeling of fear, as well as a decrease in the production of sweat, a physiological phenomenon accompanying the feeling of fear [83]. Many biological studies conducted on oxytocin have shown that absorption of this hormone systematically causes a drop-in heart rate and blood pressure, causing the individual to experience a feeling of calm and security. Because of its effects on the parasympathetic nervous system, oxytocin greatly reduces anxiety and is therefore a perfect anxiolytic [84]. By reducing fear and anxiety, oxytocin has direct consequences for human behavior and the perception of the outside world. The properties of oxytocin thus act directly on the social behavior of the human being, by promoting trust and closeness between individuals. It would inhibit aggressive behavior and make it more optimistic. Studies conducted by this university have shown that, under the effect of oxytocin, people would better remember happy faces than sad but also better recognize positive social indicators than threatening [85]. Evidence for the role of oxytocin in regulating anxiety is undeniable. We expect that the diverse particularities of the oxytocin system will help broaden our understanding of anxiety and stress-related disorders [86].

Atrial Natriuretic Peptide

Atrial natriuretic (ANP) factor is a polypeptide hormone essentially synthesized by the right atrium of the heart. It participates in the homeostasis of sodium, potassium, and water by acting on renal excretion and has a vasodilatory action [87].

Pretreatment of 150 μg of natriuretic peptide avoids CCK4-induced panic attacks in patients with panic disorder [88] as well as in healthy volunteers. On the other hand, ANP exerts anxiolytic-like effects on CCK4-stimulated anxiety attacks in patients with panic disorder. In addition, ANP produced an inhibition of the hypothalamic-pituitary-adrenocortical system and sympatholytic effects [89]. Future research will have to confirm or disprove these preliminary findings. To date, there is no useful synthetic derivative [90].

Antidepressants in Anxiety

The concept of antidepressants is evolving gradually since these molecules are used successfully to treat other mental pathologies than depression. They are also discouraged in the treatment of bipolar disorders [91].

Clomipramine was the first to prove an activity in the treatment of obsessive-compulsive disorder (OCD), while other imipramines and derivatives are not effective. In fact, its desmethylclomipramine metabolite is a potent inhibitor of both serotonin and norepinephrine reuptake. The combined results of clomipramine and desmethylclomipramine on the inhibition of serotonin reuptake are much greater than those of other tricyclics. Other selective serotonin reuptake inhibitors, such as fluoxetine, fluvoxamine, sertraline, and paroxetine, have also been shown to be effective in the treatment of OCD. Their effectiveness in treating this condition is clearly not related to their antidepressant properties as these drugs reduce obsessive-compulsive symptoms in patients who are not depressed.

Since the 1960s, several studies have demonstrated the effectiveness of MAOIs in anxiophobic states. These molecules have been shown to be particularly effective in the treatment of social phobias [92], but the difficulty of using these derivatives has led to them being used only in severe cases or even to abandoning them.

These results have been confirmed more recently, and new studies have been developed thanks to the use of potentially fewer toxic derivatives such as the MAO A-specific inhibitors.

Klein and Fink were those who first observed that imipramine was able to prevent panic attacks. Later Klein showed that imipramine was effective in treating phobias with panic attacks but not effective in phobias. These observations led to the treatment of subjects with panic attacks with low doses of imipramine as a preventive measure; the high doses exaggerate the phenomenon. The dose is increased in steps until, after 3 months; doses of imipramine are similar to those usually used in depression. Authors have shown that imipramine and trazodone are effective in the treatment of generalized anxiety. Imipramine also has better results than trazodone and diazepam compared to placebo after 6 and 8 weeks. This work confirms previous work that had been conducted in patients with anxio-depressive pathology. From now on, the various IRRS have been granted marketing authorization for generalized anxiety and other anxiety disorders.

The fact that antidepressants are active in the treatment of anxiety disorders led us to seek the explanation of their mechanisms of action in these pathologies. It turns out that 5-HT2A receptors can participate in this activity. It seems more and more obvious that this action would be exercised at the level of the amygdala, a cerebral structure that seems to be a "filter" for the perception of emotions and is rich in 5-HT2A receptors.

Conclusion

The fact that the pathophysiology of anxiety is complex has led to very different drug developments in this area. The first were benzodiazepines discovered by serendipity. It turns out that animal models of anxiety have been developed as to their effectiveness in "screening" BZDs. BZDs decrease serotonin in the brain; they have a more disinhibitory action than really anxiolytic. The SSRIs also reduce the cerebral serotonin in a less abrupt way than the BZDs which contributed to their therapeutic success; it remains to know if more specific derivatives of a type of serotoninergic receptor could have a better benefit/risk. The glutamatergic track appears from time to time, but we do not know how to control the glutamate/GABA balance. As for the neuropeptides, they are not effective in themselves but seem to be modulators of the serotoninergic activity; used alone they are of limited effectiveness. There are used as biological markers for anxiety disorders [93].

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19 From Benzodiazepine Prescription to Dependence: Learning Processes Involved

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Benzodiazepines: Mechanism of Action, Clinical Use, and Side Effects

Benzodiazepines (BZD) are among the most commonly prescribed psychotropic medications worldwide because of their multiple uses and are taken by about 4% of the population [1–3]. They potentiate the actions of γ-amino-butyric-acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS), acting through ionotropic $GABA_A$ and $GABA_C$ receptors [4, 5], and metabotropic $GABA_B$ receptors [6]. $GABA_A$ receptors are heteropentameric ligand-gated, anion selective, chloride channels that open upon GABA binding [7]. These receptors are composed of five subunits with a specific stoichiometry of 2α, 2β, and 1γ (among others); and each subunit family presents several isoforms: α1–6, β1–3, γ1–3, δ, ε, π, Θ, and ρ1–3 [8].

The pharmacological actions of BZD are mediated by a high-affinity site on GABA-A receptor, widely distributed on neurons into the CNS, acting as allosteric modulators, which means that they only act in the presence of the endogenous ligand GABA [9]. Based on efficacy, BZD can be positive and negative allosteric modulators, with different potencies (partial positive or negative modulators) or antagonists. The positive modulators potentiate $GABA_A$ receptor-evoked currents, whereas the negative modulators decrease these currents. The antagonists prevent and reverse the effect of both types of allosteric modulators and with no consequences on the channel activity on their own [10]. Nevertheless, only positive allosteric modulators such as clonazepam and diazepam (DZ) and the antagonist flumazenil are used therapeutically. All BZD bind to a specific cleft located between α and γ subunit of the $GABA_A$ receptor [11, 12], but only α1-, α2-, α3-, and α5-containing $GABA_A$ receptors are sensitive to BZD [13].

BZD are prescribed extensively to treat anxiety and sleep disorders and used as adjuvant therapy in depression, pain management, and as muscle relaxants [14–16], usually requiring long-term treatment. They are widely used for their rapid onset of action and clinical efficacy, as well as low toxicity and decreased risk of suicide [14]. BZD are safe and effective for short-term treatment. However, long-term use is controversial due to the development of tolerance and their liability for physical dependence [17]. Even with correct treatment duration and doses, BZD use has both short- and long-term side effects. Short-term, untoward effects include sleepiness that may interfere with daily function, increased risk of motor vehicle accidents, falls that may be

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accompanied by fractures especially in the elderly, and potential for abuse or misuse [14]. Long-term drawbacks include tolerance and physical dependence, cognitive and memory impairment leading to withdrawal, or rebound symptoms following treatment discontinuation, which may be greater in severity than pretreatment levels and may persist for extended periods [14, 15].

Benzodiazepines Abuse, Misuse, and Wrong Prescription

Misuse of prescription drugs means taking a medication in a manner or dose other than prescribed; taking someone else's prescription, even for a legitimate medical complaint; or taking a medication to feel euphoria. The term nonmedical use of prescription drugs also refers to these categories of misuse [18]. Increases in prescription drug misuse over the last 15 years are reflected in increased emergency room visits [19], overdose deaths associated with prescription drugs [20–23], and treatment admissions for prescription drug use disorders, being addiction the most severe form of them. The reasons for the high prevalence of prescription drug misuse vary by age, gender, and other factors but likely include ease of access [24]. Nonmedical use of prescription drugs is highest among young adults, aged 18–25 [18]. Youth who misuse prescription medications are also more likely to report use of other drugs [25–28]. More than 80% of older patients use at least one prescription medication on a daily basis [29]. This can potentially lead to health issues resulting from unintentionally using a prescription medication in a manner other than how it was prescribed, or from intentional nonmedical use [30].

Consumption of BZD is becoming a public health problem because of the indiscriminate use. The Drug Abuse Warning Network (DAWN) monitored emergency department (ED) visits in selected US areas through 2011. DAWN reported that more than 1.2 million ED visits in 2011 could be attributed to nonmedical use of prescription drugs; this represents about half of all ED visits related to drug misuse and 28.7% of those involved BZD [21], being BZD one of the two most frequently reported prescription medications in drug abuse-related cases along with opioid-based pain relievers (http://www.nida.nih.gov). Furthermore, BZD abuse often occurs in conjunction with the abuse of another substance (e.g., alcohol or cocaine), making treatment approaches even more difficult.

Aside from physical and psychological dependence, other potential problems associated with improper BZD use or abuse are suicidal thoughts or actions, worsening of depression, sleep disorders, and aggression [31]. While CNS depression and cardiorespiratory depression rarely characterize oral BZD overdoses [32], in 2013, an estimated 22.767 people died of an overdose involving prescription drugs in the United States, and BZD were involved in approximately 31% of these fatal overdoses [33]. Furthermore, overdose mortality involving BZD rose at a faster rate than did the percentage of individuals filling prescriptions and the quantity filled [34]. It may be probably because the increases in the total quantity filled reflected both an increase in the number of individuals filling BZD prescriptions as well as substantial increases in the amount each individual received. Furthermore, among physicians prescribing BZD, the median quantity prescribed over the year is reported to be more than doubled between 1996 and 2013, suggesting either a higher daily dose or more treatment days, which potentially increased the risk of fatal overdose [35]. Moreover, people at high risk for fatal overdose may be obtaining diverted BZD (i.e., not directly from medical providers). The proportion of fatal overdoses involving diverted versus prescribed BZD is unknown. Finally, increases in alcohol use or combining BZD with other medications could increase the risk of fatal overdose and explain this rise [34].

Inappropriate BZD prescriptions mainly among the elderly population are common [36, 37] and represents a health problem, considering that in some cases approximately 70% of the patients received an inappropriate prescription [38]. Other studies showed that prescriptions were adequate for only 1.9% for adults and 5.8%

of older adults, drawing the attention to inappropriate indication for use, non-recommended procedures for the age group and/or patient; risk of severe drug interactions; and problems related to dose, frequency, and mainly period of treatment [39]. Although the use of BZD is justified in many situations, their long-term use and the presence of potentially inappropriate prescriptions are frequent phenomena that could significantly affect older adults' functioning [40–43].

Because older adults have less muscle mass, an increased body fat percentage, and a reduction in total drug elimination mainly due to a decrease in renal efficiency, they are exposed to the extended action of BZD [44]. Furthermore, the half-life of BZD can be tripled in elderly patients [45], therefore long-acting drugs (such as clonazepam and DZ) are not recommended for these patients because they increase the risk of side effects [46–48] affecting stability, memory, and concentration [49, 50]. Also, they have been associated with hypertension, coronary and renal diseases [51, 52], as well as with falls, risk of fractures, and hospitalizations [51, 53–55]. Additionally, older adults are also susceptible to develop physiological and psychological dependence on BZD [50]. Then, when BZD prescription must be considered for these patients, intermediate or short-acting drugs such as lorazepam and alprazolam at the lowest effective dose are recommended [45, 47]. The high proportion of inappropriate BZD prescriptions observed among the older adult population suggests that prevention strategies should be developed to inform older adults, doctors, pharmacists, and other healthcare providers about the appropriate use of these drugs [38].

Conditioned Benzodiazepines Tolerance and Dependence

As it was stated before, the problems associated to BZD use include diversion, misuse, driving impairment, morbidity and mortality related to overdose, tolerance, and dependence [1, 2]. Tolerance is manifested when drug dosage must be increased in order to achieve the same pharmacological effects. Dependence is reflected by the appearance of a series of symptoms when the drug is abruptly withdrawn, and for BZD the risk of dependence increases after long-term use [56]. The most frequent physiological symptoms characterizing BZD dependence include insomnia, anxiety, gastric problems, tremors, agitation, fearfulness, and muscle spasms [57]. Less frequently observed are irritability, sweating, depersonalizations, hypersensitivity to stimuli, depression, suicidal behavior, psychosis, seizures, and delirium tremens [58]. On the other hand, addiction is considered a brain disease defined by the World Health Organization as compulsive substance intake despite negative consequences, and it is also characterized by relapse after a prolonged period of abstinence. Most often, people who recreationally abuse BZD also abuse other drugs such as alcohol or opioids (www.nida.nih.gov).

It has been demonstrated that 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 associative learning processes [59, 60]. The environmental context of an experience induced by a drug of abuse is crucial for drug-seeking behavior and relapse in human addicts [61, 62]. This fact relays in the Pavlovian conditioning principles, which consider that an associative learning occurs with the contingency between two stimulus events: a neutral stimulus (conditioned stimulus, CS) followed by a biologically relevant stimulus (unconditioned stimulus, US). The first one (CS) may acquire the property to induce a response in the organism (conditioned response, CR) that is similar to the one triggered by the US [63]. Repeated or chronic drug administration often results in the conditioning of physiological responses, where drug administration ritual may act as a CS that will eventually elicit a CR. These conditioned responses have been suggested to play a role in drug tolerance, dependence, and sensitization, even for BZD [64–68].

Furthermore, learning and memory, particularly contextual memories, are important for the establishment of conditioned responses to drugs

of abuse [59, 61]. It has been shown that an associative learning process is underlying the development of tolerance and dependence to DZ, because pre-exposure to DZ administration context impairs the development of tolerance to the sedative effects of DZ [65]. Additionally, changes in contextual cues presented during DZ long-term treatment avoid expression of the anxiety-like behavior after drug discontinuation [64]. Alternatively, withdrawal expression can be attenuated or avoided by pre-exposure to the administration context during the last days of long-term treatment [69]. The anxiety-like behavior expressed by dependent animals is evident until 5 days after discontinuation [70], but when these animals are re-exposed to the context in which they experienced DZ withdrawal, 2 weeks after DZ discontinuation, this withdrawal symptom is expressed over again [70]. Furthermore, alterations in contextual cues experienced during withdrawal prevent its expression after long-term withdrawal [71]. Then, the environmental cues present during the drug administration procedure or withdrawal are able to modulate the behavioral expression of both tolerance and dependence to DZ, by affecting the conditioned responses previously described.

Repeated Benzodiazepines-Induced Alterations in Brain Structures Related to Learning and Memory

As it was stated before, BZD are positive modulators of GABA-A R function. Different subtypes of this receptor are widely spread in the CNS, particularly in brain areas related to learning and memory (hippocampus, medial prefrontal cortex (mPFC), amygdala) as well as to abuse and physical dependence (nucleus accumbens (NAc) and ventral tegmental area (VTA)), among others [72]. Repeated long-term BZD administration induces neuroadaptations in these and other brain structures affecting their function and thereby impact in behavioral expression after treatment discontinuation (Fig. 19.1).

Hippocampus

The hippocampus is a brain structure crucial for episodic memory formation [73–76]; in fact,

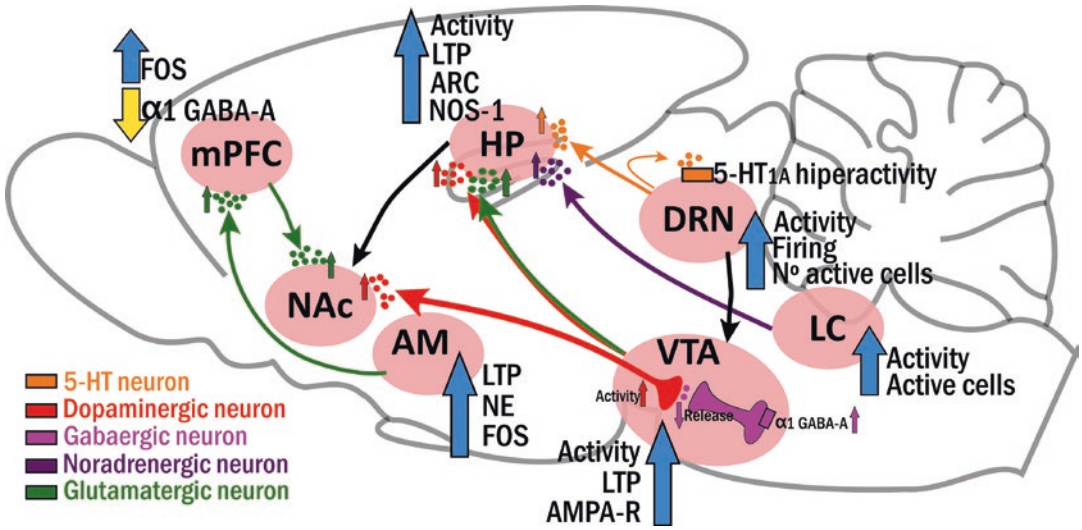

Fig. 19.1 BZD effects after chronic administration in *mPFC* medium prefrontal cortex, *HP* hippocampus, *DRN* dorsal raphe nucleus, *LC* locus coeruleus, *AM* amygdala, *NAc* nucleus accumbens. Acute BZD effects in *VTA* ventral tegmental area. *LTP* long-term potentiation, *NOS-1* nitric oxide synthase-1, *NE* neuronal excitability, *ARC* activity-regulated cytoskeleton-associated protein, *FOS* transcription factor FOS, *GABA-A* gaba A receptor type, $5HT_{1A}$ serotoninergic 1A receptor type, *α1* GABA-A receptor subunit alfa 1. Colored dots (green, red, orange, and magenta) indicate different neurotransmitters release

hippocampal (HP) neurons encode configuration items and events in the spatial and temporal context in which they were experienced, a central feature of episodic recollection [77]. Specifically, the dentate gyrus (DG) has been described as a HP subregion with high rates of plasticity, and it is targeted by different psychoactive drugs modulating synaptic plasticity [78]. The long-term potentiation (LTP) in the hippocampus, an enduring increase in the efficacy of glutamatergic synaptic transmission, is accepted as a molecular mechanism for memory storage in the brain in which contextual cues are relevant [79–82].

It has been described that development of tolerance and dependence to DZ administration are caused by learning and memory phenomena, where the hippocampus has an essential role [83]. Rapid tolerance to the sedative effects of DZ has been linked to an enhanced HP DG synaptic plasticity, measured as a reduced threshold to induce LTP [84]. It has been also related to an increased mRNA expression of NR1 and NR2B N-methyl-D-aspartate receptor (NMDA) subunits [85]. NMDA receptors constituted by these particular subunits are the most efficient type, because overexpression of the recombinant NR1/NR2B subunits results in a prolonged opening of the NMDA receptor channel and in an enhanced NMDA receptor activation in individual synapses [86].

Similar results have been found after development of DZ dependence by repeated long-term administration (18 days). During dependence, manifested by drug discontinuation, in which animals exhibit withdrawal signs such as anxiety, a facilitated threshold to induce LTP in the HP DG was also present [87]. Furthermore, increased immediate-early gene activity-regulated cytoskeleton-associated protein (Arc) positive cells were observed in this brain structure [70]. Arc is one of the proteins involved in processes of synaptic plasticity that are induced by neuronal activity linked to spatial learning and memory [88], promoting local expansion of the actin cytoskeleton [89]. Moreover, persistent activation of the brain-specific atypical protein kinase M zeta (PKMz) is also necessary and sufficient for maintenance of an HP LTP [90, 91]. Sustained Arc synthesis is necessary for LTP maintenance, and it has been suggested as a sequential mechanism of LTP maintenance in which Arc-dependent actin stabilization promotes PKMz synthesis and expression of enduring LTP [89, 92].

Another important player in neuronal excitability and synaptic plasticity in HP and other brain areas is the gaseous neurotransmitter nitric oxide (NO) [93]. NO is a diffusible neuromodulator synthesized in the brain mainly by the neuronal NO synthase enzyme (NOS-1) following NMDA receptor activation [94], and it is involved in a variety of physiological and pathophysiological processes including learning and memory [95], anxiety [96], and neuroadaptations induced by different addictive drugs [97–103], among others [104–106]. Furthermore, inhibition of NO synthesis attenuates pentetrazole-induced withdrawal syndrome in DZ-dependent mice [99]. Recent findings showed that pre-exposure to drug administration context at the end of long-term DZ treatment not only prevented withdrawal symptoms but also reduced NOS-1 protein levels within the dorsal HP [69]. The dorsal HP is a region primarily involved in the cognitive process of learning and memory [107], and it is important to note that the decrease in NOS-1 protein levels was only observed in this region and not in ventral HP. Furthermore, these reduced NOS-1 protein levels and probably a diminished NO availability within dorsal HP could be a compensatory mechanism to reduce HP synaptic plasticity and to avoid the expression of the "anxiety-like" behavior [69].

Most of the changes induced by DZ after tolerance or dependence within the hippocampus described above were prevented or reversed by either in vivo NMDA antagonist systemic administration [108], intra-HP PKMz inhibition [71], pre-exposure to the drug administration context [65], or alteration in the contextual cues present during the withdrawal experience [64]. All together, these results have pointed out that the increased plasticity observed in the hippocampus, concomitant to the development of tolerance, dependence, and withdrawal, may be the

neurobiological substrate underlying the behavioral alterations observed as a consequence of repeated DZ administration.

Locus Coeruleus

Locus coeruleus (LC), the major noradrenergic (NA) nucleus in the pons, is also activated during DZ withdrawal after repeated long-term administration. This structure participates in a wide range of brain functions and behaviors, such as the sleep-waking cycle [109], neural plasticity [110–112], drug abuse [113], and stress responses [114]. The NA system has been proposed to modulate several cognitive functions, including learning and attention involved in arousal and vigilance [112, 115]. Furthermore, the participation of the NA system in promoting a full withdrawal syndrome after the long-term use of BZD has been well established in many studies [116]. It has been shown that DZ dependent rats after chronic administration increased the in vivo number of spontaneously active cell/track and the firing rate within LC neuronal spontaneous activity when compared to controls or to rats without physical signs of withdrawal [87].

This enhanced LC activity taken together with biochemical studies in both humans and animals showing that BZD withdrawal was associated with increased NE levels [117] may account for both physical withdrawal symptoms (involving the autonomic function) and the increased HP synaptic plasticity. Accordingly, it has been demonstrated on HP slices from adult rats that the perfusion of adrenergic agonists promotes lasting synaptic plasticity in the adult CNS [118].

Prefrontal Cortex

It is known that the mPFC, hippocampus, and amygdala are key structures necessary for the execution of memory- and anxiety-related behaviors. The CA1/subicular region of the ventral hippocampus plays an important role in the acquisition of spatial memory [119]. Furthermore, this region is connected to the mPFC [120], and this connection could contribute to the encoding of spatial information [121]. Parts of the prefrontal cortex, including the prelimbic region, have been reported to be involved in the production of fear- and anxiety-related behaviors [122, 123]. Then, the connections in the HP-mPFC-amygdala pathway may be necessary for memory-dependent emotional regulation, processes that occur during BZD withdrawal. Nevertheless, only a few studies were performed until now showing the impact of BZD long-term treatment within the mPFC structure or physiology.

It has been shown that withdrawal from chronic DZ administration in rats induces an increase in neuronal activation only in the prelimbic cortex from the mPFC, assessed by Fos-like immunoreactivity as a marker of the neuronal activation [124]. Furthermore, a marked decrease in the density of dendritic spines in apical dendrites from pyramidal neurons of the cingulate cortex was observed specifically in layer V after similar treatments in mice [125].

On the other hand, long-term treatment of rats with increasing doses of diazepam or triazolam for 10–14 days proved to induce anticonvulsant tolerance and decreased expression of mRNA and protein encoding for the α1 $GABA_A$ receptor subunit in prefrontal cortex [9, 126, 127]. This receptor subunit has also suggested mediating the sedative, amnesic, and anticonvulsant actions of classical benzodiazepines [128, 129]. In line with these results, an association between alterations of acetylated histone H3 and long-term BZD-induced downregulation of $GABA_A$ receptor subunit expression has been reported. Specifically, a decrease in the expression of α1 $GABA_A$ receptor subunit mRNA and promoter acetylation in mPFC was associated with long-term treatment with DZ. Also, 10 days of DZ treatment increased the expression of histone deacetylase (HDAC) 1 and 2 in mPFC. Thus, the increased expression of HDAC1 and HDAC2 (class 1 HDACs) and consequently increased histone deacetylation mechanism of this class 1 HDACs may underlie long-term DZ-induced decreased expression of the α1 $GABA_A$ receptor subunit mRNA in frontal cortex [130].

Considering the importance of the mPFC both in the expression of the BZD withdrawal symptoms and in the mechanism underlying dependence, withdrawal, and probably addiction to BZD, it is important to arrow new studies in this particular brain area.

Dorsal Raphe Nucleus

The forebrain 5-HT system, commonly referred to the dorsal raphe nucleus (DRN), is implicated in many aspects of the cerebral function, including emotion and fear processing, cognition, movement, and regulation of the sleep-wake cycle [131–133]. 5-HT has been implicated in the control of a wide variety of psychiatric functions, with dysfunction of the 5-HT system thought to be involved in the development and/or progression of neuropsychiatric disorders including depression and anxiety [134, 135]. Furthermore, it has been postulated that the anxiety occurring during DZ withdrawal is mediated by an increased 5-HT release in the hippocampus [136]. In fact, DZ withdrawal after chronic administration in rats induced increased number of spontaneously active cells per track on DRN and the number of neurons firing between 1.01 and 1.50 spike/sec [137]. Additionally, hypersensitivity of somatodendritic serotonin 5-HT1A receptors was observed after repeated DZ [138], while the 5-HT1A agonist, 8-OH DPAT, during DZ withdrawal not only dose-dependently reversed anxiety but also decreased the DRN firing rate and hence the release of 5-HT in the dorsal hippocampus. These results provide clear evidence that the DRN-dorsal HP 5-HT pathway is at least one of the pathways playing an important role in mediating DZ withdrawal-induced anxiety [139]. Furthermore, pretreatment with MK-801 during DZ chronic administration impaired both the development of anxiety signs during DZ withdrawal and the increase in electrical activity within the DRN [137]. Serotonin systems have been implicated in the regulation of HP synaptic transmission [111, 140, 141]. Then, the increased DRN neuronal activity may result in a facilitation of the HP synaptic transmission during withdrawal from chronic DZ administration previously described [141–143].

Amygdala

Amygdala is a key brain structure in the modulation of emotional behavior [123, 144–147] and particularly in the Pavlovian aversive learning [148–150]. The basolateral complex (BLA) receives relevant information from the environment via HP, thalamic, and cortical afferents [151, 152]. In turn, the BLA projects to the central nucleus of the amygdala, which then projects to midbrain and brainstem areas involved in the coordination of endocrine, somatic, and behavioral responses to aversive stimulation characteristic of fear and anxiety [146, 152, 153]. Withdrawal from repeated BZD administration may induce exaggerated emotional responses and stress-induced behavioral deficits lasting several days after the interruption of chronic BZD [154–156]. The long-lasting occurrence of exaggerated emotional reactions to mild aversive stimulation could be an additional component of BZD withdrawal [154]. It has been demonstrated that withdrawn rats from chronic DZ display enhanced fear learning and also show increased neuronal excitability and facilitated LTP in BLA together with a disinhibition of BLA projection neurons, caused in pyramidal neurons by suppression of IPSPs generated by recurrent activation of GABAergic interneurons [157]. All this changes favor multiple firing in response to cortical afferent activity and greater synaptic plasticity that probably underlies the increased aversive learning observed during DZ withdrawal [158]. This neuronal hyperactivity is consistent and enhanced Fos-like immunoreactivity [159] as well as increased glucose use following flumazenil-precipitated withdrawal [160] and reduced mRNA levels of glutamatergic AMPA receptors subunits in the amygdala nuclei [161].

On the other hand, a recent study showed that chronic DZ administration on the mouse brain induced alterations in the expression levels of 177 genes corresponding to protein-coding genes and also non-protein-coding genes. Most of the non-protein-coding genes detected in the hippocampus and amygdala (78.4% and 90%, respectively) were downregulated. Interestingly, the lipocain-2 (Lcn2), known member of the lipocalin family of transport proteins [162–164], which are related to iron transport, were upregulated in both the amygdala and hippocampus [165]. In the CNS, Lcn2 released from injured neurons activates microglia and astrocytes [166]. Activated microglia secretes Lcn2 and sensitizes themselves to apoptosis [167] and promotes neu-

ronal death [168] and also is involved in the structural plasticity of neurons such as reductions in spine density [125]. All these data suggest that chronic DZ administration may also disrupt iron homeostasis particularly in brain areas related to cognition and learning and further support recent clinical studies revealing that BZD overuse is associated with an increased risk of Alzheimer's disease [169–171]

Benzodiazepines Effects Within the Natural Reword System

As it was mentioned before, 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 [172]. The VTA and the NAc are part of the mesolimbic system, which is an integral component of brain reward system. Dopamine (DA) neurons within the VTA provide one of the major sources of DA to limbic structures, including the NAc. Anatomically, VTA is constituted of a heterogeneous population of DA, GABA, and Glu neurons. DA neurons represent the majority of the VTA cells, while GABA and Glu neurons account for 35% and 2–5% of the total population, respectively [173–179]. Moreover, it seems that some VTA neurons co-release multiple neurotransmitters, jeopardizing their neurochemical classifications. For instance, VTA DA neurons that project to the NAc and dorsal striatum can co-release DA and GABA or DA and Glu from the same axons [176, 180–182]. Interestingly, VTA neurons that project to the lateral habenula have been shown to co-release GABA and Glu, thus sending mixed inhibitory/excitatory signals [183]. Similarly, a functional connection in which VTA neurons co-release both GABA and Glu in the HP DG has been recently described [184]. In spite of the complexity of the VTA neurotransmission, it is a fact that DA participates in the encoding of reinforcement and learning [185].

A common particularity of addictive drugs is that they all target the VTA and acutely increase extracellular levels of DA within the VTA and target regions [186, 187]. Drugs of abuse may induce these DA increments by reducing GABA interneurons activity within the VTA leading to a disinhibition of DA main VTA neurons (i.e., opioids [188] and cannabinoids [189]); by activating directly DA neurons (nicotine [190]); by affecting the DA transporter located at the terminals of DA neurons either by blocking it (cocaine) or reversing its activity (amphetamines) [191]; or by inhibition of the DA metabolic enzyme, the monoaminooxidase (MAO; like amphetamine) [192]. Unlike natural rewards, addictive drugs always cause an increase in DA levels upon drug exposure even after repeated trials [193]. This interruption of normal DA signaling mechanisms may allow addictive drugs to hijack the reward system and lead to the malfunction of mechanisms controlling learning and memory [10].

Recent findings have demonstrated that BZD engage pharmacological and cellular mechanisms in the mesolimbic DA system [194, 195] that are similar in nature to those which have previously been identified for other drugs of abuse, because they are able to induce an indirect increase in extracellular DA levels by reducing GABA interneurons activity and thereby disinhibiting DA neurons [195]. The pharmacological effects of BZD depend crucially on α subunit isoform identity. For example, much of the literature suggests that subtypes containing α1 subunits mediate their reinforcing and sedative effects, whereas subtypes containing α2/α3 subunits have been implicated in the anxiolytic effects [195–198]. For these reasons, novel $GABA_A$ positive alosteric modulator compounds selective for α2/α3-containing $GABA_A$ receptors might have a lower abuse liability while still producing therapeutic effects such as anxiolysis [12, 196]. Indeed, the acute effects of BZD depend on the brain area in which they are administered. For instance, injection of dipotassium chlorazepate has a differential behavioral profile, leading to anxiolytic effects (mediated by its injection within the amygdala) or anxiogenic effects (mediated by its administration within PFC or Hip) [199].

Various non-selective positive alosteric modulators such as DZ and midazolam are self-

administered across a wide range of reinforcement schedules in multiple species [195, 200–203]. They are rewarding in conditioned place preference paradigm [204] and able to increase the rate of responding for and to decrease the threshold of the intracranial self-stimulation reward [198]. Then, reinforcers such as DZ and zolpidem, similarly to the other drugs of abuse, were shown to induce plasticity in the glutamatergic synapses contacting VTA DA neurons [194]. Particularly, BZD induced an LTP that was prevented by co-administration of the BZD antagonist flumazenil and by the NMDA receptor antagonist dizocilpine [194]. BZD-induced LTP in VTA DA neurons was associated with insertion of new AMPA receptors with specific subunit composition (GluA2-lacking), and intra-VTA local network was sufficient for the LTP induction via inhibition of VTA GABA interneurons [195].

The studies related to how chronic BZD exposure can affect remodeling and plasticity of brain reward circuitry have not been investigated as deeply as for many drugs of abuse, including cocaine, morphine, and nicotine. It is necessary to know how they can affect the physiology not only of the VTA but also the NAc, the PFC, and many other brain regions. Those experiments will be important for determining the commonalities that BZD share with other addictive drugs, as well as for identifying the specific attributes that make them unique. Recent data described below reveal how BZD, acting through specific $GABA_A$ receptor subtypes, activate midbrain DA neurons and how this may hijack the mesolimbic reward system. Such findings have important implications for the future drug design with reduced or even absent addiction liability [10].

Pharmacological or Behavioral Strategies to Treat Benzodiazepines Withdrawal

When clinical data are observed regarding management of BZD withdrawal, physicians prefer to utilize a gradual dose taper to avoid withdrawal symptoms in order to favor patient compliance to BZD discontinuation, although withdrawal symptoms occur even with slow dose taper from relatively low doses of BZD [205–207]. However, it can be seen a lot of effort focused on potential pharmacological treatments, considering the extensive list of investigated compounds for the treatment of BZD withdrawal, but unfortunately there is not an effective pharmacological therapy yet available.

Pharmacological Treatments

A large number of drugs have been used to alleviate, reverse, or prevent BZD withdrawal symptoms in animals (Table 19.1), considering all the evidence regarding the mechanisms that underlie BZD dependence and withdrawal and the brain structures involved (see section "Repeated Benzodiazepines-Induced Alterations in Brain Structures Related to Learning and Memory"). It has been shown that drugs with good anxiolytic and anticonvulsant effects such as the partial positive modulators abecarnil and Ro 19-8022 are also effective in reducing withdrawal symptoms from traditional BZD [207]. Mice withdrawn from 12-day daily alprazolam treatment showed increased anxiety-like behavior, muscle tone, and seizures between day 1 and day 28 after drug discontinuation. The development of these withdrawal symptoms was prevented if alprazolam treatment was followed by 1-week treatment with abecarnil. On the other hand, mice withdrawn for 72 h from 8-day alprazolam treatment showed less social behavior and were more aggressive. An acute administration of Ro 19-8022 reversed these behavioral withdrawal signs, without causing side effects such as sedation or ataxia [208]. However, these replacement treatments should be examined carefully before being recommended for therapy of BZD dependence and withdrawal in humans, because BZD might have addictive potential themselves [207], as it was previously discussed.

On the other hand, serotoninergic partial agonists have been also tested considering that this neurotransmitter system has also a role in BZD withdrawal expression, because 5-HT1A agonists reverse anxiety and decrease the DRN firing rate. Co-administration of buspirone, a 5-HT1A partial agonist, with DZ for 7 days increased the incidence of withdrawal-induced convulsions [209]. Other study also reported that withdrawal from 7-day DZ treatment, buspirone failed to

Table 19.1 Experimental pharmacological strategies to treat BZD withdrawal signs in animals after different BZD chronic exposure

Citation	BZD treatment and duration	Withdrawal treatment (WT)	WT duration	Withdrawal signs or behavioral test	Experimental subject
Pinna 1997 [100].	Alprazolam 6 mg/kg Twice daily for 12 days Subcutaneous injections	Abecarnil 6 mg/kg	7 days Daily subcutaneous injections	Anxiety-like behavior Muscle tone Seizures	Male mice 20–24 g
Krsiak, M. 1998 [208]	Alprazolam 1 mg/kg Twice daily for 8 days Oral administration	Ro19-8022 10 mg/Kg	One oral administration	Anxiety-aggression Social and aggressive behavior	Male mice 18–20 g
File SE. 1991 [213]	Diazepam 2 mg/Kg/day 21 days Intraperitoneal injection	Buspirone 200 μg/Kg	One s.c. administration	Anxiety/social interaction Elevated plus maze	Male hooded Lister rats 180 g
Tsuda M. 1998 [222]	Diazepam oral administration in DZ-admixed food Increasing concentrations (1–12 mg/g) 30 days	MK-801 0.05 and 0.1 mg/Kg	One i.p. administration	Motor, emotional, and autonomic withdrawal signs Body weight loss	Male Fischer 344 rats
Galpern WR. 1991 [226]	Alprazolam 2 mg/kg/day 7 days	Carbamazepine, 25 or 100 mg/kg/day	7 days	Open-field activity	Mice
Martijena ID. 1997 [155]	Diazepam 2 mg/Kg/day 21 days Intraperitoneal administration	Carbamazepine, 7.5 mg/kg, i.p.	One administration	Forced swim test. Active avoidance test	Male Wistar rats 220–260 g

reverse anxiety-like behavior in the social interaction test in rats, in the light/dark transition test in mice, and in the withdrawal-induced weight loss [210]. These findings are in agreement with clinical studies as buspirone failed to reverse or prevent BZD withdrawal symptoms in humans [211, 212]. However, positive results have been obtained when the administration of low doses of buspirone reversed anxiogenic responses in the elevated plus maze and in the social interaction test in rats withdrawn for 24 h from 21-day DZ treatment, while higher doses were anxiogenic in both withdrawn and control rats [136, 213]. Physicians would be unlikely to prescribe 5-HT1A agonists to outpatients if there is a risk of aggravation of withdrawal symptoms at higher doses. Moreover, buspirone has been shown to have no benefit [212] and sometimes to aggravate BZD withdrawal symptoms in humans [211]. In addition, clinical practice has shown that prior BZD experience may attenuate the anxiolytic actions of buspirone and other 5-HT1A agonists [212]. These evidences suggest that 5-HT1A agonists are probably of limited value in the treatment of BZD dependence and withdrawal [207].

Another neurotransmitter involved in BZD withdrawal is NA. In humans, propranolol, a βNA receptor antagonist, has been shown to relieve autonomic withdrawal symptoms such as palpitations and tremor, all mediated by increased noradrenergic activity, with little effects on anxiety [214, 215]. Furthermore, propranolol and clonidine, an α2NA receptor agonist, failed to suppress the anxiogenic withdrawal reaction detected 24 h after cessation of 4-week chlordiazepoxide

treatment in rats [216]. Thus, blockade of NA neurotransmission with α2 agonists and β antagonists appears to have little effect on anxiety as the predominant symptom of BZ withdrawal and limits their use as add-on therapies for BZD withdrawal.

The excitatory amino acid neurotransmitter Glu, particularly acting on NMDA receptors, is believed to contribute to the neurobiology of anxiety disorders [217–219]. NMDA receptor antagonists evidenced anticonvulsant, muscle relaxant, and anxiolytic properties in animal models [217, 220]. Moreover, DZ chronic treatment has been reported to induce upregulation of NMDA receptors [64, 221]. Rats withdrawn from DZ for 72 h showed tremor, muscle rigidity, reduced body weight, and emotional responses such as vocalization and aggression and also had reduced seizure threshold. Administration of MK-801, a non-competitive NMDA antagonist, during the withdrawal period had a protective effect on the withdrawal signs expression [222, 223]. Although NMDA antagonists showed to be effective in the treatment of BZD withdrawal in animals, they can produce muscle relaxation and ataxia [220, 224], that might limit their use for treatment of any condition where motor impairment is undesirable. In fact, it is difficult to establish whether the decrease in withdrawal-induced anxiety following treatment with NMDA antagonists actually reflects a therapeutic success [225] or whether these effects are merely secondary to the muscle relaxation and ataxia caused by these drugs [207]. Although a low dose of MK-801 was able to prevent development of tolerance to the sedative effects of DZ when it was administered together with DZ, without inducing motor effects [108].

The anticonvulsant carbamazepine has also been shown to have benefit in reducing the symptoms of BZD withdrawal in humans. Furthermore, carbamazepine given for 7 days following withdrawal from 7-day alprazolam treatment attenuated manifestation of withdrawal-induced hyperactivity in open-field test and BZD receptor upregulation in the cortex and hypothalamus. Interestingly, large variability in the response between animals was found in this study [226], similar to that found in humans [227]. Also, a single carbamazepine administration to rats withdrawn for 96 h from 21-day DZ treatment reversed impairments in the forced swim test and in the active avoidance paradigm [155].

The mechanism of action of carbamazepine on BZD withdrawal symptoms remains unknown. The ability of carbamazepine to inhibit electrical excitation in the limbic system may play an important role [228]. Another possible mechanism that has been proposed belongs to inhibition of sodium channel-mediated neurotransmitter release [229]. Carbamazepine is an anticonvulsant and thus can protect against withdrawal-induced seizures. It has also been found to increase open-arm activity in the elevated plus maze in rats [230], suggesting that it may also possess anxiolytic properties.

It also appears to be effective in treating BZD withdrawal in several clinical studies [227, 231, 232]. Patients receiving carbamazepine prior to or during withdrawal from BZD had more rapid and better-tolerated discontinuation with less intense withdrawal symptoms than if gradual taper was used alone [227, 233]. These results support carbamazepine as a possible pharmacological tool in the therapy of BZ withdrawal. On the other hand, the variability in responses to carbamazepine in mice [226] and in humans [227] might indicate that it can be useful only in some individuals. Unfortunately, the characteristics of patients most likely to respond to carbamazepine in the treatment of BZ withdrawal remain unknown.

Behavioral Strategies

Since pharmacological strategies showed partial or non-efficacy for treatment of BZD dependence, behavioral or cognitive approaches are the focus of alternative strategies to treat or prevent symptom of BZD withdrawal (Table 19.2). In the case of the insomnia, guidelines recommend limiting the use of BZD hypnotics to 4 weeks [234, 235]. Nevertheless, BZD use is often prolonged because insomnia is a chronic problem [236]. The usual clinical management of BZD dependence is gradual tapering, but 50–60% of users resume use of their medication during the month following the end of the withdrawal period [237].

Table 19.2 Behavioral treatments used in clinical studies to treat BZD withdrawal signs

Citation	BZD withdrawal sign	BZD drug factors		Study size (n)	Mean age Years (SD)	WT used
		Duration (SD)	Dose mg/day (DE)			
Baillargeon L. 2003 [238]	Insomnia	152 [122] months	6.3 [4.1]	65	67.4 [6.8]	Efficacy of taper ± CBT
Morin CM. 2004 [239]	Insomnia	19.3[10.3] years	9.54 [6.13]	76	62.5 [6.3]	Effectiveness of taper or CBT alone or in combination
O'Connor K. 2008 [241].	Anxiety disorder Insomnia	11.45 [9.96] years	14.77 [12.6]	41	48.22 [9.5]	Effectiveness of treatment-as-usual group
	Anxiety and insomnia	6.55 [4.99] years	11.84 [13.2]	22	48.19 [9.8]	Effectiveness of group support only
		7.84 [8.01] years	12.98 [10.6]	23	47.35 [9.9]	Effectiveness of cognitive behavioral therapy plus group support

Some evidences indicate that simply encouraging patients to reduce medications has little effect; rather, setting up a medication taper schedule with weekly visits to assess progress is necessary to achieve significant reductions of hypnotic use. In addition, while such a supervised medication tapering regimen is essential in the initial discontinuation phase, it may not be sufficient to keep patients from taking these medications in the long term because of persistent sleep disturbances.

The addition of behavioral treatment specifically targeting insomnia symptoms may attenuate withdrawal symptoms (e.g., rebound insomnia) and prevent relapse [238]. On the other hand, comprising cognitive-behavioral therapy (CBT) and a gradual tapering of benzodiazepine hypnotics have demonstrated the superiority of a combined intervention, over the usual clinical approach (tapering alone) for people with chronic insomnia who had been using medication for a long-term period. The benefits in terms of BZD cessation were still present 1 year after the end of the intervention. The CBT was provided in small groups rather than individually, and the intervention involved behavioral, cognitive, and educational components. The goal was to reinforce the bed as a contextual cue for sleep and to regularize sleep rhythm. Participants were instructed to go to bed only when sleepy, to go into another room when they could not fall asleep, to use the bedroom only for sleeping, and to get up at the same time every morning and to avoid day time naps. Sleep restriction consisted of curtailing the time spent in bed to the amount of time actually spent sleeping. At the end of treatment, the 77% of participants in the combined treatment group reported a completed reduction of the BZD hypnotic dosage, while in tapering-alone group only 38% of participants reported the same result [238].

Another similar study evaluates the efficacy of three interventions (supervised medication taper, CBT, and a combination of the two approaches) for BZD discontinuation in older adults and to examine their short- and long-term effects on subjective and objective sleep patterns, as recorded by patients' sleep diaries and electroencephalography, respectively. They demonstrated that all three groups had significant decreases in the frequency of medicated nights, being lower in the combined CBT and medication taper group than in the group that received medication taper alone. Furthermore, the 63% of the patients were drug-free at post-treatment. The proportion of drug-free patients was significantly higher in the combined CBT and medication taper group (85.2%) than in the medication taper group (48%) and the CBT group (54.2%) [239]. It is important to highlight that participants were healthy and relatively "young" older adults who were willing and motivated to discontinue their BZD medication. With one or two exceptions, the participants were not

abusing BZD medication in the sense of exceeding the recommended dose [240].

BZD are not only used for sleep disorders, and different strategies for discontinuation of BZD were evaluated when the participants have been treated for insomnia, panic with agoraphobia, generalized anxiety disorder, social phobia, specific phobia, and adaptation problems with anxious mood. Participants received treatment as usual (taper only) plus physician counseling in the same clinic setting, CBT plus taper, or group support (GS) plus taper [241]. In this study CBT was divided into three sections, covering preparation, severance, and maintaining abstinence. The therapy aimed to enhance self-efficacy principally through normalizing expectancies of withdrawal and attributions of withdrawal; through boosting confidence in coping without BZD, coping with anxious inhibiting situations; and through developing a belief in capacity to function autonomously from BZD. In the GS no CBT strategies were presented, and exchanges took the form of open-ended discussion on themes such as "What is anxiety?" Here no direct action or strategy to deal with any problems was suggested. Any requests for specific help were deflected back to the group. The principal difference with CBT was in the lack of any specific directions for changing thoughts and behaviors. The outcomes in both the CBT and the GS subgroups were equivalent and showed a significant increase in state of quality of life, although the CBT group had less abandons and showed higher self-efficacy after BZD taper. Over all 86 participants, a high-baseline level of psychological distress, anxiety, and dosage predicted a poor outcome, but increase in self-efficacy achieved with the CBT therapy contributed to a successful outcome [241]. Therefore, it can be observed that CBT strategy has a preponderant effect in generating increases in the confidence of individuals to make decisions in difficult situations and in this way facilitate BZD discontinuation. Applying this multidisciplinary approach in the community could help reduce BZD use by older people and prevent health problems related to these medications [238].

Final Remarks

At the beginning of BZD discovery and pharmacological optimization, these compounds were considered safe because they had no apparent side effects. Chlordiazepoxide (Librium) was the first BZD approved for use in 1960, and rapidly they were the most prescribed drugs in America, with more than 2.3 billion tablets sold in 1978. Their high therapeutic index, availability of the antagonist flumazenil in case of overdose, and their rapid onset of action make these compounds particularly versatile and difficult to replace in clinical psychiatry. Nevertheless, their prolonged use may induce tolerance to some of their effects, liability for physical dependence, and abuse potential. Then, physicians should evaluate to prescribe BZD under the light of recent advances in the knowledge of the risks and benefits of these compounds, also considering potential misuse on the basis of the patient's attitude, personality, and pathological behavior [242, 243]. However, many patients could misuse or abuse BZD by themselves due to dependence generated by chronic use or to attenuate symptoms of the withdrawal syndrome without proper medical guidance.

In order to reduce BZD side effects, in the literature it can be found that a considerable amount of studies focused on developing effective treatments for BZD therapy discontinuation, dependence, withdrawal, or even addiction. Nowadays, even though there are not 100% effective therapies, some strategies have been able to demonstrate positive results for many patients. Combined gradual tapering and cognitive-behavioral therapies are considered first line of treatment chosen in the clinics, rather than the available pharmacological strategies, that may be used as add-on therapy together with the first line.

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Beyond Acute Traumatic Brain Injury: Molecular Implications of Associated Neuroinflammation in Higher-Order Cognitive Processes

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Traumatic Brain Injury: Definition, Epidemiology, and Classification

Traumatic brain injury (TBI) is the result of an external mechanical force applied to the cranium and the intracranial contents, leading to temporary or permanent impairments, functional disability, or psychosocial maladjustment [1, 2]. TBI can adversely affect a person's quality of life in numerous ways, including cognitive, behavioral/emotional, and physical effects that affect interpersonal, social, and occupational functioning. In addition to the TBI impact to the individual, TBI can negatively impact families, communities, and also the country's economy. One case of TBI occurs every 15 seconds in the USA, generating 1.7 million new head injury victims per year. These events are responsible for 50,000 deaths, leave 80,000 individuals with permanent disabilities, and cost more than US$77 billion on average per year. The frequency of brain injury is currently higher than that of any other diseases, including complex diseases such as breast cancer, acquired immunodeficiency syndrome (AIDS), Parkinson's disease, and multiple sclerosis. In the USA, children aged 0–4 years, adolescents aged 15–19 years, and adults aged 75 years and older are among the most likely to have a TBI-related emergency department visit or to be hospitalized for a TBI. Adults aged 75 years and older have the highest rates of TBI-related hospitalizations and deaths among all age groups. The leading causes of nonfatal TBI in the USA

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are falls (35%), motor vehicle-related injuries (17%), and strikes or blows to the head from or against an object (17%), such as in sports injuries [3]. The incidence of sport-related concussions is estimated to be 130,000 per year among children 5–18 years of age [4]. Among active military personnel, blast injury is the most common cause of TBI [5]. The prevalence (i.e., the existing cases at any given time) of TBI is not well documented, because most cases (i.e., mild TBI; mTBI) are not fatal and patients may not have been hospitalized. Estimates often are based on existing disabilities. Estimates by the National Institutes of Health Consensus Development Panel on Rehabilitation of Persons with TBI showed that 2.5–6.5 million Americans live with TBI-related disabilities [3, 6].

TBI may be penetrating or non-penetrating and diffuse or focal and vary in severity, location, and patient characteristics. *Impact loading* causes TBI through a combination of contact forces and inertial forces. *Inertial force* ensues when the head is set in motion with or without any contact force, leading to an acceleration of the head. *Contact force* occurs when impact injury is delivered to the head at rest. *Static or quasistatic loading* is rare and occurs when a slowly moving object traps the head against a fixed rigid structure and gradually squeezes the skull, causing many comminuted fractures that may be enough to deform the brain and lead to fatal injury (See Fig. 20.1).

TBI-related injuries are divided into two subcategories: (1) *primary injury*, which occurs at the moment of trauma, and (2) *secondary injury*, which occurs immediately after trauma and produces effects that may continue for a long time.

- *Primary Injuries*: can be manifested as focal injuries (e.g., skull fractures, intracranial hematomas, lacerations, contusions, penetrating wounds) or they can be diffuse (as in diffuse axonal injury, DAI) [7, 8]. DAI is due to shear strain caused by acceleration/deceleration forces that ultimately lead to disconnection and Wallerian degeneration.
- *Secondary Injury*: attributable to further cellular damage from the effects of primary injuries and are related to ischemia, brain swelling, and other complex mechanisms such as inflammation and may in themselves have severe consequences. Secondary injuries may develop over a period of hours or days following the initial traumatic assault [9].

The early phase of damage usually occurs within 24 h of injury and is directly related to tissue damage and deregulated physiological functions. The intermediate phase takes place in the days following TBI and entails neuroinflammation. The late phase is primarily associated with seizures and epileptogenesis and arises days to weeks after TBI. Early damage following TBI often stems from the ischemic cascade. There is a

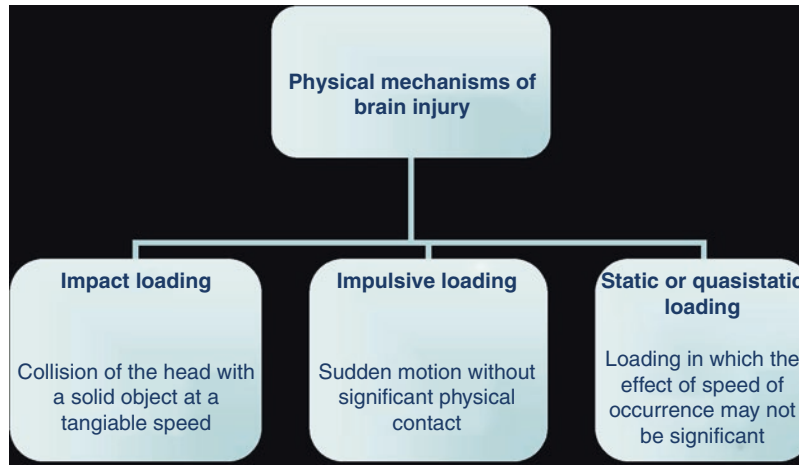

Fig. 20.1 Physical mechanisms of brain injury

fine interplay regarding normal energy processes and disruption of this intricate path. It leads to decreased glucose utilization, lactic acid accumulation, reduced ATP and activity of ATP-reliant ion pumps, Ca^{2+} −induced depolarization, excitotoxicity, and cellular death.

The severity of TBI can be classified as mild, moderate, or severe on the basis of clinical presentation of a patient's neurologic signs and symptoms. Currently, the severity of TBI is categorized based on the Glasgow Coma Scale (GCS), in which patients are scored on the basis of clinical symptoms, and the resulting overall score classifies their injury as mild (score: 13–15), moderate (score: 9–12), or severe (score: <9). Moreover, duration of posttraumatic amnesia (PTA) has proved to be a strong predictor of TBI outcome [10, 11], considering that mild TBI may be associated with PTA for up to 24 h [12], and about 70–90% of TBI cases are considered mild, while 10–20% of cases are moderate to severe [13]. Symptoms of mTBI can include headaches, dizziness, nausea, and amnesia. These injuries usually resolve within days to weeks of the insult. However, occasionally, these injuries can result in long-term cognitive and behavioral deficits.

Furthermore, there is evidence to suggest that moderate to severe TBI, and even repeated mTBI, might be associated with increased risk of neurodegenerative diseases such as Alzheimer's disease [14], chronic traumatic encephalopathy [15], and Parkinson's disease [16].

Cognitive Deficits Induced by TBI

After moderate to severe TBI, cognitive dysfunction is common, with more than half of the patients experiencing chronic cognitive impairment that produces lifelong disabilities [17]. These deficits include slowed information processing and impaired long-term memory, attention, working memory, executive function, social cognition, and self-awareness [for extensive review, see [18]].

Following a concussion, the expectation of a full functional recovery within 7–10 days may be realized for most people [19], but others continue to experience symptoms for an extended period of time [20–22]. The cluster of symptoms that commonly present together in these individuals has been recognized as post-concussive syndrome (PCS) [21, 23]. Post-concussive symptoms may develop following a TBI of any severity and are generally grouped into three categories. The first are cognitive symptoms (e.g., attention, executive function, memory, mental slowing, speech changes) [24]. The second group are physical and somatic symptoms (e.g., headache, fatigue, dizziness and nausea, pain headaches, sleep disruption, sensitivity to light or sound, hearing problems, and visual disturbances, even seizures) [24, 25]. The third group is integrated by emotional/behavioral symptoms (e.g., depression, anxiety, irritability, attention deficit, and executive dysfunction) [26, 27]. The deficits produced by mTBI are frequently more subtle and poorly recognized than those resulting from severe TBI [28–31]. They include physical problems such as headache, dizziness, and visual disturbances; cognitive impairments such as attention, memory, and executive dysfunction; and emotional or behavioral problems such as irritability, anxiety, depression, affective liability, apathy, and/or impulsivity [32, 33].

Many individuals with TBI have chronic and debilitating communication problems [34, 35]. These problems are most commonly characterized as impairments in the ability to communicate appropriately and effectively across contexts [34, 35]. Social communication deficits may include impaired comprehension of indirect language [36–38] and poor organization of spoken and written discourse [39–41] and are thought to reflect underlying cognitive rather than linguistic impairments [42]. Moderate-to-severe TBI may reduce the processing ability available for social communication or, alternatively, that communication tasks may be more taxing for individuals with TBI than for their uninjured peers [43–45]. With the standard clinical assessment used, it is very difficult to identify those individuals at risk for post-concussive syndrome. Moreover, since working memory is one of the domains primarily affected by mild TBI, a combination of clinical

and fMRI measures of working memory early after injury (within the first week) may predict the patient outcome. Individuals who do not recover at 1 week after injury showed an increased activation in the right [46, 47], often prefrontal [48], cortex when the activation of individuals who have sustained a TBI is compared with controls. However, this pattern is not evident in individuals recovering. Furthermore, the pattern of activity change in areas of the "default network" (DN) (posterior cingulate cortex and medial prefrontal cortex) have the potential to be predictive of recovery. When the activation in these areas was low during the working memory task (hypoactivation), individuals with an mTBI did not recover. When activation in these areas was relatively high during the task, individuals with mTBI recovered. This result raises the possibility that the activation in the DN may eventually be useful to identify individuals who will require more intensive rehabilitation after mTBI [49]. In line with these results, another study performed more than 1 year after TBI showed that working memory and information processing speed was significantly impaired in mTBI participants with persistent PCS compared to mTBI participants without PCS and all non-head injured participants. Correlations between cognitive performance and symptoms were only observed for mTBI participants, with worse performance correlating with lower sleep quality, in addition to medium effect size associations (which do not reach statistical significance) with higher PCS symptoms, posttraumatic stress disorder (PTSD), and anxiety [50].

An important issue to consider in the TBI outcome and persistence of PCS symptoms is to clarify pre-injury developmental, medical, neurological, psychiatric, substance, academic, and employment histories, particularly as regards conditions that may influence recovery following mTBI. One third of TBI patients may present one or many of these conditions [51], and their presence may also offer explanation of relatively poor recovery after an apparently mTBI.

It is important to take into account substance abuse and/or intoxication during TBI, since the association of substance abuse with brain injury and the relatively poor psychological and functional outcome after TBI are well described [32, 52–55].

Depression, anxiety disorders, posttraumatic stress disorder, and sleep disturbances may develop after TBI, and premorbid disorders of these types may be exacerbated by mild TBI [27].

Animal Models of TBI

To study TBI preclinically, researchers have developed several experimental animal models to replicate human pathophysiology (see Table 20.1, Fig. 20.2).

Molecular Mechanisms Involved in Secondary Injury Contributing to Neurodegeneration

As it was mentioned before, secondary injury appears as a consequence of the primary damage, and it can occur in a period from days to weeks or even months. It triggers a complex cascade of intracellular signaling, which includes ATP depletion, neuroinflammation, oxidative stress, and cytoskeleton damage. These events are associated to glutamate receptor-mediated excitotoxicity, altering membrane's permeability by the disruption of the ionic balance, thus inducing cellular edema [60]. This, in turn, favors the calcium massive entrance into the cell due to the augmentation of extracellular potassium [61], which can induce endoplasmic reticulum (ER) stress, and cellular death by apoptosis and/or necrosis [62]. These events induce the production of toxic and pro-inflammatory mediators, such as prostaglandins, oxidative metabolites, chemokines and pro-inflammatory cytokines, which can lead to lipoperoxidation, enhanced BBB disruption, and brain edema [60]. In addition, compensatory mechanisms are triggered, such as the unfolded protein response (UPR) and autophagy, aiming for the restoration of the cellular homeostasis [63, 64].

The UPR is triggered in response to the ER stress, mainly because of the loss of calcium

Table 20.1 Characteristic lesions induced by different animal models of traumatic brain injury

Animal models of traumatic brain injury	Characteristics
Fluid percussion injury (FPI)	*FPI produces a TBI that is characterized by cerebral edema, intraparenchymal hemorrhage, and cortical neuronal injury. Lately, the FPI model has been modified to lateral FPI model, which creates not only focal cortical contusion but it also transmits the traumatic injury into subcortical structures such as the hippocampus and thalamus. The neuronal loss starts immediately after the impact and progresses up to 7 days post-TBI. The cascade of the molecular changes continues for months in the subcortical structures such as the septum pellucidum, thalamus, amygdala, and striatum. This TBI model produces similar symptoms to humans and manifests severe neurobehavioral deficits that persist more than 1 year after TBI* [56, 57]
Controlled cortical impact injury (CCI)	*CCI is a TBI model that provides a more controlled injury in terms of velocity force, time, and depth of injury as compared to the FPI model. This model creates cortical injury, SDH, axonal injury, and subcortical injury in the thalamus and hippocampus. The CCI model-induced brain injuries cause long-term neurobehavioral deficits that persist more than a year and are associated with cortical atrophy and reduced brain perfusion* [56, 58]
Weight drop impact acceleration injury (WDIAI)	*WDIAI is a model that generates an open or closed head injury. The result of the first impact is cortical contusion with possible subcortical intracerebral hemorrhage. This leads to the formation of a necrotic cavity within 2 weeks after injury. In this model, the recovery phase is ranged from 2 weeks to 3 months. The closed head injury of this model causes neurologic deficits, neurodegeneration, an inflammatory response with microglia activation, blood-brain barrier (BBB) breakdown, and DAI. The pathology features of this model contain similar components as seen in human TBI that is caused by motor vehicle accidents or sports injury* [56]
Blast injury	*Blast injury model is created by high-velocity ballistic penetration or a stub of a blast. The anatomical and morphological characteristics of the blast injury are related to the trajectory of the injury with intraparenchymal hemorrhage and subsequent cavity formation. Moreover, this model also generates additional TBI components such as inflammation with BBB breakdown, cerebral edema, neurodegeneration, tauopathy, and axonal degeneration* [56, 59]

homeostasis, in order to prevent accumulation of altered proteins [64]. It has been described that ER stress contributes to the injury spread observed in postmortem human brains of patients who suffered chronic traumatic encephalopathy as well as to behavioral deficits present in adult rodents TBI models [65]. On the other hand, there are basal (quality control) and stress-induced levels of autophagy. While basal autophagy is necessary for the turnover of cellular components, stress-mediated autophagy may occur as a compensatory response to oxidative stress, organelles dysfunction, or when ATP levels need to be restored. When autophagy is unable to restore cellular energy, apoptosis or necrosis are triggered [66]. In mTBI, autophagy upregulation has a protective role, whereas in severe injury, it seems to be inhibited, contributing to cell death [63].

Excitotoxicity and Calcium Imbalance

The alteration of ionic balance after TBI induces an extensive depolarization, promoting a massive glutamate release, increasing the extracellular levels of the neurotransmitter. This phenomenon is exacerbated by impairment in glutamate buffering and clearance, mainly because of posttraumatic reduction of excitatory amino acid transporter expression [67]. These transporters, located at neurons and astrocytes, are responsible for removing glutamate from the extracellular space, but when their activity is reduced further, increases in extracellular glutamate levels occur, self-perpetuating excitatory signaling [68]. The glutamate overstimulation and its reuptake deplete ATP cellular storage, increasing extracellular potassium about six times. Moreover, the latter events are enhanced

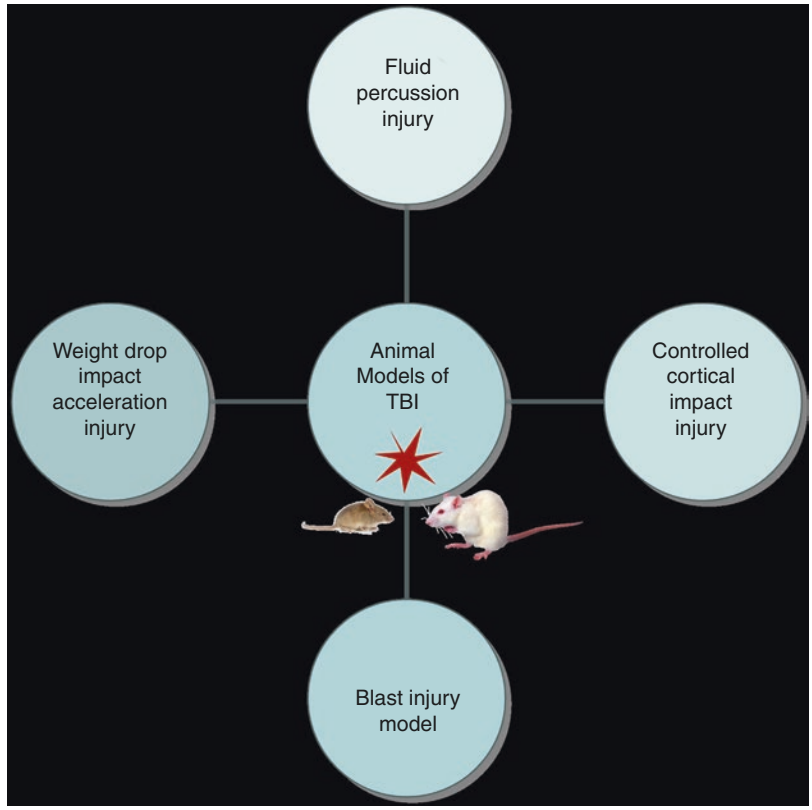

Fig. 20.2 Animal models of TBI that have been frequently used for research

by alterations in the potassium conductance in astrocytes after TBI [69]. Cytotoxic effects of glutamate are mainly mediated by the influx of large amounts of calcium through N-methyl-D-aspartate (NMDA) receptors from extracellular pools, as well as from intracellular calcium stores [70]. These effects are exacerbated by the hypoxic opening of other plasma membrane calcium-permeable channels, due to an increase in oxidative stress and the decreased of extracellular pH [71].

Several mechanisms are involved in the damage induced by elevated levels of intracellular calcium in neurons, such as the activation of calcium-dependent enzymes and mitochondria calcium overload, which may lead to necrotic cell death or apoptosis [72]. For instance, in neurons there are specific calpain isoforms which are activated by micromolar calcium concentrations, reached after excitotoxic insults such as TBI. Furthermore, cytoskeletal proteins such as tubulin, microtubule-associated proteins, neurofilaments, and spectrin are substrates for calpains; thus, their activation also induces structural alterations [70]. In addition, calpains are also related to the degradation of membrane proteins and other cellular enzymes, such as kinases and phosphatases, altering cellular normal activity. Nevertheless, calpain activation only occurs when neuronal physiology is compromised; therefore, calcium homeostasis restoration appears to be one of the main targets for diminishing TBI-induced damage [73].

Another family of enzymes associated to increased intracellular calcium levels are caspases, which are related to the induction of apoptotic death, and their activation has been observed after TBI [70, 74]. In addition, TBI-induced caspases activity has been related to mitochondrial swelling, to increases in oxidative damage and pro-apoptotic factors, to reduced expression of BBB tight junction proteins, and to the cleavage of axonal membrane structural proteins [74]. There are also calcium-dependent

endonucleases that have been shown to be activated after TBI, generating DNA damage, characteristic for apoptotic cell death [71]. These morphofunctional impairments increase membrane disruption, altering its permeability to ions and macromolecules and finally contributing to cell damage as well as to the abnormal physiology in neurons after TBI [70].

On the other hand, calcium can also activate enzymes associated to inflammatory and oxidative stress cascades, such as cytosolic phospholipase A2 (cPLA2) and phospholipase C (PLC), which levels are also increased after TBI [70]. The cPLA2 activation induces the release and cellular accumulation of soluble arachidonic acid (AA), the eicosanoids precursor, involved in inflammatory processes, platelet aggregations, and vessels' smooth muscle regulation. In addition, PLC mediates the production of both diacylglycerol (DAG) and inositol trisphosphate (IP3), inducing calcium release from the endoplasmic ER [70]. Finally, upon NMDA receptors activation, calcium activates neuronal nitric oxide synthase (NOS-1), by stimulation of the calcium-dependent enzyme calmodulin, which has been involved in panic-like behavior in mice [75]. Moreover, increased NO levels combined with superoxide generate the highly toxic free radical, peroxynitrite [72], that along with the high metabolism of AA and impaired mitochondria may end up saturating endogenous scavengers.

Therefore, there is strong evidence in the literature supporting the role of glutamate in secondary injury. Microdialysis-based studies in rodents showed an increase in extracellular glutamate, which was proportional to the severity of the damage [76, 77]. In addition, it has been shown an increase of glutamate in the cerebrospinal fluid of patients suffering TBI, persistent for periods longer than 1-week posttrauma, where the severity of the injury also was correlated to worst patient's outcome [78]. Finally, a persistent deficit of the extracellular regulation of glutamate may set the basis for the cognitive and emotional impairment of patients affected for TBI, probably by loss of fidelity and specificity of synaptic transmission [69].

Mitochondrial Injury and TBI

Mitochondria regulates energy metabolism, calcium homeostasis, and intracellular trafficking [79]. In the central nervous system (CNS), they are one of the main sources of physiological reactive oxygen species (ROS) production, whereby their membrane is constantly submitted to oxidative events. However, in normal conditions, neurons remain protected by constant detoxification processes and for the CNS isolation by the BBB [80]. In addition, given the continuous ROS exposure, mitochondria are strictly regulated by their dynamics and turnover. These processes aim to preserve or restore the normal functioning, by fusing healthy mitochondria with dysfunctional ones [81]. In the case of the persistence of dysfunctional fractions, these mitochondria are separated from functional ones by fission, to be then recycled by mitophagy (the selective mitochondria turnover by autophagy) [80]. Nevertheless, long-term pathological conditions inhibit fusion and stimulate fission, causing mitochondrial fragmentation, finally triggering apoptotic cell death [81].

In TBI, axolemma disruption favors a massive calcium entry into the cytoplasm, which is actively sequestrated by mitochondria [79]. This might lead to mitochondrial swelling and bioenergetic deterioration, following impairment in axonal transport, axon degeneration, and neuronal disconnection [82]. In addition, the interruption of axonal transport produces accumulation and aggregation of toxic proteins, similar to what is observed in several neurodegenerative diseases, which may potentiate the deleterious effects over neurological functional outcome [80].

On the other hand, outer mitochondrial membrane permeability is a key factor for the determination of the cell's outcome after a noxious event, since within the intermembrane space there are several proteins involved in apoptotic cell death signaling. Furthermore, mitochondrial inner membrane disruption results in a decreased ATP production [82]. The reduction of ATP is mainly due to the impairment of the electron transport chain, where physiological electron leak is

enhanced, generating a greater production of superoxide radicals ($O_2^{-\bullet}$), which can activate mitochondrial NOS-1, as described above. High reactive species derived from the electron leak, such as hydroxyl radical (•OH) and nitrogen dioxide (NO2•), induce lipoperoxidation of mitochondrial membrane fatty acids. Lipoperoxidation contributes to the self-propagating pathogenesis following TBI, as the result of neurotoxic aldehydes generation, which are capable of forming adducts with mitochondrial proteins, inducing their malfunctioning [83]. Finally, these processes may lead to an alteration in mitochondrial turnover (mitophagy) and dynamics (fusion/fission), potentially leading to cellular apoptosis [80, 81].

Following acute neuronal injury, there has been observed the induction of mitochondrial permeability transition, defined as an abrupt augmentation of inner mitochondrial membrane permeability for molecules smaller than 1.5 KDa [82]. This, combined with the increase of intramitochondrial calcium, will lead to the opening of the mitochondrial permeability transition pore, inducing loss of the mitochondrial membrane potential and ATP production, as well as calcium release toward the cytosol. These events, altogether, can activate different proteases inducing an increase in lactate levels, cytoskeletal degradation, and neurodegeneration [84].

Molecular Mechanisms of Secondary Injury as Potential Pharmacological Targets for TBI Treatment: A General Overview

It has been shown that beta-amyloid (Aβ) oligomers can interrupt glutamate reuptake, favoring an extrasynaptic NMDA receptor activation, thus leading to a reduction of long-term potentiation and the enhancement of long-term depression of synaptic transmission in many brain areas. Therefore, treatment with NMDA receptors antagonists has been studied as a molecular target to ameliorate TBI-induced cognitive impairment. In rodents subjected to TBI, it has been observed that memantine (an extrasynaptic NMDA receptor antagonist) favors an improvement in cognition and neuroprotection. Nevertheless, this approach has failed in human clinical trials, probably due to the relevance of glutamate in normal excitatory signals. In addition, the use of inhibitors of glutamate release, such as N-acetylaspartylglutamate (NAAG) peptidase inhibitor, ZJ-43, was shown to significantly improve cognition in rats submitted to TBI, by limiting the hydrolysis of NAAG [85].

On the other hand, several studies have shown an attenuation of brain edema, by inhibiting interleukin 1β (IL-1β) after TBI. Specifically, by treating mice subjected to CCI with antibodies anti IL-1β, it was observed a reduction in the extension of the damage, tissue loss, microglial activation, inflammatory infiltration, and cognitive deficit, in comparison to control. In addition, in mice subjected to CCI and microvascular endothelial cell monolayers treated with a calpain inhibitor, a decrease in calpain-dependent IL-1β activity was observed, as well as an attenuation in BBB tight junction dysfunction [86].

It is known that TBI stimulates the production of several neurotrophins including the insulin-like growth factor-1 (IGF-1) [87, 88]. However, TBI appears to induce only transient increases in IGF-1 and its signaling molecules, which are probably not sufficient to provide neuroprotection or stimulate subacute repair or regenerative mechanisms. Therefore, exogenous administration of IGF-1 may supplement and extend the actions of endogenous IGF-1. In fact, administration of exogenous IGF-1 after experimental TBI promotes neuroprotection and improved functional outcomes. Although IGF-1 administration stimulates regenerative events including neurogenesis and angiogenesis in other CNS injury models [87], its brain repair potential after TBI is not yet known. Importantly, IGF-1 was well tolerated by patients in early-phase clinical trials for TBI, and IGF-1-treated patients showed improved metabolic outcome compared to placebo-treated patients [89, 90].

Finally, the use of glutathione intracranial administration reduces the disruption of glial barrier by a 70% parenchymal cell death, and neuroinflammation, and in a lesser extent (50%)

meningeal cell death [91]. Moreover, the administration of N-acetylcysteine to patients within 24 hours post-mild TBI has shown to exert an improvement on their recovery after 7 days of treatment, regarding to placebo. In addition, similar results were obtained in weight drop and fluid percussion rodent models of TBI, where it was observed a behavioral recovery under the treatment with n-acetylcysteine after mild and moderate TBI [91].

Molecular Mechanisms in TBI Underlying Development of Neurodegenerative Pathologies

As it was stated above, secondary injury induced by TBI can lead to long-term neurological and neuropsychiatric sequelae. These sequelae include, but not are limited to, neurodegenerative pathologies. Some of the neurodegenerative pathologies induced by TBI include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and chronic traumatic encephalopathy [25, 92, 93]. Symptoms of these neurodegenerative processes include behavioral disturbances, cognitive dysfunction, and/or motor-related symptoms. The common feature of these pathologies is a degeneration of certain groups of neurons associated with the clinical symptoms [92]. However, the exact molecular mechanisms by which a TBI can develop any of these pathologies are not yet known, or there is no consensus among the scientific community.

Alzheimer's Disease

AD is mainly characterized for cholinergic neuron loss in the brain [92] and an accumulation of Aβ peptides in plaques in brain tissue [93]. Additionally, an intracellular aggregation of microtubule associated protein tau in neurofibrillary tangles occurs [93, 94]. Despite these findings, what is the common denominator between Alzheimer's disease and TBI? The answer is chronic inflammation. The discussed and not accurate mechanism of pathogenesis in AD induced by TBI could be mediated by oxidative stress, inflammation, and excitotoxicity. All these processes occur during TBI-induced secondary injury.

Figure 20.3 summarizes the possible pathways for neurodegeneration and AD induction by TBI. Inflammation and oxidative stress gated by TBI are closely related to inflammatory cytokines, which in turn downregulates Aβ clearance genes, promoting the deposition on protein plaques [92]. In fact, activated microglia during inflammation can cause excitotoxicity, stimulating neurofibrillary tangle deposition due to release of toxic levels of cytokines [93]. Besides, non-enzymatically glycated tau contributes to oxidative stress [95]. On the other hand, high levels of glutamate in synaptic cleft are responsive for excitotoxicity. The apparent result is the development of free radicals with consequent cell death [92]. In combination, all these processes ensue synergistically, enhancing the severity and progress of the disease.

The abnormal proteinopathies could be vie wed as part of the injury process instead of a result of the injury [93], because some studies found that the accumulation of amyloid precursor protein (APP) in the traumatized axons after TBI promotes the Aβ plaque formation [96]. Although many others enunciate that in numerous studies with animal models, this deposition of Aβ from APP is not observed [97–100]. Further potential pathogenesis mechanisms include neural network disruption, abnormal calcium signaling, and vascular abnormalities [94]. Moreover, BBB damage is implicated in pathophysiology of AD, due to the slower clearance of senile plaques [101].

Parkinson's Disease

Parallel to AD disease, PD is governed by the formation of α-synuclein-rich Lewy bodies and characterized by death of dopaminergic neurons, specifically in the substantia nigra [102]. The α-synuclein accumulation is the prevailing point among TBI and Parkinson's disease [92] due to the damage of axonal transport by injuries in white matter. Additionally, in this disease it is

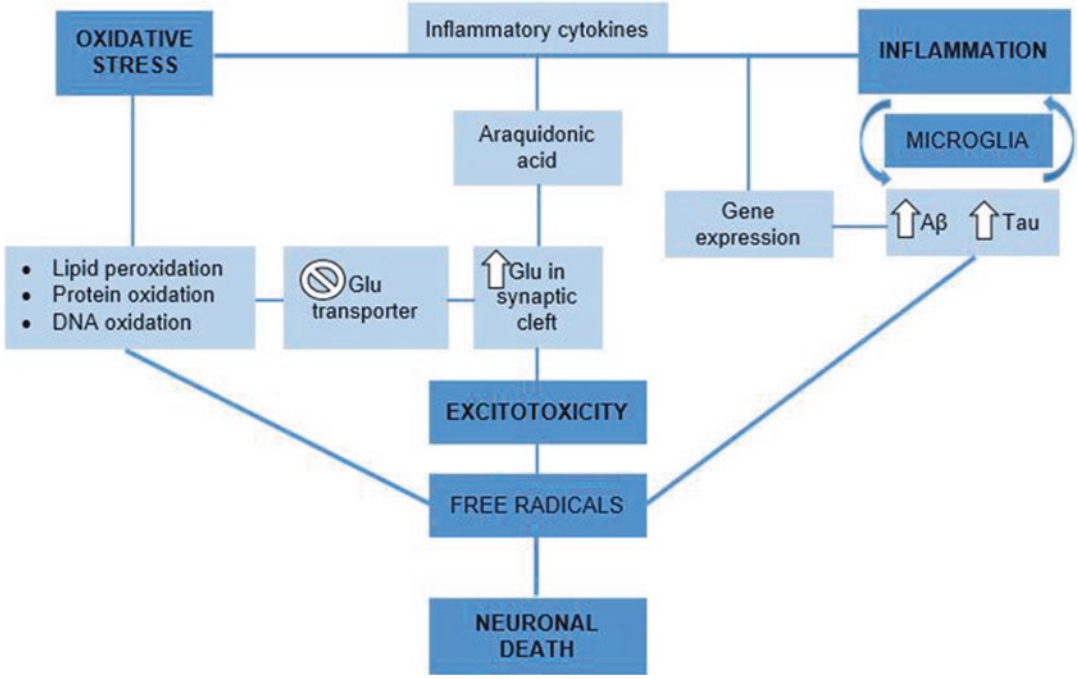

Fig. 20.3 Neurodegenerative pathways in AD

also observed inflammation, oxidative stress, deficient handling of proteins and mitochondrial dysfunction [103].

Clinical studies have emphasized the incidence of PD in animal models of TBI [104] and humans with previous TBI [105], and the evidence is probably strongest for PD, instead of a TBI association with AD [106], though a recent research reported that TBI is not a potential risk factor for PD, compared with individuals that never suffer a TBI [107]. The difficulties to establish a consensus on these associations are probably due to the complexity of epidemiology and pathology of AD and PD.

Amyotrophic Lateral Sclerosis

ALS is a neurodegenerative disorder where upper and lower motor neurons are affected [108]. The incidence of this disease is 3–5 per 100,000 people [109, 110]. Furthermore, there are several features of its pathogenesis waiting to be unveiled. Only 2 percent of all cases are due to mutation in the superoxide dismutase (SOD1), and similarly to AD and PD, cytotoxic TDP-43 proteins aggregates are developed [103]. Additionally, some evidence point out the oxidative stress, glutamatergic excitotoxicity, microglial activation, and inflammation as promoters of disease progression and severity [111, 112].

Neuroinflammation as a Putative Cause of Neurocognitive Deficits Induced by TBI

Extensive evidence point out neuroinflammation as a major pathological process in the secondary response following injury, that may cause many of the neurocognitive deficits and behavioral changes observed after TBI. A major component of the neuroinflammatory response to brain injury is release of IL-1α or β, the prototypical pro-inflammatory cytokine that binds to the type 1 IL receptor (IL-1RI), and IL-1 signaling is a major mediator of secondary neurologic injury. In fact, the IL-1R-blocking drug anakinra, currently approved in many countries for rheumatoid arthritis, was also been studied for off-label use in other disorders with an inflammatory component, including stroke and TBI [113–117].

Anakinra was able to induce significant improvement in learning during the acquisition phase of the Barnes maze in mice under the TBI induced by FPI, compared with saline-treated FPI mice, indicating that therapeutically targeting IL-1RI may be more likely to provide effective treatment for patients suffering cognitive deficits after TBI than drugs that specifically target individual IL-1 or their signaling [118]. In a different TBI model in rats, frontal CCI at multiple levels of severity resulted in substantial, persistent deficits in several domains of function, namely, attention, impulse control, ability to complete trials, choice, and reinforce collection latencies. The long-term neuroinflammation caused by brain injury was strongly associated with chronic impulsivity (evident over 3 months post-injury, even in mildly injured animals) as well as the degree of recovery in impulse control, even when accounting for gross tissue loss [119]. Additionally, by using the same TBI model, sensorimotor deficits at 3 days post-TBI were observed, and late cognitive flexibility disorder was evidenced by the reversal learning task of the Barnes maze 3 months after injury. These data give an overall invaluable overview of time course of neuroinflammation that could be involved in demyelination and late cognitive disorder over a time scale of 3 months in a model of mild TBI [120].

Pomalidomide is an immunomodulatory agent with a reported tumor necrosis factor-α (TNF-α) inhibitory action of up to 50,000-fold greater than thalidomide [121]. It is clinically available with suppressive effects on angiogenesis, and tumor cell proliferation underpins its use and efficacy in the treatment of multiple myeloma and other cancers [122]. Interestingly, pomalidomide attenuates the size of the resulting cortical lesion by reducing the neuronal degeneration and improves multiple motor functional outcomes in an animal model of a penetrating form of TBI. Additionally, mRNA and protein levels of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 were significantly blunted by pomalidomide when it was administered up to 5 h after TBI [123].

On the other hand, high mobility group box 1 (HMGB1) proteins, non-histone nuclear proteins which contribute to the architecture of chromatin DNA [124, 125], are highly conserved. It has been reported that immediately after neuronal injury, there is a passive release of significant amounts of HMGB1 from the nucleus into the extracellular space [126]. HMGB1 initiates several cell responses including inflammation as well as mediate the activation of inflammatory process via binding with different types of receptors, including RAGE and TLR4 [127, 128]. HMGB1 acts as a pathogenic inflammatory agent. It is involved in the response mediating ranges of conditions such as epilepsy [129, 130], septic shock [131], ischemia [132, 133], TBI [134], PD [135], AD [136], and MS [137]. HMGB1 can influence synaptic function in the brain regions such as the hippocampus, which is involved in hyperexcitability and cognitive decline in epilepsy [138]. HMGB1 participates in cognitive decline probably by causing disruption of the BBB leading to cognitive deficits in aged rats [139]. Interestingly, accumulating evidence suggests that neuroinflammation is highly associated with epilepsy and cognitive dysfunction after TBI and HMGB1 exhibits a key role as an initiator and amplifier of neuroinflammation as well as in neuronal excitation [140], while inhibition of HMGB1 expression exhibits protective effects in animal model of TBI [141]. Similar line of results has been reported where anti-HMGB1 monoclonal antibodies improved neurological deficits in ischemic-induced brain injury [142] and prevent cognitive dysfunction after TBI [143], while antagonists competing with HMGB1 for receptor binding significantly ameliorated the blood-brain barrier breakdown and brain edema induced by CCI, and these effects were associated with the decrease in expressions of inflammation-related factors as well as improved neurological functions [144]. Taken together, all these findings suggest HMGB1 as a potential candidate to be a common biomarker of TBI, neuroinflammation, epileptogenesis, and cognitive dysfunctions which can be used for early prediction and progression of those neurological diseases [145].

Current Clinical and Pharmacological Management of TBI

Medical management of TBI should be tailored to the severity of each case [146]. In milder cases the treatment is focused in the control of the symptoms. There is strong interest to encourage healthy lifestyle strategies such as maintaining the circadian rhythm, having four-meals-a-day diet [147], and doing aerobic exercise training [148]. All these activities are effective in diminishing the common symptoms of mTBI. Headaches, depending on their pattern and intensity, can be managed with nonsteroidal anti-inflammatory drugs (NSAIDs), serotonin receptor agonists (triptans), and phenothiazine antipsychotics. Nausea and vomiting are treated with anti-emetics as well [147].

In order to treat cognitive symptoms of mTBI, pharmacological and nonpharmacological strategies have been assessed. Physical exercise was effective in improving the post-concussion symptom scale and diminished the reaction time when evaluated and days off work [149]. In terms of neurorehabilitation, results are controversial. Patients with mTBI who received multidisciplinary treatment (supportive psychotherapy, physiotherapy, and occupational therapy) did not show improvements in neurobehavioral outcomes after 6 months. These results contrasted only when subjects with psychiatric history prior to TBI were taken into account. They showed a significant decrease in depressive symptoms after this supportive therapy [150]. On the other hand, cognitive rehabilitation achieved better results when working attention deficits were assessed [151].

When pharmacological strategies are considered for the patient management, amitriptyline, a tricyclic antidepressant, has been widely used in patients with mTBI because it prevents migraine, although majority of the studies failed to prove effectiveness in diminishing depressive symptoms. Sertraline, a selective serotonin reuptake inhibitor, in contrast to amitriptyline has shown improvement not only in depression but also in cognition and impulsivity [152]. Moreover, methylphenidate, a psychostimulant that blocks the reuptake of norepinephrine and dopamine into the presynaptic neuron commonly used to treat patients with attention deficit hyperactivity disorder, is useful for enhancing attention in patients with mTBI but fails in improving memory or processing speed [153, 154]. In severe TBI cases, amantadine (dopamine agonist) and CP-0127 (bradykinin antagonist) showed a markedly improvement in arousal, according to GCS, but no effects were seen in attention, memory, general cognition, and global outcome according to Glasgow Outcome Scale (GOS) [155], proving that there is still insufficient pharmacological armamentarium for the management of cognitive disorders induced by TBI.

Severe TBI has a different management strategy because elevated intracranial pressure (ICP) is often encountered and it becomes the main problem to treat in order to get an adequate cerebral perfusion pressure (CPP). When increased ICP is because of a lesion with mass effect, surgery for evacuation of the lesion is indicated. However, when the cause of raised ICP is cerebral edema, medical management turns challenging and often frustrating, because there is not currently effective medical treatment that focuses on the secondary injury causes. The first pharmacological step in the treatment of a patient with severe TBI is sedation. The goal is to decrease cerebral metabolism and oxygen consumption and also protect against seizures. Propofol and morphine are commonly used. Morphine is more effective decreasing ICP, but mortality and GOS were similar when both agents were compared [156]. Because propofol can induce hypotension and depression of cardiac output, a strict control of these parameters must be continuously checked [157]. On the other hand, even though neuromuscular blockade is widely spread, there is still lacking data to support or reject its use. The benefice of nondespolarazing neuromuscular blocking agents (NBA) is the facilitation of mechanical ventilation [158], but it has been shown that patients with severe TBI who received NBA during their stay in intensive care unit had more elevated ICP records than controls without it [159].

Once the patient is under mechanical ventilation and sedated, a placement of an external ventricular drain (EVD) should be the next step in order to control ICP. It has been demonstrated that continuous drainage of cerebrospinal fluid (CSF), commonly from the frontal horn of the ventricle, is more effective in decreasing ICP than when it is done intermittently [160]. If the patient persists with ICP over 20 mmHg, the use of hyperosmolar agents plays an important role. It is known that administration of hypertonic saline and mannitol increases plasma osmolarity by reducing cerebral edema, due to the passage of water from the interstitial and intracellular space to the intravascular compartment. Significant differences between these two agents in terms of global outcomes have not been found, and both are effective reducing the ICP [161]. Moreover, a close control of electrolyte balance and renal function must be performed in order to prevent cardiac and renal complications.

The last pharmacological stage in the stepwise ICP management is the use of barbiturates. They achieve neuroprotection reducing ICP by getting burst suppression, decreasing cerebral metabolism and oxygen consumption, and altering vascular tone. Furthermore, barbiturates couple regional blood flow and inhibit ROS-mediated lipid peroxidation [162], one the main process of secondary injury. Nevertheless, barbiturates may produce severe adverse effects such as hypotension, hypokalemia, respiratory complications, infections, and hepatic and renal dysfunction [163]. Finally, when ICP persists uncontrollable, risking the normal CPP, a surgical procedure called decompressive hemicraniectomy, which implies the removal of an important amount of the calvarium, should be performed in order to give space to the brain and prevent herniations. This procedure, of no less than 12x15cm, demonstrates to reduce mortality but with higher rates of disability and vegetative states [164–166]. Other classic procedures that have not been shown to be beneficial like hypothermia [167], prolonged early hyperventilation [161], and bifrontal craniectomy [168] are not further recommended.

Nanomedicines for Brain Delivery and TBI Applications

The BBB plays the key role in regulating the access of molecules to the CNS. After TBI, it is known that access to the brain of bioactive molecules is limited, and the associated pathological process itself makes it difficult to achieve effective treatment or diagnosis. During this pathological process, an alteration of the BBB has been reported. It includes its disruption, leakage, and alterations in the receptor expression, allowing proteins and red blood cells to enter within the cerebral parenchyma. These changes may provide opportunities to treatment with nanomedicines [169, 170]. The field of nanomedicine involves interdisciplinary research and development related to the application of nanotechnology in medicine on diagnosis, monitoring, treatment, or prevention taking the chemical, physical, and biological advantages that nanometric scale systems possess [171–177]. The nanomedicines used for drug administration have a diameter between 1 and 1000 nm which are able to lead and control release of active molecule in the target site improving its bioavailability and effectiveness [173, 177, 178]. These systems not only minimize the degradation of the drug but also increase the accumulated fraction in the pathological area, preventing the release in cells of healthy organs and more importantly reducing effective doses as well as side effects.

The design of the nanoparticles should focus on achieving a high load capacity and long circulation times, protecting the drug from degradation or its premature or anticipated release. In addition, the system must be nontoxic and biocompatible [173, 179–181]. Nanoparticles can incorporate hydrophobic and hydrophilic drugs, proteins, biological macromolecules, and genes, among others. Moreover, they can improve drug solubility and present high stability as well as controlled and directed release, allowing their administration by different routes [182, 183]. In addition to these properties, nanoparticles intended for the administration of drugs into the brain must have the ability to cross the BBB and possess sufficient physical stability in blood to

achieve prolonged blood circulation [169]. Generally, a bioactive molecule or drug can be adsorbed, encapsulated, trapped, or covalently bound to a matrix or surface of nanoparticles [177] and, thus, modify its pharmacokinetics and pharmacodynamics [173]. A large variety of nanostructures have been described and used with different composition and properties including liposomes, polymeric nanoparticles, lipid nanoparticles, inorganic nanoparticles, nanocrystals, cyclodextrins, dendrimers, and different hybrid nanoparticles. The use of different technological tools can impact the nanoparticle properties such as its diameter, morphology, surface charge, and drug loading capacity, among others [173, 179]. In turn, these properties may influence nanoparticle brain delivery across the BBB.

Liposomes are composed of phospholipids that spontaneously form unilamellar or multilamellar concentric bilayers [184, 185]. The polymeric nanoparticles are composed of biodegradable polymers, and its core can be formed by several natural polymers such as cellulose, gelatin, chitosan, and alginate or synthetic polymers as polylactic (PLA), poly (lactic-co-glycolic acid) (PLGA), polyanhydrides, and poly-ε caprolactone [186]. Dendrimers are well-organized nanoscopic macromolecules that have high aqueous solubility, biocompatibility, polyvalence, and precise molecular weight. They are composed of a central molecule associated with repeated units and reactive groups on the surface [186, 187]. In relation to lipid-based nanoparticles, we can name lipid nanoparticles, lipid nanocapsules, and nanostructured lipids where it is possible to find a solid lipid core at room temperature, in the liquid state or a mixture of both, respectively [188–190]. Inorganic nanoparticles include mainly those based on metals (magnetic or not), carbon (nanotubes and fullerenes), and silica. In addition, different types of these nanoparticles have been combined to obtain hybrid nanoparticles, either those that combine lipids and polymers or organic and inorganic components [169]. Thus, the combination of hydrophilic polymer polyethylene glycol (PEG) and nanoparticles showed benefits in the TBI treatment. Particularly, PEG-decorated silica nanoparticles administered following TBI were able to effectively and efficiently enhance resealing of damaged cell membranes compared with uncoated particles or PEG alone [191–193].

In the design of nanosystems intended for CNS targeting, different aspects must be analyzed. The small size of the nanoparticles which confer a large specific surface exchange allows passage through the smaller capillaries to access a wide range of tissues and cells and prevents rapid elimination by phagocytes. It has been described that nanoparticles with sizes from 20 to 100 nm could reach the brain because their size is smaller than the capillaries thus producing a minimum clearance [173, 194]. The PEGylation process (coating of nanoparticles with PEG) is used to avoid opsonization and to achieve a longer blood circulation time. Furthermore, strategies of passive and active delivery are also used to allow nanoparticles to cross the BBB and release drugs into the brain. In some cases of TBI, the pathological process involves a leaky BBB and permeable blood vessels that offer an opportunity of extravasation and accumulation for circulating nanoparticles in brain areas where injury lead to permeability changes of the BBB, like enhanced permeability and retention (EPR) effect [170, 195]. PEG-decorated liposomes have shown selectively accumulation in the affected brain areas after induction of TBI. These liposomes could deliver drugs directly into the affected site in traumatized brain due to an EPR-like effect, suggesting that they are promising systems to enhance central drug delivery after TBI [196]. Additionally, nanoparticles can be functionalized with positive charge in order to use the BBB active transport, facilitating adsorptive-mediated transcytosis mechanism by electrostatic interaction with negative surface of BBB. Another functionalization strategy is related to the surface inclusion of cationic biomolecules, proteins, or specific ligands which can be bound to receptors of cells of BBB [169]. These strategies are implemented to achieve uniform, longer-lasting, and effective delivery of drugs into the brain. For instance, PLGA nanoparticle core coated with a PEG layer was coupled to imaging agent (800CW) which specifically binds to intracellular

proteins of cells that have lost membrane integrity, thus revealing the extent of the damaged area into a mouse brain and greater penetration with smaller nanoparticles [197].

Different nanoparticles loaded with broad active molecules were designed for treatment and diagnosis of TBI that showed promising results. Thus, cerebrolysin-loaded PLGA nanoparticles were able to reduce brain pathology following TBI and superior neuroprotective effect in comparison to free cerebrolysin in a rat model. A rapid access of nanoparticles into the brain and a prolonged drug release was observed [198]. In another study, brain-derived neurotrophic factor-PLGA nanoparticles coated with poloxamer 188 showed an increase of molecule levels in the central nervous system with a consequent improvement in neurological and cognitive outcomes in an animal model of TBI [199]. Two PLGA-based formulations (microspheres and nanoparticles) loaded with stromal derived factor were studied as strategy for local brain delivery when injected in mouse brain, showing different in vivo response probably related to the particle size and long-term sustained release. Although both particles preserved chemokine bioactivity, nanoparticles showed a more sustained release than microspheres inducing migration of recruiting neural stem cell to the damaged area after their injection in mouse brain with TBI [200]. In another study, it was shown that a dendrimer loaded with pentobarbital significantly increased the absorption after TBI [201]. Furthermore, ROS-reactive thioether core-cross-linked nanoparticles showed a reduction of neuroinflammation and outcome improvement in a mouse model of TBI. These nanoparticles were able to accumulate in damaged brain areas quickly after injection and reduced the spread of damage during the early phase of the disease [202]. Additionally, neurotrophin elastin-like peptide-based fusion proteins maintained the biological activity of the neurotrophin and increased bioavailability by limiting protein loss due to diffusion and allowing controlled spatio-temporal delivery. These nanoparticles have impact in the field of neuronal regeneration and treatment of neurodegenerative diseases, including TBI [203].

Nanoparticles are also used for diagnosis purposes, such as the superparamagnetic iron oxide nanoparticles, which is one of the most used contrast agents for the detection of cells transplanted by nuclear magnetic resonance. Furthermore, the superparamagnetic iron oxide nanoparticle labeling via DNA hybridization was explored to achieve a fast and specific cell labeling method to monitor transplanted cells in the CNS by magnetic resonance imaging in vitro as well as in vivo [204]. A recent work used nanoparticles to demonstrate that the magnetic particle imaging modality can be applied to imaging TBI events with an excellent contrast [205]. Another novel nanosystem was reported as a wireless sensing device to monitor the intracranial brain deformation in real time during the event of a blast-induced TBI. This system consists of an implantable soft magnet composed by iron oxide nanoparticles and an external head-mounted magnetic sensor that is able to measure the field in three dimensions. The change in the relative position of the soft magnet with respect to the external sensor induces changes in the magnetic field, which are used to extract the temporal and spatial motion of the brain under the blast wave in real time [206].

On the other hand, iron-based magnetic nanoparticles were designed to label mesenchymal stem cells as well as to track the fate of cells by magnetic resonance imaging [207]. In the same way, a delivery system using a surface modified solid gold nanoparticle was studied modifying the protein expression of therapeutically beneficial biomolecules. The results offer a simple efficient approach to transfection without complication from viral methods and illustrate that gene transfer on a gold nanoparticle peptide fusion can induce an efficient one-step transformation of rat mesenchymal stem cells [208].

Strategies to enhance survival and direct the differentiation of stem cells in vivo following tissue transplantation are critical to realize the potential of stem cell-based therapies. A nanoparticle system to deliver neurogenin-2 to human fetal tissue-derived neural stem cells resulted effective to promote neuronal differentiation and maturation in the brain area that needs to be

repaired. A larger number of neurogenin-2-transfected human neural stem cells, when transplanted with a tailored hyaluronic acid hydrogel into the TBI lesion site in a rat model, were more effectively differentiated into neuronal lineage compared with non-transfected cells [209]. In the same way, a nanosystem was developed to encapsulate retinoic acid, a potent morphogen, to effectively induce neural stem cells differentiation in vivo. However, a limited number of transplanted neural stem cells can finally accumulate at the TBI site. The transplanted neural stem cells partially reconstructed the damaged neuron circuit and improved the axon signal conductivity, helping ameliorate the negative impact of TBI and partially rescued gene transcripts regulating brain functions [185].

Concluding Remarks

All evidences presented in this chapter suggest that the neuroinflammatory nature of TBI, as a vicious circle, is the gating factor that may lead to the development of long-term disabilities including cognitive, affective, and physical detriments as well as neurodegenerative pathologies. Clinical evidences indicate that management of TBI is difficult and limited, because the insufficient pharmacological or procedural armamentarium together with their frequent adverse effects and complications must be taken into account when deciding which therapy is more convenient to apply. Therefore, it is essential to generate new scientific evidence to solve the questions posed by the mechanisms gated by TBI that contribute to long-term disabilities and draw attention to them as potential pharmacological targets of newly designed and more effective drugs or systems.

Finally, it is important to highlight that the use of nanomedicine in TBI is incipient and a strong impact of this type of development is projected in the coming years. It is particularly visualized that nanometric systems will have prevalence in the future on the design of novel pharmacotherapies that allow reaching the CNS more efficiently and diagnostic devices that can improve the prognosis of this pathology.

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Physiology and Pharmacology of Melatonin

21

Michel Bourin

Introduction

Melatonin is a natural hormone that was discovered by chance in 1958 by Aaron B. Lerner at Yale University. Conducting research on the treatment of vitiligo, a depigmenting skin disease, the latter has been experimenting with administering to his sick patients a bovine epiphysis extract, thinking to obtain an effect on the pigmentation of the skin. Instead, Dr. Lerner made his patients sleepy. He identified the substance and named it N-acetyl-5-methoxytryptamine [1]. This hormone, derived from serotonin, is produced mainly in the pineal gland and is secreted at night, with a peak around 3 am. It follows a circadian rhythm, controlled by the suprachiasmatic nuclei, biological clock of our organism [2]. This clock works itself rhythmically, and it is driven by the synchronizers of the environment.

The pineal gland is located at the back of the brain and belongs to the epithalamus, on the roof of the third ventricle [3]. Organ richly vascularized, its irrigation is by arteriolar ramifications from the posterior choroidal arteries. The vascularization of the mammalian pineal gland is characteristic of that of a tissue with intense secretory activity. The rat epiphyseal blood flow, calculated per gram of tissue, is greater than that of most endocrine glands and is equivalent to that of the post-hypophysis. The connection pathway includes retinohypothalamic suprachiasmatic nuclei (SCN) binding, which plays a key role in the generation of circadian melatonin rhythms and body temperature in particular. Dubbed sleep hormone, melatonin is the key hormone regulating our chronobiological rhythms, such as circadian rhythms, including temperature and sleep-wake rhythms, indicating to the body its position in alternation day-night and allowing him to adapt to his environment [4]. Its secretion can be disturbed by artificial light. There are other extrapineal sources of secretion of melatonin, especially in the retina, intestine, liver, kidneys, cochlea, thyroid, and some blood cells [5]. Melatonin is present in all biological fluids, including cerebrospinal fluid, saliva, bile, synovial fluid, amniotic fluid, and breast milk. In many of these fluids, melatonin concentrations are higher than those of blood [6].

Biosynthesis

Melatonin is mainly synthesized in the cells of the pineal gland, pinealocytes, from its precursor, tryptophan. In the absence of intraepiphyseal storage after its production, it is directly

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released into the venous blood through the blood-brain barrier, passively and in a nictemeral rhythm [7]. The biosynthesis of melatonin is mainly at night and lasts about 10 hours. Firstly, tryptophan is converted to 5-hydroxytryptophan by tryptophan hydroxylase and then serotonin (or 5-hydroxytryptamine) by the action of a decarboxylase enzyme. This serotonin undergoes acetylation by the arylalkylamine N-acetyltransferase (NAT) which gives N-acetylserotonin, followed by methylation with hydroxy-indole O-methyltransferase to give N-acetyl-5-methoxytryptamine or melatonin [8]. (Fig. 21.1) After maturation in the first year of life, melatonin production peaks between the ages of 3 and 6, and the nocturnal peak gradually decreases to 80% to reach adulthood levels. As it ages, calcification of the pineal gland results in decreased secretion of melatonin [9]. It should be known that NAT is the limiting enzyme in the rate of synthesis of melatonin. This enzyme has an activity at night which is up to 100 times higher than during the day, hence the important secretion of nocturnal melatonin. In the adult male, nocturnal endogenous melatonin production is 10 to 80 µg per night, and the concentration of melatonin in the blood is highly variable; it ranges from 50 to 200 µg/mL [10]. Melatonin has the particularity of being both hydrophilic and lipophilic, which give it the possibility of crossing different cell barriers, in particular, the blood-brain barrier after its synthesis [11]. The half-life of melatonin is around 30 minutes [12]. It is interesting to note that the plasma concentration of melatonin is very variable from one individual to another but very reproductive in the same individual from 1 day to the next, which raises a very important interindividual difference.

Regulation of Biosynthesis

The secretion of melatonin is regulated by the central nervous system at the level of suprachiasmatic nuclei (SCN), location of the internal clock [13]. When the decrease in brightness is captured by the retina, a neuronal message is sent from the SCN to neurons producing norepinephrine. The latter stimulates the β1-adrenergic receptors present on pinealocytes [14]. A cascade of reactions then activates the key enzyme producing melatonin, N-acetyltransferase. Melatonin secreted into the blood will transmit photoperiod information to all structures with melatoninergic receptors. Conversely, light inhibits the secretion of melatonin if certain conditions are met: sufficient illumination and prolonged at an optimum time, which is to say between 2 and 4 o'clock in the morning [15]. In addition, the blue light will have more effect on its secretion, while the red light has no effect. Indeed, light inhibits noradrenergic transmission by hyperpolarization of the retinohypothalamic pathway. The effect of artificial light on the melatonin plasma secretion curve shows that if light is given once in the middle of the night, the secretion rate falls sharply. Then the secretion resumes with an offset peak. Evening light delivery shifts the peak in the morning, while morning administration moves the peak in the evening. The plasma profile may be narrowed if light is administered in the morning and evening.

Pharmacokinetics

The interindividual variability being very important, the melatonin plasma concentrations and its half-life depend on several factors such as the

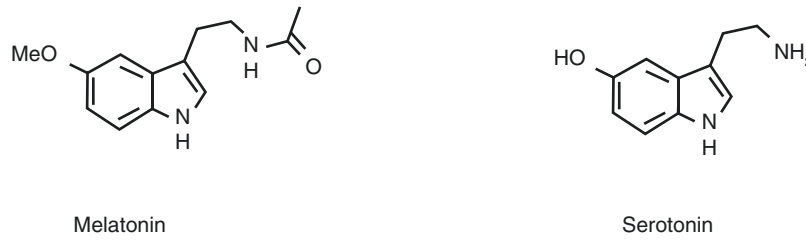

Fig. 21.1 Comparison between the chemical structure of melatonin and serotonin

dose of administration, the time, and the type of Galenic presentation. Absorption of oral melatonin is complete in adults and can be halved in the elderly [16]. There is a major hepatic first pass effect. Sustained-release preparations will be preferred when endogenous secretion is decreased to achieve a lower blood pressure peak and a relatively high concentration over a longer period than with immediate release preparations [17]. Blood melatonin is 70% bound to albumin and to a lesser extent to orosomucoid [18]. The half-life of absorption is 0.4 hours, and the rates increase in the plasma after 30 minutes to 3 hours. It has been observed that melatonin receptors are more sensitive between 17 and 20 hours.

Melatonin is a very lipophilic hormone that is eliminated up to 90% by the effect of first pass through the liver. It is then metabolized to 6-hydroxy-melatonin, an inactive metabolite, and then transformed by sulfation and glucuronidation before being eliminated in the urine [19]. A small part of melatonin is also degraded in the brain (15%). In the liver, it is CYP1A2 that essentially metabolizes melatonin. CYP2C19 is involved to a lesser extent [20]. This metabolism decreases with age. We can measure the main urinary metabolite, 6-hydroxymelatonin sulfate, which has a half-life of 20 minutes, for example, to help diagnose certain pathologies.

Only a small proportion of melatonin, which is not metabolized, is eliminated directly via the kidneys. Its bioavailability is 40–70% for doses ranging from 2.5 to 100 mg. This implies that it is best to start at the smallest possible dose, especially that a dose as low as 0.5 mg gives higher concentrations than physiological concentrations. Food seems to increase absorption, but the clinical impact seems weak [21]. Exogenous melatonin has a very rapid distribution phase (half-life time, 2 minutes) followed by a slower elimination phase (half-life time, 20–50 minutes). As a result, the sleep-inducing effect is rapid and transient, lasting for 3–4 hours.

Melatonin has a large circadian rhythm with very low levels during the day and high levels (10 times) at night, the peak of secretion being around 02–03 h, whether the species is nocturnal or diurnal [22]. Other extrapineal gland sources exist in particular in the retina, the digestive tract, and certain blood cells [23]. The circadian rhythm of melatonin is, for the same individual, very reproducible from day to day, which makes the hormone an important marker of circadian synchronization [24], but the interindividual differences are important. The nocturnal elevation of melatonin concentration is related to the nocturnal release of norepinephrine (NE), since a neuronal message, initiated in suprachiasmatic nucleus when neurons are subtracted from the light-inhibiting effect, induces the release of norepinephrine. This stimulates the beta-1-adrenergic receptors present on the pinealocytes (and to a lesser extent, the α-adrenergic receptors), then the adenylate cyclase via the guanine nucleotide-related proteins (GS proteins), which results in an intracellular rise of cyclic AMP followed by rapid release of melatonin by activation of the key enzyme, N-acetyltransferase. While hydroxyindole-O-methyltransferase, the enzyme catalyzing the last stage of synthesis, is very weakly rhythmic, the N-acetyltransferase of the pineal gland has a very high amplitude rhythm with nocturnal activity 50–100 times higher than that presented the day. Beta-adrenergic control of melatonin secretion results in inhibition of the hormone by β-adrenergic receptor antagonists such as propranolol and atenolol [25]. On the other hand, we observed that the administration of beta-adrenergic agonists to humans, in the morning when melatonin levels are very low, does not stimulate the secretion of the hormone, because its synthesis is almost null during the light phase. The goal, during the therapeutic use of melatonin, is therefore always to preserve the natural circadian rhythm of the hormone with its high concentrations at night and low during the day.

Control of Secretion by Light

The light-dark cycle is the main synchronizer of the hormone: it drives suprachiasmatic nucleus and consequently a number of circadian rhythms, including those of melatonin and body temperature (Fig. 21.2). Transmitted through the retina,

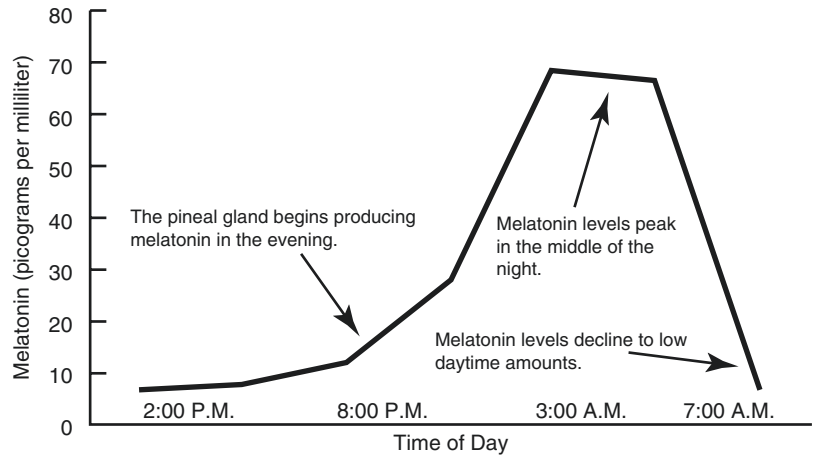

Fig. 21.2 The amount of melatonin secretion from human pineal gland during various time of day

light is the most important synchronizer for the training of the clock, which will be used in the treatment of chronobiological disorders of which we will see some applications [26]. The synchronization of the endogenous clock by photoperiod begins in fetal life in mammals in relation to the secretion of maternal melatonin [27]. In all cases where the light-dark cycle is modified, such as during transmissions over more than three time zones [28], night shift work [29], or in case of blindness, the profile secretory of melatonin is disrupted. Light acts differently on the secretion of melatonin depending on the time of exposure. When the exposure occurs at night at the peak of secretion (02–03 h), secretion of melatonin is completely inhibited throughout the duration of exposure. When exposure is in the morning, there is phase advance, i.e., the peak circadian rhythm of melatonin is advanced over time. When the exposure takes place at the end of the afternoon, there is a phase delay that is to say that the peak is delayed compared to its usual temporal location. In addition to the exposure time, the effect of light depends on the species and its characteristics (intensity, duration, and spectrum). In the total absence of light (in the dark), the circadian rhythm of melatonin is no longer synchronized with the environment and is out of phase with the outer light-dark cycle (free-ride phenomenon) [30]. As light is the visible portion of the electromagnetic spectrum, it is conceivable that other wavelengths are also likely to affect the secretion of melatonin. We have recently shown the absence of effects in humans exposed to magnetic fields of 50 Hz, even when exposure lasted 20 years.

Melatoninergic Receptor

The action of melatonin is mediated through receptors on cell membranes. Their modulation by melatonin varies according to the exposure of people to light. There are three binding sites for melatonin in the human body: MT1, MT2, and MT3. The two subtypes MT1 and MT2 are G protein-coupled receptors [31] and belong to a group of high-affinity pharmacological sites, while MT3 has been characterized as an enzyme involved in protection against oxidative stress and belongs to a group of low-affinity sites. These three subtypes are encoded by genes located on different chromosomes. In humans, MT1 and MT2 receptors are found in several central nervous structures, including the suprachiasmatic nuclei of the hypothalamus and the pars tuberalis of the adenohypophysis. It is currently known that MT1 and MT2 receptors have numerous peripheral locations [32], such as the gastrointestinal tract, lungs, spleen, thymus, kidneys, prostate, blood vessels, lymphocytes, neutrophils, tissue adipose and hippocampus, and in other unidentified structures still today.

The melatonin receptors MT1 and MT2 belong to the superfamily of seven transmembrane domains coupled to heterotrimeric G

proteins (GPCRs). Central melatonin binding sites were first described in the bovine [33] following autoradiography with [3H]-MLT. Other experiments have demonstrated melatonin binding sites at peripheral organs in the rat [34]. Pharmacological studies as well as the cloning of melatoninergic receptors have allowed the functional characterization of the latter. Initially, the melatoninergic receptors were divided into two classes, ML1 and ML2, based on the affinity differences and kinetics of binding with the radioligand of 2-[125I]-iodomelatonin (2-[125I]-MLT), as well as differences in the picomolar, whereas ML2 binds the radioligand with a lower affinity, of the order of nanomolar. In mammals, the low-affinity binding site, initially identified as ML2, has been pharmacologically characterized and renamed MT3 [35]. Melatonin and one of its precursors, N-acetylserotonin, compete for binding of 2-[125I]-MLT to the picomolar binding site, whereas ML2 binds the radioligand with a lower affinity to order of nanomolar.

The MT3 binding site was purified from hamster kidney tissue and identified as an enzyme, quinone reductase II (QR2). This QR2 protein has a binding profile of 2-[125I]-MLT identical to the MT3 binding profile in the hamster brain [36]. Historically, the QR2 enzyme has been considered as a detoxification enzyme, by analogy with the QR1 enzyme. For the moment, it has not been clearly demonstrated that the QR2 enzyme has these detoxifying properties [37].

In 1994, the first true melatoninergic receptor (Mel1c) was cloned from *Xenopus melanophores* (*Xenopus laevis*) [38] then in *Ornithorhynchus anatinus* [39]. This discovery allowed revealing different receptor subtypes with class-related pharmacology ML1: Mel1a, currently MT1; Mel1b, corresponding to MT2; and Mel1c, found in birds, fish, and *Xenopus* but not in mammals. It is in all cases GPCRs. A phylogenetic study has demonstrated that the mammalian orphan GPR50 receptor is an orthologous Mel1c receptor [40]. This GPR50 receptor is related to the family of melatonin receptors, despite the loss of capacity binding of melatonin [41]. The GPR50 receptor has a distribution in many neuroendocrine tissues such as the dorsomedial hypothalamus, the lateral hypothalamus, the arcuate nucleus, or the layer of ependymal cells lining the third ventricle. This expression appears to be conserved in several species (sheep, mouse, rat, and hamster) suggesting a biological function for this receptor [42].

The coding region of the MT1 and MT2 receptors is composed of two exons separated by an intron about 10 kB. Exon 1 codes for the N-terminal part and up to the first intracellular loop of the receptor. Exon 2 codes for the rest of the protein [43]. The MT1 and MT2 receptors have a strong sequence identity in amino acids: 55% for the whole sequence and 70% for only the transmembrane domains. Receivers have about 300 amino acids with a molecular weight of about 40 kDa. As mentioned earlier, MT receptors are related to the A family of Gprotein-coupled receptors (GPCRs). Twenty to 25 highly conserved amino acids represent the signature of this class of GPCRs. The high degree of conservation of this amino acid sequence suggests that they would play an important role in maintaining structural integrity and/or functional of these receptors. Of these amino acids, the aspartate of TM 2 and three residue hydrophobic properties of TM 6 and TM 7 (proline, phenylalanine, and asparagine) would be important for activation of the receptors as well as arginine of the DRY motif (Asp-Arg-Tyr) between the passage TM 3 and the second intracellular loop. Two cysteines on the first two loops extracellular cells involved in a disulfide bridge and a possible site of palmitoylation after the short helix 8 located directly at the exit of the TM 7 are also very well preserved during of evolution [44].

This posttranslational modification site represents a possible point anchoring to the plasma membrane that can form a fourth intracellular loop. It was shown by site-specific mutagenesis that these cysteines are necessary for the activation of G proteins as well as signaling pathways [45]. The ligand binding site primarily involves transmembrane domains as well as extracellular loops [46]. The pattern E/DRY/W, and more particularly the aspartate residue, seems important in protein coupling G. Only the ovine MT2 receptor has a DRY pattern. MT2 receptors of other species have an NRY motif, where asparagine is

replaced by aspartic acid. This motive participates in the inactivated state of GPCRs [47]. The N-terminus of the MT1 receptor has two potential glycosylation sites on asparagine residues (Asn), while that of the MT2 receiver has only one. The C-terminus of the two receptors contains a site for casein kinase 1α, casein kinase II, and protein kinase C (PKC). This site would participate in the membrane anchoring and the installation of the tracks of MT signaling system. MLT activates different molecular cascades depending on the cellular context.

The way signaling mainly described for MT receivers is the so-called "cAMP" pathway of the secondary signaling pathways are also described. Coupling of MT1 receptors can be done with inhibitory heterotrimeric proteins Gi, sensitive to pertussis toxin (PTX), specific inhibitor, and with Gq/11 proteins, insensitive to the PTX. When binding melatonin to its type 1 receptor, a Gi protein activates to inhibit the activity of adenylate cyclase (AC) and thereby decrease the production of cAMP from ATP. The activity of protein kinase A (PKA) and the phosphorylation of cAMP-response element-binding (CREB) protein are therefore inhibited [48]. In parallel with the cAMP pathway, the MT1 receptors can directly activate the phospholipase C (PLC) pathway or indirectly via the βγ subunit of the G protein. Activation of the MT1 receptors can also regulate certain ion channels. In sheep pars tuberalis cells, activation of MT1 receptors results in increased intracellular calcium (Ca2 +) concentration via PTX-insensitive G proteins [49]. In contrast, in neonatal rat pituitary cells and in AtT20 cells stably expressing MT1 receptors, melatonin acts via PTX-sensitive G proteins and inhibits calcium flow. MT1 receptors can also be coupled to calcium-activated potassium channels (BKCa2 +) as well as to G-protein-activated (GIRK Kir3)-compatible incoming potassium channels. Activation of this receptor may also modulate arachidonic acid formation, stimulate c-Jun N-terminal kinase activity, and modulate MAP kinase activity.

MT2 receptors are also coupled to inhibition of cAMP formation [50]. Furthermore, activation of this receptor subtype induces inhibition of nucleotide formation, the cyclic guanosine monophosphate (cGMP). In SCN, melatonin by binding to MT2 receptors activates protein kinase C (PKC), a response that is abolished following the administration of 4-phenyl-2-propionamidotetralin (4P-PDOT). These results suggest that MT2 receptors are coupled to the inositol signaling pathway triphosphate (IP3). Indeed, the binding of melatonin on MT2 induces the activation of a Gq protein that activates phospholipase C (PLC). This one is produced from a lipid transmembrane, phosphatidylinositol diphosphate (PIP2), diacylglycerol (DAG), and second messenger, the IP3. IP3 has receptors expressed at the membrane of the endoplasmic reticulum, thus modulating the intracellular release of Ca^{2+} [51].

Pharmacology of Melatoninergic Receptors

Many pharmacological molecules have been developed to specifically mimic the effects of melatonin. The following paragraph presents several of these molecules well known for their selectivity on MT receivers. The MT1 and MT2 receptors have a picomolar affinity for the reference radioligand, 2-[125I]-iodomelatonin. MT1 and MT2 receptors, expressed as transiently in the COS-7 cell line [52] or stably in CHO cell lines and NIH-3 T3, are characterized by the following classification of affinities: 2-iodomelatonin ≥ melatonin > > N-acetylserotonin > > serotonin.

In addition, the MT1 receiver differs from the receiver MT2 by a higher affinity for 2-iodomelatonin and ramelteon than for melatonin and a very low affinity for 6-chloromelatonin. The MT2 receiver is characterized by similar affinities for 2-iodomelatonin, ramelteon, melatonin, and 6-chloromelatonin [53]. The ligands with a better affinity for MT2 than for MT1 are as follows: luzindole (2-benzyl N-acetyltryptamine) (15–25 times stronger), GR128107 (113 times), IIK7 (90 fold), K185 (140 fold) [54], and a luzindole analogue, DH97 (90-fold) [55]. 4-Phenyl-acetamidotetraline (4P-ADOT) is a selective ligand for MT2. However, the affinity ratio MT1/MT2 (300/22000) varies according to the level of expression of the receptors

or current signaling pathways. Many melatoninergic ligands are competitive antagonists with a variable degree of selectivity.

Luzindole, the first competitive antagonist discovered, shows a better affinity for MT2 than for MT1. The specific and selective ligands of MT2, 4P-PDOT, and its derivatives, 4P-ADOT (4-phenyl-2-acetamidotetraline) and 4P-CADOT (4-phenyl-2-chloroacetamidotetraline), are competitive antagonists in native tissues, where the level of receptor expression is low. In contrast, the 4P-PDOT and luzindole behave as agonists or partial agonists in recombinant systems. 4P-PDOT also has a strong affinity for the heterodimer MT1/MT2 [56]. 5-Hydroxyethoxy-N-acetyltryptamine (HEAT), a ligand nonselective MT1 and MT2, shows different efficiencies by acting as a full agonist for MT1 and as an MT2 antagonist [57]. IIK7 is a selective MT2 agonist, which inhibits forskolin-induced cAMP formation, when the receptors are expressed in the NIH-3 T3 line. All of these studies indicate that the pharmacological effects of luzindole and 4P-PDOT are complex, both in native tissues and in recombinant systems [58].

The recombinant MT1 receptors exhibit constitutive activity, producing a spontaneous regulation of effectors in the absence of ligand [59]. As indicated previously, inverse agonists stabilize the free forms of the receptor and reduce thus the activity of the ligand-independent receptor. As a result, luzindole and 4P-PDOT may be considered inverse agonists of MT1. The inverse agonist behavior of luzindole and 4P-PDOT is called into question in several works [60]. In the CHO lineage expressing a high concentration of hMT1 receptors, these molecules would have a higher affinity for MT1 in the presence rather than absence of GTP. In addition, luzindole and 4P-PDOT would decrease basal [35S]-GTPgS binding and increase training cAMP.

Human MT receptors have been localized in the brain and in peripheral tissues by various techniques such as 2-[125I]-MLT autoradiography, the RTPCR technique, as well as immunohistochemistry. In birds and lower vertebrates, MT receptors are widely distributed in the central nervous system. In mammals, these receptors are more locally distributed. Pars tuberalis, subdivision of the anterior lobe of the pituitary gland, has a very high level of MT receptor expression [61]. The MT1 and MT2 receptors are found in the retina and the hypothalamus, especially at the level of the SCN and the HPM, but in the hippocampus and the cerebellum. The MT1 receptors are also found in the thalamus, cornea, and cerebral cortex [62]. MT1 and MT2 receptors have also been localized in peripheral tissues, such as the heart and arteries [63], lung [64], liver [65], and skin [66]. The MT1 receptor is found in the adrenal glands [67], kidneys [68], and T and B lymphocytes [69]. The MT2 receptor is also detected at the level of the intestines [70].

Physiological Involvement of Melatonin Receptors

Although melatonin receptors are well known, the specific function of the MT1 receptor versus that of the MT2 receptor is not well established. In many species, including man, the mRNAs encoding the MT1 and MT2 receptors and the receptors themselves are expressed in the peripheral and cerebral arteries [71]. At the level of the rat caudal artery, important for the mechanism of thermoregulation, activation of the MT1 receptor induces vasoconstriction, whereas that of MT2 receptor leads to vasodilation.

The action of melatonin would be mediated by the inhibition of calcium-activated large-conductor potassium channels ($BKCa^{2+}$) [72]. These results, however, are controversial. Invalidation studies of genes encoding MT receptors make it possible to understand the physiological implication of each receptor subtype. In the pars tuberalis of deleted mice of the gene coding for the MT1 receptor, the expression of the clock genes (mPer1, mCry1, Clock, and Bmal1) is drastically decreased. These results make it possible to identify the role of the MT1 receptor expressed in pars tuberalis in mice [73]. Indeed, acting through MT1 receptors, melatonin is an important regulator of the rhythmic expression of clock genes.

Double studies invalidating the gene coding for the MT1 and/or MT2 receptors, this time in the SCN, show in vitro that phase advances in the circadian rhythm would involve activation of the receptor subtype 2, whereas in vivo activation of both subtypes (MT1 and MT2) seems to be necessary for the expression of these phase advances induced by melatonin [74, 75]. On the other hand, during a forced swim test on invalidated mice for one of the genes encoding MT receptors, the antidepressant effects of luzindole pass only through MT2 receptors [76].

Effects of Melatonin

Melatonin is present in all the liquid compartments of the body, and all the cells are exposed to it [77]. It influences a number of functions of the body, although its physiopathological and pharmacological effects are not fully understood, as well as its mechanism of action, although melatonin receptors (or high-affinity binding sites of melatonin) have been found in different tissues [78].

Most mammals, including humans, have been able during evolution to use photoperiodic information, that is to say, the daily duration of illumination, to adapt to the environment and seasonal changes. Many species are able to respond to these changes in photoperiod by adapting some of their physiological and behavioral functions to anticipate future seasons. The hibernation cycle is a specific example of these photoperiod-dependent physiological processes that involve a very complex neuroendocrine control system. The pineal gland, the terminal organ of the visual system, is a neurochemical transducer, and its function is to inform the body about the photoperiod (or the length of the night) thanks to the secretion of melatonin. Highly lipophilic, melatonin rapidly passes the cell membranes and plays its role as a chemical messenger. All cells and cell organelles are therefore exposed to the circadian rhythm of melatonin. Provided that it can interpret this signal by a mechanism involving a receiver or other means, each cell of the body can be informed of the photoperiodic situation of the environment.

Suprachiasmatic nucleus of the hypothalamus is considered as the main biological clock of the body, controlling many circadian rhythms, including that of melatonin [79]. However, melatonin can intervene on this endogenous clock, since melatonin receptors have been found in the suprachiasmatic nucleus in rats, hamsters, sheep, and humans. Thus, melatonin is able to directly inhibit the activity of protein metabolism and synthesis in the rat SCN [80].

Interrelation of Melatonin with Different Endocrine Axes

The pineal gland has an inhibitory role on most endocrine glands. Thus, pinealectomy in rats results in increased circulating levels of gonadal steroids, follicle-stimulating hormone (FSH), and prolactin. Ablation of the pineal in rats also causes changes in pituitary, gonadal, thyroid, and parathyroid function. In studies with rat pineal perfusions, pharmacological concentrations of corticosterone and dexamethasone have been shown to cause inhibition of melatonin production while at physiological concentrations; testosterone and 17b-estradiol stimulate the secretion of the hormone [81]. While there appears to be evidence of a direct interaction between the pineal gland and the pituitary gland, gonads, and hypothalamus in various laboratory animals, in humans, the effects on these endocrine functions are less obvious and controversial [82].

Gonadotropic Axis

The interrelation of melatonin with the gonadotropic axis is the one most studied [83]. In animals, the pineal gland is an important link in the neuroendocrine system that regulates circannual rhythms of the reproductive function. The epiphysis influences the maturation and cyclic activity of the gonads as a function of light exposure and allows synchronization of the animal's reproductive system with its environment. Melatonin transmits photoperiodic information to the reproductive function; this influence is most often inhibitory [84]. The action of melatonin is manifested differently according to the mode of

administration (the duration and the time of administration), the doses injected, and the characteristics of the subject (age, sex, and so on).

Corticotropic Axis

There appears to be an inhibitory effect of melatonin on adrenal function in rodents [85]. Pinealectomy induces hypertrophy of the adrenal gland with hypersecretion of adrenocorticotropic hormone (ACTH), corticosterone, and/or aldosterone, whereas administration of melatonin or pineal extracts may neutralize these effects [86]. An inhibitory effect of melatonin on ACTH secretion has been suggested [87]. In vitro, the effects of pineal gland extracts on the adrenal glands of mice depend on the circadian stage of gland collection: according to this, the response of corticosterone to ACTH can be amplified or attenuated [88].

Thyrotropic Axis

The secretion of the thyroid is influenced by photoperiod, and melatonin has an antithyrotropic effect. The suppression of circulating melatonin by injection of antimelatonin antibodies into the rat results in an increase in plasma thyroid-stimulating hormone (TSH) concentration. This effect depends on the circadian stage and age of the subject. Melatonin decreases plasma thyroxine and TSH levels if administered at the end of the photoperiod [89].

Pancreas

Melatonin participates in the regulation of insulin secretion. The experiments in mice and rats in vitro and in vivo show that melatonin has a significant depressant effect on insulin only if the concentration of insulin is in the plasma or in the islet incubation medium. Isolated Langerhans are increased by a glucose load [90].

Melatonin Sleep and Desynchronization

There is no formal experimental evidence that physiological concentrations of melatonin are able to intervene in the sleep cycle although the hypothesis has been made that the increase in the frequency of sleep disorders in the subject is related to the significant decrease in the concentration of nocturnal melatonin [91] (Fig. 21.3). Its main effect is thus to improve the quality of sleep and to allow the resynchronization of its own rhythm.

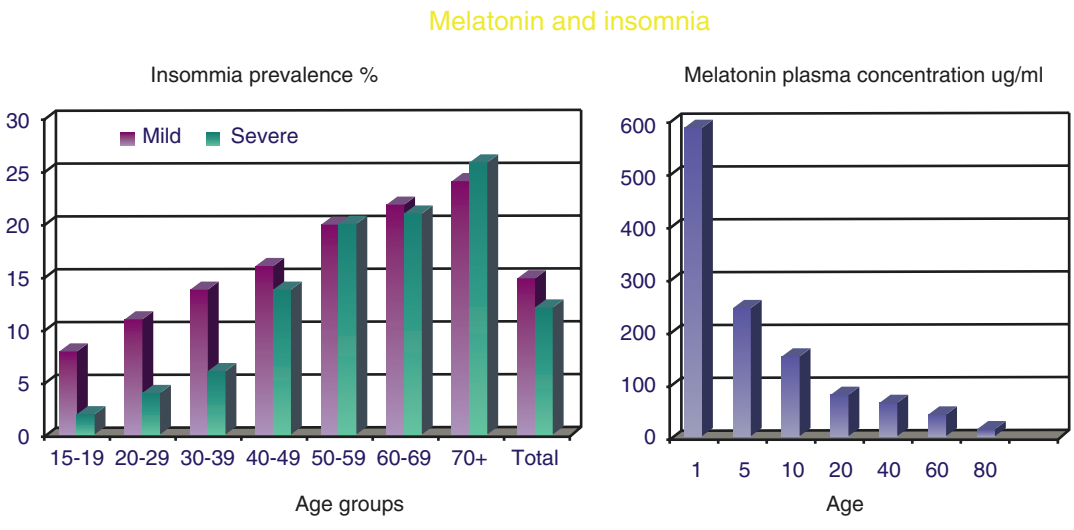

Fig. 21.3 Comparison between insomnia prevalence and melatonin plasma concentration depending of patient age

Some studies have suggested its effectiveness in transmeridian flights to combat the effects of jet lag [92]. The pharmacological administration of melatonin reduces sleep latency and increases the quality and total duration of sleep [93], lowers body temperature [30], and does not alter the architecture of sleep [94]. The duration of paradoxical sleep is not affected, while the results are controversial for slow sleep [95]. As melatonin lowers body temperature, it can regulate the sleep/wake cycle through thermoregulatory mechanisms [96]. Melatonin has been used to treat sleep disorders in subjects with total blindness [97], in Alzheimer's disease [98], and in delayed phase sleep disorders, administered to the patient. Adequate time, it advances the phase of sleep. A relationship between the oral dose of melatonin and the degree of phase shift of endogenous melatonin has been shown. It is also interesting to note that the administration of the hormone facilitates benzodiazepine withdrawal [99].

Desynchronization occurs when the biological clock is no longer in sync with the environment, leading, among other things, to sleep disturbances. Desynchronization is thus observed in night work and shift work, transmeridian flights [100], blindness [101], depression [102], and hormone-dependent cancers [103]. Disruption of circadian organization has been proposed as one of the possible explanatory factors for certain mood disorders including depression. The nocturnal peak of melatonin is decreased in depressed patients, and the circadian profile of the hormone has, according to the patients, either a phase advance or a phase delay (Fig. 21.4). The antidepressant activity of agomelatine has been shown on the treatment of major depression [104] and on forced swimming test a behavioral model of depression [105]. Agomelatine adjunctive therapy was not superior to placebo adjunctive therapy for acute bipolar depression [106].

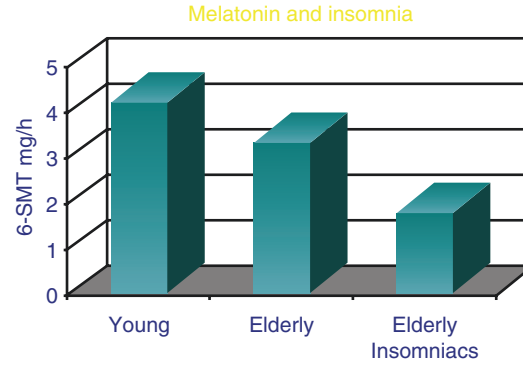

Fig. 21.4 Plasma concentrations of 6-SMT the main metabolite of melatonin depending of age and insomnia

Melatonin, Free Radicals, and Aging

The plasma concentration of melatonin is halved in the elderly and even more pronounced in Alzheimer's disease [107] according to a process that probably involves different mechanisms such as calcification of the pineal, the decrease in the number of or the sensitivity of β-adrenergic receptors in the pinealocyte membrane, and the decreased activity of the key NAT enzyme. As melatonin, which is lipophilic, easily crosses the cell membrane, all the actions it is likely to have do not necessarily involve its specific membrane receptors. Many studies have reported its action antiradical even more powerful than vitamin E, reference in the field [108]. The suspected role of free radicals in the processes of cellular aging has therefore made melatonin a means of combating age-related neurodegenerative processes without formal experimental evidence yet being provided. It has also been tested in other pathologies accompanied by an increase in free radicals, particularly ischemia. This decrease in melatonin in the elderly, its potent antiradical action, and results indicating that cross-pineal transplants of young mice to aged mice (and vice versa) lead to an increase in the life span of animals [109] have led the American public following a hype, an abusive self-medication that we can only regret. Although melatonin does not appear to have significant side effects in acute administration, long-term toxicity is not known, nor are possible interactions with other drugs taken together.

Melatonin and Cancer

In vivo pinealectomy in mice increases the rate of tumor mitosis, and in vitro the growth of breast cancer cells in culture has been found inhibited

[110]. A significant correlation between lowered levels of plasma melatonin and endometrial cancer has been demonstrated, as in breast cancer, with positive estrogen receptors [111, 112]. In addition, the toxic effects of chemotherapy with interleukin-2 or tamoxifen can be reduced by the concomitant administration of melatonin as an adjunct [113]. These results remain to be confirmed for larger groups of patients. Another aspect of the possible relationship between melatonin and cancer is evidenced by recent epidemiological studies reporting a significant increase in the relative risk of breast cancer in women working regularly at night. The advanced mechanistic hypothesis is the reduction of the nocturnal melatonin peak, which is related to illumination at night, leading to an increase in estrogen, estradiol in particular, which increases the growth and proliferation of hormone-sensitive breast cells. If these data were confirmed, they would pose a major public health problem. It remains to be specified, however, if other factors of confusion than those sought by the authors are not likely to explain these results. The melatonin hypothesis has also been advanced in epidemiological studies that report a potential link between exposure to an electromagnetic field of 50–60 Hz and the incidence of diseases, depression, and cancers [114]. However, recent work on workers exposed daily for 1–20 years to the magnetic field in their work and home shows that this exposure does not result in alterations in melatonin secretion, which refutes the so-called advanced melatonin hypothesis as a biological explanation of the supposed effects of magnetic fields on human health.

Melatonin and Autism

Research into the circadian secretion of melatonin in autism seems all the more interesting as abnormalities in the circadian rhythm of cortisol have been described in children with autism [115]. Studies have investigated the production of melatonin in autistic disorders, either by measuring serum or plasma levels or by measuring urinary excretion or that of its major metabolite, 6-MS [116]. Early studies, although limited by the small size of the samples, all reported abnormalities in melatonin production. Thus, authors [117], whose study involved ten young adults with autism, showed an increase in the diurnal secretion of urinary melatonin without a decrease in nocturnal secretion. In another study [118], it was found an abolition of the circadian difference in melatonin secretion in a 15-year-old autistic patient with hypomelanin. A research team who studied the circadian rhythm of plasma melatonin in ten young men with autism observed that plasma melatonin levels were higher during the day and lower during the night than those of control subjects [119].

In a group of 14 children with autism compared to children with normal development, it was found significantly lower mean serum melatonin concentrations, especially at night, with 24-hour stable melatonin concentrations, thus an abolition of circadian secretion difference, for 10–14 children, and, for the other 4, an increase in diurnal secretion resulting in an inversion of the nictemeral rhythm of melatonin [120]. It is remarkable that none of the autistic patients recruited by this team had a normal circadian melatonin secretion rhythm. On the other hand, it was reported a decrease in diurnal plasma melatonin in a large group of 43 autistic patients [121]; in 2010, later it was showed a decrease in urinary excretion of 6-MS over 24 hours in 10 autistic patients [122].

Melatonin dosing at bedtime improves sleep disturbances in young blind [123] or children with Angelman syndrome [124]. In the context of pervasive developmental disorders, it was investigated the treatment of sleep disorders in Rett's syndrome [125] and the treatment of these disorders in Asperger's syndrome [126, 127]. Melatonin treatment decreases sleep latency but does not change the number of nocturnal awakenings in Rett's syndrome, while it improves both in Asperger's syndrome. Some studies in autism report a therapeutic effect of melatonin on sleep disorders observed in autistic patients, with very few side effects.

Mainly melatonin is used in the autism spectrum disorder (ASD) to improve sleep disorder. A systematic review found that, when measured,

levels of melatonin or melatonin derivatives are often below average in individuals with ASD compared with healthy individuals. Furthermore, the physiology of melatonin is abnormal in many individuals with ASD, which in some cases correlates to ASD symptoms. Some individuals have abnormalities in genes involved in melatonin production or receptor function. This meta-analysis found that the use of melatonin in ASD is associated with significantly improved sleep parameters (sleep duration and sleep onset latency) [128].

Melatonin and Irritable Bowel

Melatonin relieves abdominal pain in patients with both irritable bowel syndrome and sleep disorders, according to a placebo-controlled study [129]. The study included 40 patients who received a randomized, double-blind dose of 3 mg melatonin before bedtime or placebo for 2 weeks. It showed that melatonin significantly reduced the average score of abdominal pain – from 2.35 against only 0.7 in the placebo group – and increased the average threshold of rectal pain, from 8.9 mm Hg, compared with a decrease of 1.2 mm Hg in the placebo group. There was no significant difference between the two groups in terms of bloating, stool type, and frequency, as well as anxiety and depression scores. In patients with both irritable bowel syndrome and sleep disorders, administration of 3 mg melatonin before bedtime for 2 weeks significantly attenuated abdominal pain and reduced the sensitivity of rectal pain without improving sleep disorders or psychological suffering. These data suggest that the beneficial effects of melatonin on abdominal pain (of this type of patients) are independent of its action on sleep disorders and on psychological profiles. Sleep data obtained from both questionnaires and polysomnography showed that the 2 weeks of melatonin administration did not influence sleep-related parameters, including total sleep time, latent sleep, time latency and sleep efficiency, awakenings, the duration of stages 1–4, paradoxical sleep, and latency of paradoxical sleep [130]. More recently a paper made a review of melatonin for the treatment of irritable bowel syndrome by the same authors, without new data [131].

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22 Cytoprotection by Melatonin: The Metabolic Syndrome as an Example

Daniel P. Cardinali and Daniel E. Vigo

Introduction

A series of risk factors for cardiovascular diseases including hyperinsulinemia, glucose intolerance, dyslipidemia, obesity, and high blood pressure are known as the metabolic syndrome (MS). The prevalence of MS varies from 15% to 30% depending on the region of the world considered [1, 2]. An increase of 1.5–2.5 times in cardiovascular mortality occurs when MS is present. Therefore, MS represents one of the main public health problems nowadays.

MS is preventable. Excessive accumulation of fat, whether in white adipose tissue or in other organs, is the consequence of hypertrophy and hyperplasia of white adipocytes in a context of positive energy balance. Poor diet, lack of exercise, and chronic resistance to insulin are important factors that contribute to excessive fat accumulation [3, 4]. Abnormal nutritional balance is controlled mainly at the hypothalamic level by a complex circuit of orexigenic and anorexigenic signals and by an endogenous clock that establishes a circadian rhythm for appetite-satiety, a function highly affected by modern lifestyle habits.

In the last decade, there has been a considerable increase in our understanding of the cellular and molecular factors that contribute to MS. A basic function that seems to be strongly influenced by (and influences) obesity and metabolic disease is the internal timing system [5–7]. The correlation between the higher incidence of obesity and the ubiquity of modern social habits, such as light at night, unusual meal times, irregular sleep/wake schedules, and travel across different time zones, all encompassed in the "24/7" lifestyle of the modern society, strongly suggests that sleep deterioration and circadian system disruption are involved in the etiology of MS. Several clinical surveys have shown a higher prevalence of MS among night shift workers, indicating that artificial lighting may contribute to the higher prevalence of metabolic disorders [8–11].

Both animal and human data have shown that circadian and sleep disturbances lead to insulin resistance and MS. In a study conducted in 593 patients with type 2 diabetes mellitus, sleep debt was associated with long-term metabolic disruption, which may promote the progression of the disease. For every 30 min of sleep debt during a week, the risk of obesity and insulin resistance at 12 months increased by 18 and 41%, respectively

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[12]. Taken together, these findings indicate the need to improve the quality of sleep to prevent the development of obesity and insulin resistance, as well as its progression to diabetes.

As a chronobiotic/cytoprotective agent, melatonin may occupy a special place in the prevention and treatment of MS [13, 14]. Melatonin improves sleep efficiency and has antioxidant and anti-inflammatory properties, in part because of its function as a metabolic regulator and mitochondrial protector [15–17]. This review summarizes what is known about this subject. Medical literature was identified by searching databases including (MEDLINE, EMBASE), bibliographies from published literature, and clinical trial registries/databases. Searches were last updated on January 12, 2019.

Inflammation and the MS

Obesity in MS is associated with low-grade inflammation of white adipose tissue, which can subsequently lead to insulin resistance, impaired glucose tolerance, and diabetes [18, 19]. Adipocytes actively secrete proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, resistin, and leptin, which triggers a vicious circle that leads to additional deposition of fat. The increase in circulating levels of C-reactive protein and other inflammatory biomarkers also indicate the occurrence of inflammation in obesity [20, 21].

Early MS is characterized by an increase in systemic markers of lipid oxidation, such as oxidized low-density lipoproteins, which contribute to the development of insulin resistance [22–24]. In the mitochondria, lipid peroxidation particularly affects cardiolipin, a phospholipid located at the level of the inner mitochondrial membrane, which is required for several mitochondrial bioenergetic processes, as well as in mitochondria-dependent apoptosis steps, e.g., it prevents the opening of the transition pore of mitochondrial permeability [25].

Several studies have shown that the altered production of proinflammatory cytokines in obesity modulates the size and number of adipocytes through paracrine mechanisms that play an important role in the regulation of fat mass [26–28]. The amounts of proinflammatory molecules derived from adipose tissue in obese patients decrease after weight loss [29]. Therefore, fat cells are both a source and a target for proinflammatory cytokines. When the mass of white adipose tissue increases due to hypertrophy of the adipocyte, large adipocytes develop a secretory dysfunction characterized by overproduction (synthesis and release) of proinflammatory adipocytokines that decrease the sensitivity of the tissue to insulin and promote oxidative stress (leptin, resistin, TNF-α, inhibitor of plasminogen activator-1, IL-1, IL-6). In addition, adipocytes release less amounts of adiponectin (an insulin-sensitizing adipocytokine). Consequently, obesity is the result of a multifactorial combination of genetic, metabolic, endocrine, inflammatory, and circadian dysfunctions, whose long-term maintenance is favored by behavioral disorders [30].

Levels of inflammatory mediators generally increase with age, even in the absence of acute infection or physiological stress. Such stress leads to the inflammatory damage of cellular components, including proteins, lipids, and DNA, and contributes to the decline of physiological functions related to age. The term “inflammaging” was introduced to underscore the importance of inflammation in senescence and its role in the development of age-related diseases such as MS [31–34]. The type of persistent oxidative stress that progresses slowly, as a result of increased production of reactive oxygen and nitrogen species and which is reinforced by damage to the mitochondria, is typical of inflammation [34–36].

Basic Biology of Melatonin Relevant for Cytoprotection in MS

Melatonin, an unusually phylogenetic conserved molecule present in all known aerobic organisms, is effective both as a chronobiotic and as a cytoprotective agent. “Chronobiotic” defines a drug that can synchronize and increase the amplitude

of circadian rhythms, with melatonin being the prototype [37, 38].

The light-dark variation in the synthesis of melatonin by pinealocytes is the essential fact that explains the role of melatonin as a chronobiotic that coordinates the physiology of biological rhythms [39]. The action of melatonin as a chronobiotic is twofold: on the one hand, it "opens the doors of sleep" by inhibiting the promoting activity of late awakening driven by suprachiasmatic nuclei (SCN) [40, 41]. On the other hand, melatonin is the "hormone of darkness," a chemical code of the duration of the night, and has established itself as crucial in the transmission of information from light to the neuroendocrine system. Melatonin represents a "hand" of the biological clock in the sense that it responds to the signals of the SCN, the temporal variation of the melatonin rhythm indicating the state of the clock, both in terms of phase (time in the internal clock in relation to external time) and amplitude [42].

In all mammals, circulating melatonin is derived almost exclusively from the pineal gland [43]. In addition, melatonin is synthesized locally in many cells, tissues, and organs, including the lymphocytes, bone marrow, thymus, gastrointestinal tract, skin, and eyes, where it can play an autocrine or paracrine role [44]. In both animals and humans, melatonin participates in diverse physiological functions that not only indicate the duration of the night but also improve the elimination of free radicals and the immune response, showing relevant cytoprotective properties [45].

The chronobiotic action of melatonin is mediated via the melatonin receptors, which have been identified both in the CNS and in the periphery [46]. MT_1 and MT_2 receptors all belonging to the superfamily of membrane receptors associated with G proteins (G-protein-coupled receptors, GPCR) have been cloned. These receptors mediate melatonin inhibition of adenylate cyclase (and in the case of the MT_2 receptor, guanylate cyclase) and participate in the action of melatonin on the phase and amplitude of circadian rhythms. By using receptor autoradiography with the nonselective 2-[^{125}I]-iodomelatonin ligand and real-time quantitative reverse transcription-polymerase chain reaction to label melatonin receptor mRNA, MT_1 and MT_2 receptors have been identified in the retina, SCN, thalamus, hippocampus, vestibular nuclei, and cerebral and cerebellar cortex. At the level of the hippocampus, MT_2 receptors were detected in CA3 and CA4 pyramidal neurons, which receive glutamatergic excitatory inputs from the entorhinal cortex, whereas MT_1 receptors were predominantly expressed in CA1 [47].

More recently, another member, GPR50, was included in the melatonin receptor subfamily. GPR50 shows high-sequence homology to MT_1 and MT_2 but does not bind to melatonin or any other known ligand. An interesting feature of these receptors is their capacity to form homo- and heteromers between each other and also with other GPCRs [48]. The following heteromers have been described: MT_1/MT_2, MT_1/GPR50, and heteromers composed of MT_2 and the serotonin 5-HT_{2c} receptor or the orphan GPR61, GPR62, and GPR135 receptors. These heteromers represent novel pharmacological entities as they exhibit functional properties that are different from those of the corresponding homomers. For example, MT_2/5-HT_{2c} heteromers are targeted by the clinically relevant antidepressant agomelatine, and MT_1/MT_2 heteromers regulate nocturnal retinal light sensitivity [49].

Due to its liposolubility, melatonin penetrates all membranes and is associated with cytoplasmic proteins such as calmodulin and tubulin, which cause important changes in the cytoskeleton [50]. Melatonin also accesses the cell nucleus where the receptor sites were supposed to belong to the orphan receptor superfamily RZR/ROR [45]. However, RZR/ROR demonstrably does not bind melatonin. Rather, melatonin may act indirectly via this transcription factor, e.g., by affecting the circadian accessory oscillator component RORα through sirtuin-1 activation [51].

The cytoprotective activity of melatonin exceeds that mediated via receptors. Almost every cell in the human body contains melatonin, in quantities much higher than those circulating in blood derived from the pineal gland [44]. The mitochondrial capacity to synthesize melatonin is now confirmed, but for reasons that remain

unexplained, intracellular melatonin does not get the extracellular space. To modify intracellular melatonin levels, doses much higher than those employed as a chronobiotic are needed [52, 53].

In cell cultures, physiologically relevant effects of melatonin are revealed at doses in the range of 10^{-8} to 10^{-9} M, these concentrations being enough for almost complete or total receptor saturation. However, most studies on neuroprotective and anti-inflammatory effects in animals employ pharmacological doses, which clearly exceed the saturation of the receptor.

In both the cytoplasm and the cell nucleus, melatonin has important antioxidant and scavenging effects on free radicals, which are largely independent of receptors [54]. These effects are exerted in three ways: (a) melatonin is a free radical scavenger; (b) melatonin is metabolized to compounds with high antioxidant activity; (c) melatonin is an indirect antioxidant, which stimulates the synthesis of antioxidant enzymes and inhibits that of prooxidant enzymes. Melatonin has a proven superiority to vitamin C and E in protection against oxidative damage and in the elimination of free radicals [55]. In addition, melatonin potentiates the effects of other antioxidants, such as vitamin C, Trolox, and NADH. Several antiapoptotic and cytoprotective effects of melatonin are exerted under conditions of ischemia (unrelated to free radicals) and can be attributed to the stabilizing activity of the mitochondrial membrane [53].

Melatonin is an immunological modulator that shows proinflammatory and anti-inflammatory properties [56, 57]. The anti-inflammatory actions are of medicinal interest, since they are observed in high-grade inflammation such as sepsis, ischemia/reperfusion, and brain injury, as well as in the low-grade inflammation seen in neurodegenerative disorders and aging. Melatonin has significant anti-inflammatory properties presumably by inhibiting the binding of nuclear factor-κB (NF-κB) to DNA, thus decreasing the synthesis of proinflammatory cytokines, by inhibiting cyclooxygenase (Cox) [58] in particular Cox- 2 [59], and by suppressing the expression of the inducible gene of nitric oxide synthase (iNOS) [60]. In addition, other pathways of secondary signaling are involved, including inhibition of high-mobility group box-1 signaling and toll-like receptor-4 activation, prevention of inflammasome NLRP3 activation, and upregulation of nuclear factor erythroid 2-related factor 2. These effects of melatonin are also reflected by downregulation of proinflammatory and upregulation of anti-inflammatory cytokines. A particular role in the actions of melatonin is associated with the positive regulation of sirtuin-1, which shares several known effects of melatonin and also interferes with proinflammatory signaling [57].

Melatonin and Inflammation

Reversal of inflammation by melatonin occurs at different levels. One of them is the correction of metabolic dysregulation, including the prevention of insulin resistance. Melatonin reduces serine phosphorylation of the insulin receptor substrate 1 (IRS-1) and upregulates IRS-1 expression [61]. Both melatonin and the melatonergic agonist piromelatine reverse the blocking of this key step in the transduction of insulin signals [62–64].

An additional level of action of melatonin refers to the prevention of processes that favor or lead to inflammation. These include calcium overload and excessive release of nitric oxide (NO) that result in peroxynitrite formation, peroxynitrite-derived free radicals, tyrosine nitration, and mitochondrial dysfunction [16]. It is known that all these changes initiate low-grade inflammation in various organs.

The immunological effects of melatonin represent a third relevant area for inflammation. The multiple functions of melatonin as a modulating immune agent comprise proinflammatory and anti-inflammatory actions (acting as a "buffer" of the immune system) [16, 36, 56]. In the liver of aged and ovariectomized female rats (an animal model of MS), melatonin downregulates proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, and upregulates the anti-inflam-

matory cytokine IL-10 [65]. Reductions of TNF-α and IL-1β and increased levels of IL-10 were observed in the liver [66], pancreas [67], and heart [68] of the accelerated senescence mouse strain SAMP8.

Peripheral oscillators exist in cells relevant to MS, such as pancreatic β-cells [69], hepatocytes, adipocytes, cardiomyocytes [70], and leukocytes [71, 72]. In all these cell types, melatonin modulates factors involved in metabolic sensing of the circadian apparatus like peroxisome proliferator-activated receptor-γ coactivator-1α, peroxisome proliferator-activated receptor-γ, phosphoinositide 3-kinase, protein kinase B, and the accessory oscillator components AMPK, nicotinamide phosphoribosyltransferase, and sirtuin 1 [36, 73]. Melatonin reduced proinflammatory factors by suppressing the expression of nuclear factor-κB via recruitment of a histone deacetylase to its promoter [74]. Other aspects of epigenetic modulation by melatonin via circadian oscillators have been recently summarized [75].

Evidence for the Therapeutic Value of Melatonin in the MS: Animal Studies

Treatment with melatonin in rats can reduce obesity, type 2 diabetes, and hepatic steatosis [76, 77]. In several animal models of hyperadiposity, the injection of melatonin could normalize most of the observed alterations and correct the altered biochemical proinflammatory profile (Table 22.1).

In addition, melatonin is effective in animal models of ischemic and nonischemic heart failure, an important comorbidity of MS (Table 22.2). From the doses of melatonin used in these different animal models, the human equivalent dose of melatonin for a 75 kg adult was calculated by normalization of body surface area [78] (Tables 22.1 and 22.2). Body surface area correlates well across several mammalian species with several parameters of biology, including oxygen utilization, caloric expenditure, basal metabolism, blood volume, circulating plasma proteins, and renal function, and has been advocated as a factor to be used when converting a dose for translation from animals to humans [78]. Noteworthy, theoretical human equivalent doses calculated from Tables 22.1 and 22.2 results ranged from 2 to 3 orders of magnitude greater than those employed in humans.

Melatonin treatment of streptozotocin-induced type 1 diabetic rats induces the regeneration and proliferation of β-cells in the pancreas leading to a decrease in blood glucose [143]. Loss of melatonin in circulation after pinealectomy results in hyperinsulinemia and accumulation of triglycerides in the rat liver [144]. The long-term administration of melatonin improves lipid metabolism in type 2 diabetic rats via restoring insulin sensitivity [145]. Melatonin treatment increases glycogen content in the liver of rats [146], while in high-fat diet-induced diabetic mice, the intraperitoneal injection of 10 mg/kg melatonin improved glucose utilization and insulin sensitivity and ameliorated hepatic steatosis [147].

As shown in Table 22.1, melatonin administration is usually very effective in reversing hyperadiposity in animal models of MS. The reasons for the decrease in body weight after melatonin in the absence of significant differences in food intake is worthy to be explored. A key piece of evidence in this regard is the observation that melatonin plays a role in seasonal changes in adiposity by increasing the activity of the sympathetic nervous system innervating white and brown fat [148]. Melatonin not only affects white adipose tissue but also increases the recruitment of brown adipocytes and increases their metabolic activity in mammals [149–151]. It was speculated that the hypertrophic effect and functional activation of brown adipose tissue induced by melatonin can likely be applied to treatment of human obesity.

Table 22.2 summarizes the effect of melatonin on animal models of ischemic and nonischemic heart disease. One of the first observations was derived from this laboratory. In a model of rat myocardial infarction (by ligation of the left anterior descending coronary artery for 3 h

Table 22.1 Effect of melatonin on animal models of MS. The human equivalent dose (HED) of melatonin for a 75 kg adult is calculated by normalization of body surface area [78]

Findings	Melatonin dose	Daily HED for a 75 kg adult	Ref
In rats fed from weaning with a high-fat diet, melatonin decreased body weight gain, feed efficiency, and plasma glucose, leptin, and triglyceride levels	30 mg/kg/day p.o.	365 mg	[79]
In high-fat diet-fed mice, melatonin improved insulin sensitivity and glucose tolerance	100 mg/kg/day p.o.	610 mg	[80]
In ovariectomized rats, melatonin was effective to reduce obesity	2–3 mg/kg p.o.	25–36 mg	[81–83]
In olanzapine-treated rats, melatonin was effective to reduce obesity	0.05 mg/kg p.o.	0.6 mg	[84]
Melatonin and its analog piromelatonin inhibited weight gain and improve insulin sensitivity in high-fat-fed rats	5 mg/kg p.o.	60 mg	[62]
In high-fat-fed rats, melatonin attenuated body weight increase, the increase in plasma glucose, insulin, adiponectin, leptin, triglycerides, and cholesterol levels, and counteracted disrupted 24-h patterns	2.3 mg/kg p.o.	25 mg	[85]
Melatonin improves inflammation processes in the liver and pancreas of senescence-accelerated prone male mice (SAMP8)	1 mg/kg p.o.	6 mg	[66, 86]
Melatonin reduced body weight gain, visceral adiposity, blood triglyceride, insulin levels, and TBARS under a high-calorie diet in rats.	4 mg/kg p.o.	48 mg	[87]
In young male Zucker diabetic fatty rats, melatonin treatment reduced mean weight gain without affecting food intake, decreased in a nonsignificant way blood pressure, and improved dyslipidemia	10 mg/kg p.o.	120 mg	[88]
Melatonin improves MS induced by high fructose intake in rats without affecting food intake	2.3–20 mg/kg p.o.	25–120 mg	[89–93]
Melatonin and its analog piromelatonin reduced blood pressure in spontaneously hypertensive rats	5 mg/kg p.o.	60 mg	[94]
Melatonin prevents the development of the MS in male rats exposed to different light/dark regimens	120 mg/kg p.o.	1.45 g	[95]
Melatonin attenuates high-fat diet-induced fatty liver disease in rats	5–10 mg/kg p.o.	60–120 mg	[96]
Melatonin ameliorates low-grade inflammation and oxidative stress in young Zucker diabetic fatty rats	10 mg/kg p.o.	120 mg	[97]
Melatonin improves hyperglycemia, hypertriglyceridemia, polyphagia, and polydipsia in streptozotocin diabetic rats	2.5–20 mg/kg p.o.	25–240 mg	[98, 99]
Protective effects of melatonin against metabolic and reproductive disturbances in polycystic ovary syndrome in rats	1–2 mg/kg i.p.	12–24 mg	[100]
Melatonin normalizes clinical and biochemical parameters of mild inflammation in diet-induced MS syndrome in rats	2.3 mg/kg p.o.	25 mg	[101]
Melatonin counteracts changes in hypothalamic gene expression of signals regulating feeding behavior in high-fat-fed rats	2.3 mg/kg p.o.	25 mg	[102]
Melatonin reduces obesity and restores adipokine patterns and metabolism in obese (ob/ob) mice	100 mg/kg p.o.	610 mg	[103]
Melatonin nephroprotective action in Zucker diabetic fatty rats involves an inhibitory effect on NADPH oxidase	2.5 mg/kg p.o.	25 mg	[104]
Melatonin prevents type 2 diabetes in high-carbohydrate diet-fed male Wistar rats	0.8 mg/kg p.o.	10 mg	[105]
Melatonin decreases fasting blood glucose, total cholesterol, LDL levels, and MDA levels and restores the vascular responses and endothelial dysfunction in diabetic, high-fat diet-fed rats	10 mg/kg p.o.	120 mg	[106]
Maternal melatonin supplementation during murine diabetic pregnancy improves the tolerance to myocardial ischemia/reperfusion injury in the offspring, via restoring cardiac IRS-1/Akt signaling	10 mg/kg p.o.	60 mg	[107]

Table 22.1 (continued)

Findings	Melatonin dose	Daily HED for a 75 kg adult	Ref
In rats with diet-induced obesity exposed to circadian disruption, treatment with melatonin alone or in combination with metformin modifies progression of metabolic dysfunction through improved adiposity, circadian activity, insulin sensitivity, and islet cell failure	20 mg/kg p.o.	240 mg	[108]
Melatonin prevents nonalcoholic fatty liver disease in high-fat diet-induced obese mice by decreasing body weight and reducing inflammation via modulation of the MAPK-JNK/P38 signaling pathway	10 mg/kg p.o.	60 mg	[109]
Melatonin reverses liver apoptosis, mainly through intrinsic pathway and reversed endoplasmic reticulum stress and mitochondrial function in rats subjected to bile duct ligation	400 mg/kg i.p.	4.85 g	[110]
Melatonin reduces body weight, liver steatosis, and low-grade inflammation and improves insulin resistance and gut microbiota in high-fat diet-fed mice	50 mg/kg p.o.	300 mg	[111]
The increased food intake, water consumption, hyperglycemia, glucose intolerance, and insulin resistance in T2DM rats were all improved by melatonin or Neu-P11 treatment Treatment increased glucocorticoid receptor expression and suppressed 11β-hydroxysteroid dehydrogenase 1 activity in the hippocampus by enhancing glucocorticoid sensitivity and HPA feedback	20 mg/kg p.o.	240 mg	[112]
Using mice fed a high-fat diet (HFD) as an obesity model, spindle disorganization, chromosome misalignment, and elevated reactive oxygen species (ROS) levels were documented in oocytes from obese animals. Melatonin administration not only reduces ROS generation but prevents spindle/chromosome anomalies in oocytes, through the SIRT3-SOD2-dependent mechanism consequently promoting the developmental potential of early embryos	30 mg/kg p.o.	180 mg	[113]
Oral supplementation with melatonin reduces oxidative damage and concentrations of inducible nitric oxide synthase, VEGF, and matrix metalloproteinase 9 in the retina of rats with streptozotocin/nicotinamide-induced prediabetes	0,32 mg/kg p.o.	4 mg	[114]
Melatonin counteracted oxidative damage, inflammation, and apoptotic cell death in lung tissue of diabetic rats	20 mg/kg p.o.	240 mg	[115]
Melatonin improves the therapeutic role of mesenchymal stem cells on glucose, insulin, total antioxidant, and malondialdehyde level in diabetic rats	10 mg/kg p.o.	120 mg	[116]
Melatonin improves insulin resistance and hepatic steatosis through attenuation of alpha-2-HS-glycoprotein in high-fat diet mice. It reduced body weight gain and improved insulin sensitivity and glucose intolerance by the upregulation of muscle p-AKT protein expression. ER stress markers in the liver and serum of HFD mice were decreased by melatonin treatment	100 mg/kg p.o.	1.2 g	[117]
In diabetic rats melatonin prevented fluorescein leakage and oxidative damage seen in the retina	20 mg/kg p.o.	240 mg	[118]

before), melatonin reduced 86–87% of the area of injury and 75–80% of the number of injured myocardial areas [119]. Several studies indicated the efficacy of melatonin to reduce cardiac damage markers, to augment cardiac antioxidant defense system, and to normalize the lipid profile in rats [120–124, 126, 127] and mice [129, 130, 152]. The same was observed in cardiomyopathy induced by streptozotocin [133] or doxorubicin [135]. In a murine model of myocardial infarction treated with cardiac progenitor cells, exposure of cells to melatonin enhances therapeutic efficacy of cardiac progenitor cells for myocardial infarction [137]. The human equivalent dose (HED) of melatonin for a 75 kg adult calculated by normalization of body surface area [78] of Table 22.2's data yielded values greater than 60 mg/day.

Collectively, the results indicate that the administration of melatonin effectively counteracts some of the disrupting effects seen in diet-induced obesity in animal insulin resistance, dyslipidemia, and obesity and the consequences of ischemic and nonischemic heart disease.

Table 22.2 Effect of melatonin on animal models of ischemic and nonischemic heart disease. The human equivalent dose (HED) of melatonin for a 75 kg adult is calculated by normalization of body surface area [78]

Findings	Melatonin dose	Daily HED for a 75 kg adult	Ref
In a model of rat myocardial infarction (by ligation of the left anterior descending coronary artery for 3 h before), melatonin reduced 86–87% of the area of injury and 75–80% of the number of injured myocardial areas	6 mg/kg/day p.o.	70 mg	[119]
In a rat model of isoproterenol-induced myocardial infarction, melatonin reduced cardiac damage markers, augmented cardiac antioxidant defense system, and normalized the lipid profile	10 mg/kg /day i.p.	120 mg	[120]
In a rat model of severe obstructive sleep apnea, melatonin was cardioprotective by decreasing BP, oxidative stress, endothelial dysfunction, and inflammation	10 mg/kg/day i.p.	120 mg	[121]
In a rat model of myocardial infarction-induced heart failure, melatonin augmented cardiac activities of Na^+, K^+-ATPase, and SERCA, content of glutathione, and levels of caveolin-3 and reduced lactate dehydrogenase and creatine kinase, lysosomal enzymatic activities, and cardiac malondialdehyde and myeloperoxidase	10 mg/kg/day i.p.	120 mg	[122]
In a rat model of hypoxic pulmonary hypertension with intermittent hypoxia, melatonin decreased right ventricular systolic pressures, the weight ratio RV/LV + S, pulmonary vascular structure remodeling, and several signals involved in proliferation of primary pulmonary artery smooth muscle cells	15 mg/kg/day i.p.	180 mg	[123]
In a rat model of isoproterenol-induced heart failure, melatonin decreased cardiac fibrosis, oxidative stress, insoluble and total collagen, and the alteration of beta-tubulin in the left ventricle	10 mg/kg/day p.o.	120 mg	[124]
In a rat model of arterial hypertension induced by continuous light for 6 weeks, melatonin was cardioprotective by decreasing cardiac fibrosis and oxidative stress but with no effect on left ventricle hypertrophy	10 mg/kg/day p.o.	120 mg	[125]
In a rat model of pulmonary hypertension induced by monocrotaline, melatonin exerted cardioprotection both curative and preventive by decreasing right ventricular hypertrophy, systemic oxidative stress, and cardiac interstitial fibrosis	6 mg/kg/day p.o.	70 mg	[126]
In a rat model of myocardial infarction, intramyocardial injection of melatonin before mesenchymal stem cell transplantation was cardioprotective by augmenting catalase and Cu/Zn superoxide dismutase, as well modulating proangiogenic and mitogenic factors and apoptosis	Intramyocardial injection of 5 μM for 24 h before stem cells	–	[127]
In a murine model of myocardial infarction, intramyocardial injection of melatonin before mesenchymal stem cell transplantation was cardioprotective by augmenting Sirt1 signaling and the expression of antiapoptotic protein Bcl2 and by reducing Ac-FoxO1, acetylated-p53, Ac-NF-κB, and Bax	Intramyocardial injection of 5 μM for 24 h before stem cells	–	[128]
In a murine model of postinfarction, cardiac remodeling, and dysfunction, melatonin ameliorated cardiac dysfunction, adverse left ventricle remodeling, autophagy, apoptosis, and mitochondrial dysfunction	20 mg/kg/day p.o.	120 mg	[129]
In a murine model of myocardial infarction, melatonin was cardioprotective by reducing post-myocardial infarction damage, Notch1 signaling, and Mfn2 expression via melatonin receptors	10–20 mg/kg/day i.p.	60–120 mg	[130]
In a murine model of myocardial infarction (ligation of the left anterior descending coronary artery for 5 days), melatonin decreased infarction damage by augmenting PGC-1α and Tom 70 expression, preserving mitochondrial integrity, and decreasing ROS production	10–20 mg/kg/day i.p.	60–120 mg -	[131]

Table 22.2 (continued)

Findings	Melatonin dose	Daily HED for a 75 kg adult	Ref
In a murine model of pathological cardiac hypertrophy, melatonin reduced pulmonary congestion, cardiac fibrosis, and the deterioration of cardiac contractile function	20 mg/kg/day p.o.	120 mg	[132]
In a model of rat diabetes mellitus, melatonin protects against streptozotocin-induced diabetic cardiomyopathy by the phosphorylation of vascular endothelial growth factor-A	50 mg/kg/day i.p.	600 mg	[133]
In rats subjected to cardiac ischemia by coronary artery ligation for 30 min and reperfusion for 2 h, melatonin attenuated myocardial ischemia/reperfusion injury by inhibiting autophagy via an AMPK/mTOR signaling pathway	20 mg/kg i.p.	120 mg	[134]
In a rat model of doxorubicin-induced cardiotoxicity, melatonin improves cardiac and mitochondrial function via peroxisome proliferator-activated receptor gamma coactivator-1α and sirtuin activity	6 mg/kg/day p.o.	70 mg	[135]
In a murine model of heart failure with preserved ejection fraction, melatonin improves cardiac function	50 mg/kg/day p.o.	300 mg	[136]
In a murine model of myocardial infarction (ligation of the left anterior descending coronary artery) treated with cardiac progenitor cells, exposure of cells to melatonin enhances therapeutic efficacy of cardiac progenitor cells for myocardial infarction	Exposure to 10 μM and 100 μM melatonin		[137]
In a mouse model of myocarditis infected with coxsackievirus B3, melatonin counteracted effectively myocardial injuries	14.4 mg/kg/day i.p.	88 mg	[138]
In a murine model of diabetic cardiomyopathy, melatonin activates Parkin translocation and rescues the impaired mitophagy activity through Mst1 inhibition	20 mg/kg i.p.	120 mg	[139]
In a rat model of overload-induced ventricular hypertrophy caused by abdominal aortic constriction, melatonin prevented the changes in cardiac fibrosis and in gene expressions of HDAC1, HDAC2, HDAC3, and HDAC4 in cardiomyocytes	10 mg/kg i.p.	60 mg	[140]
In a murine chronic pain induced by spared nerve injury model followed by myocardial ischemia-reperfusion, melatonin attenuated chronic pain-related myocardial ischemic susceptibility through inhibiting RIP3-MLKL/CaMKII-dependent necroptosis	20 mg/kg i.p.	120 mg	[141]
In a rat model of cardiac ischemia/reperfusion after ligation of descending coronary artery, melatonin treatment maintained myocardial function and cardiomyocyte viability, and these effects were highly dependent on mitochondrial fusion/mitophagy	20 mg/kg i.p.	120 mg	[142]

Evidence for the Therapeutic Value of Melatonin in the MS: Clinical Studies

Type 2 diabetic patients have low circulating levels of melatonin [153] with a simultaneous and expected regulation of mRNA expression of the melatonin membrane receptor [154]. In addition, allelic variants for melatonin receptors were associated with an increase in fasting blood glucose levels and/or an increased risk of type 2 diabetes [155–157] and with the polycystic ovarian syndrome [158]. These findings strongly bind melatonin to the homeostasis of blood glucose.

Patients with coronary artery disease show a decrease in melatonin secretion [159–162], and among elderly hypertensive patients, nocturnal urinary melatonin excretion was inversely associated with the non-dipper pattern of hypertensive disease [163]. As summarized in Table 22.3, melatonin administration proved capable of reducing nocturnal blood pressure in hypertensives [164–167] and attenuated age-dependent disturbances of cardiovascular rhythms [168].

Table 22.3 Studies including treatment of MS patients with melatonin

Findings	Melatonin dose	Ref
Melatonin treatment decreases nocturnal BP in type 1 adolescent diabetics	5 mg/day p.o.	[169]
Melatonin treatment decreases high nocturnal BP in hypertensives	5 mg/day p.o.	[167]
	2.5 mg/day p.o.	[165]
	3 mg/day p.o.	[164]
	2 mg/day p.o.	[166]
Melatonin administration attenuates age-dependent disturbances of cardiovascular rhythms	1.5 mg/day p.o.	[168]
Melatonin treatment prevents catecholamine-induced hypercoagulability in normal volunteers	3 mg/day p.o.	[170]
Melatonin attenuates the tachycardia and improves symptom burden in patients with postural tachycardia syndrome	3 mg/day p.o.	[171]
Melatonin increases antioxidant defenses and attenuates cellular damages resulting from coronary artery bypass grafting surgery	10 mg/day p.o.	[172]
Melatonin treatment in the perioperative period decreased clinical cardiac morbidity and the occurrence of myocardial ischemia after abdominal aortic aneurism repair	50 mg melatonin i.v. intraoperatively, and 10 mg melatonin/day p.o.	[173]
Melatonin increases attenuate myocardial ischemia/reperfusion injury resulting from coronary artery bypass grafting surgery	10 and 20 mg/day p.o.	[174]
Melatonin limits the ischemia reperfusion injury and improves the efficacy of mechanical reperfusion with primary percutaneous coronary intervention in ST-segment elevation myocardial infarction treated up to 2 h of symptom onset	Melatonin 51.7 μmol i.v. for 60 min before percutaneous coronary intervention and an intracoronary bolus of 8.6 μmol within the first 60 sec of reperfusion	[175]
Melatonin did not improve the myocardial salvage index after primary percutaneous coronary intervention in patients with ST elevation myocardial infarction	Melatonin 50 mg i.v. immediately after angioplasty	[176]
Melatonin treatment ameliorates MS in obese patients	5 mg/day p.o.	[177]
	8 mg/day p.o.	[178]
	5 mg/day p.o.	[179]
	6 mg/day p.o.	[180]
	1 or 3 mg/day p.o.	[181]
	10 mg/day p.o.	[182]
	3 mg/day in the morning and 5 mg/day at bedtime p.o.	[183]
Melatonin treatment improves MS after treatment of bipolar and schizophrenic patients with second-generation antipsychotics	5 mg/day p.o.	[184]
	3 mg/day p.o.	[185]
	3 mg/day p.o.	[186]
Melatonin administration normalizes MS in elder hypertensive patients	3 mg/day p.o.	[187]
	5 mg/day p.o.	[188]
Melatonin treatment improves enzymatic profile in patients with nonalcoholic liver steatosis	5 mg/day p.o. twice daily	[189]
	5 mg/day p.o. twice daily	[190]
	5 mg/day p.o. twice daily	[191]
		[183]
The combination of melatonin and zinc acetate, when used alone or in combination with metformin, improved glycemic control in type 2 diabetic patients	10 mg/day p.o. twice daily	[192]
Treatment of preinvasive endometrial cancer in women with PCOS using female sexual hormones in combination with melatonin, antidiabetic, antidopaminergic, and anti-serotonin therapy favorably influenced female sexual hormone profile and lipid metabolism and caused the restoration of normal endometrium	5 mg/day p.o.	[193]
Melatonin improves menstrual irregularities and biochemical hyperandrogenism in women with PCOS through a direct, insulin-independent effect on the ovary	2 mg/day p.o.	[194]

A meta-analysis of randomized controlled trials suggests that a controlled release preparation of melatonin is effective and safe to improve nocturnal hypertension [195]. As a pleiotropic molecule, melatonin can exert its antihypertensive and anti-remodeling effects through its antioxidant and scavenging properties, preserving the availability of nitric oxide and having sympathoplegic effects that provide cardiovascular protection in MS.

Resembling animal experiments, clinical studies have shown that melatonin improves lipid profiles in MS patients. Melatonin treatment (1 mg daily for 30 days) increased levels of high-density lipoprotein-cholesterol in peri- and postmenopausal women [196]. Several mechanisms may explain the hypolipidemic effects of melatonin, such as reduced intestinal absorption of cholesterol [197] or an inhibited cholesterol biosynthesis [198].

The hypercoagulability induced by catecholamine in acute stress (that contributes to thrombus growth after rupture of the coronary plaque) was attenuated by the administration of melatonin [170]. This was probably mediated by the reported inhibitory effects of melatonin on platelet aggregation [199–201]. Taking these results into account, melatonin may have a protective effect in reducing atherothrombotic risk in MS.

Several studies support the beneficial role of melatonin in patients with MS (Table 22.3). Treatment with melatonin improves MS in obese patients [177, 178], as well as in bipolar and schizophrenic patients after treatment with second-generation antipsychotics [184–186]. The administration of melatonin normalizes MS in elderly hypertensive patients [187] and improves the enzyme profile in patients with alcoholic hepatic steatosis [190, 191]. The combination of melatonin and zinc acetate, when used alone or in combination with metformin, improved the glycemic control in type 2 diabetic patients [192], and an inverse relationship between the urinary excretion of 6-sulfatoxymelatonin and insulin levels and insulin resistance was reported in healthy women in the Nurses' Health Study cohort [202].

Polycystic ovary syndrome (PCOS) is a highly frequent reproductive-endocrine disorder affecting up to 8–10% of women worldwide at reproductive age. Although its etiology is not fully understood, evidence suggests that insulin resistance, with or without compensatory hyperinsulinemia, and hyperandrogenism are very common features of the PCOS phenotype. Dysfunctional white adipose tissue has been identified as a major contributing factor for insulin resistance in PCOS.

Overweight and/or obesity is very common in these women, thus suggesting that PCOS and MS female phenotypes share common characteristics. Sleep disturbances have been reported to double in women with PCOS, and obstructive sleep apnea is a common feature in PCOS patients [203].

Genetic variants in melatonin receptor have been linked to increased risk of developing PCOS, to impairments in insulin secretion, and to increased fasting glucose levels.

Melatonin may directly affect ovarian function: it is concentrated in human ovarian follicles relative to the level in plasma, and it alters granulosa cell steroidogenesis and follicular function in humans. However, only a few studies have been published on melatonin potential as a therapeutic agent in humans in the PCOS. Two of them relate to improvement of in vitro fertilization of patients with PCOS. The supplementation of in vitro culture medium with melatonin improved in vitro fertilization outcome in PCO, while melatonin and myoinositol enhanced, synergistically, oocyte and embryo quality and improved in vitro fertilization of patients with PCOS. These findings suggest that the addition of melatonin to in vitro fertilization media may improve the cytoplasmic maturation of immature oocytes.

In an open label study including 40 normal-weight women with PCOS, ultrasound pelvic examinations, hirsutism score evaluation, hormone profile assays, oral glucose tolerance test, and lipid profile at baseline and after 6 months administration of 2 mg fast release melatonin p.o. daily at bedtime were recorded [194]. Melatonin

treatment significantly decreased serum androgen and 17α-hydroxyprogesterone levels and augmented serum follicle-stimulating hormone (FSH) and anti-Mullerian hormone serum levels. Almost 95% of participants experienced an amelioration of menstrual cycle disruption. No significant changes occurred in gluco-insulinemic and lipid parameters after treatment except a significant decrease of low-density lipoprotein cholesterol [194].

Treatment of preinvasive endometrial cancer in women with PCOS using melatonin in combination with female sexual hormones and antidiabetic, antidopaminergic, and antiserotoninergic therapy favorably influenced female sexual hormone profile and lipid metabolism and caused restoration of normal endometrium [193].

In general, the results discussed above suggest that melatonin therapy may be beneficial for patients with MS, although it is evident that more studies are needed to evaluate the time/length of administration of the dose/treatment ratio of melatonin in patients with MS.

It must be noted that some results deny the capacity of melatonin to improve glucose tolerance and to reduce insulin resistance in humans. Melatonin administration decreased glucose tolerance, already in nondiabetic young individuals [202], an observation confirmed by recent studies [204, 205]. In vitro, melatonin inhibits insulin secretion, an effect that is logical in humans if one presumes that melatonin suppresses insulin during the night to sensitize the pancreatic β-cells in preparation for breakfast but is more difficult to explain in nighttime eating rodents.

Additional information concerning a glucose tolerance-reducing property of melatonin in humans came from the detection of melatonin receptor polymorphisms. To date, several single nucleotide polymorphisms (SNPs) located near or inside the gene encoding MTNR1B with an association with type 2 diabetes mellitus have been identified in Asian (Indian, Sri Lankan, Chinese, Korean, Japanese) and European ethnicities [155, 206–211]. Among these SNPs, rs10830963 appears the most strongly associated with an increase in fasting plasma glucose, glucose area under the curve, and glycated hemoglobin (HbA1C) and a decrease in pancreatic β-cell function, basal insulin secretion, and plasma insulin [212]. This G allele that carries the SNP rs10830963 is prodiabetic and is overexpressed in pancreatic β-cells, causing a more intense decrease in cyclic adenosine monophosphate (cAMP) upon melatonin stimulation and consequently suppressing more strongly the cAMP-dependent secretion of insulin [213]. It appears to affect β-cell function directly and is associated with a defective early insulin response and a decreased β-cell glucose sensitivity [213–215]. In clinical studies, the presence of the G allele worsens the decrease in glucose tolerance induced by melatonin [216].

The rs10830963 G allele may have a greater risk on the transition from normal glucose tolerance to prediabetes than on prediabetes to type 2 diabetes mellitus and is thought to have an important influence on glucose levels from childhood onward [217]. Individuals older than 45 years of age, who are carrying the rs10830963 G allele, show a higher expression of MTNR1B in pancreatic islets [213]. This has been reported in diabetic rats as well as diabetic humans. It is not known whether this is a physiological adaptive response to reduced melatonin levels or whether it is part of the pathology of diabetes. It has been proposed that an increase in MT_2 receptor expression could increase the inhibitory downstream signaling leading to an overall decrease in insulin release in type 2 diabetes mellitus [213].

Increased MTNR1B expression occurred in human islets from risk G allele carriers. Melatonin treatment reduced insulin secretion and raised glucose levels more extensively in risk G allele carriers indicating that an increased melatonin signaling may be a risk factor for type 2 diabetes [218]. Since the presence of the G allele was shown to worsen the reduction in glucose tolerance by melatonin [216], strategies of blocking melatonergic signaling in patients with diabetes have been proposed [219].

However, it must be noted that a reduction in insulin secretion is not necessarily associated with insulin resistance in the target organs, clearly improved by melatonin in most studies. In addition, other MT_2 receptor variants with entirely different properties have been found to be also associated with type 2 diabetes. Some of

them are entirely dysfunctional because of their incapability of binding melatonin, and others were found to be unable to interact with G_i proteins [220, 221]. Thus, the absence of melatonin signaling is presumably also diabetogenic.

A further important aspect concerns the contrasting findings of impairments of insulin secretion by the overexpressed MT_2 G allele and the observed reduced melatonin levels in patients with diabetes [153, 222–226]. The reduction in melatonin levels should presumably be considered as a primary change that is associated with the initiation and/or progression of the disease. In fact, the decrease in melatonin has been regarded as a risk factor for type 2 diabetes.

In young adults, the expression levels of the G allele showed a higher variability but were not statistically different from those of noncarriers, whereas a strong increase in G allele expression was observed in individuals above 45 years [213]. Based on these findings, Hardeland recently proposed an aging-related deterioration of the circadian system, which may lead to losses of rhythm amplitudes, presumably also in the pancreatic β-cell oscillators, and additionally to decreases in nocturnal melatonin secretion [227]. This suggestion gives rise to the interesting question on whether exogenous melatonin administered to carriers of the G allele or to other adults before the age of 40 years might protect the circadian system from losses in amplitudes and, thus, prevent or delay the development of type 2 diabetes. Another important point to consider in human studies is the discrimination of core symptoms (glucose homeostasis) from diabetes-associated pathologies, including those derived from an enhanced oxidative stress like liver steatosis, cardiovascular disease, retinopathy, nephropathy, or osteoporosis. In most of these associated pathologies, melatonin has a demonstrated therapeutic efficacy.

Conclusions

The clinical management of type 2 diabetes includes rigorous changes in lifestyle, insulin therapy, and treatments with medications that promote insulin sensitization (such as metformin) and insulin secretion (such as glibenclamide), new glucagon-like peptide 1 receptor agonists, dipeptidyl peptidase-4 inhibitors, and sodium glucose cotransporter inhibitors [228]. These approaches are designed to control the symptoms of insulin resistance/ß-cell dysfunction and are used alone or in combination. Many pharmacological therapies for diabetes are expensive and in some cases have been associated with adverse events, including possible pancreatitis, hypoglycemia, and osteoporosis [229]. Therefore, there continues to be a need for new cost-effective diabetes pharmacotherapies that have limited additional health risks.

Obesity is a preventable disease, but its prevalence is increasing all over the world, and because it is frequently associated with other cardiovascular risk factors and high mortality, obesity has become a major public health problem and a heavy socioeconomic burden for society in general. The environmental factors or the stressors of the so-called contemporary "24/7" societies have pronounced effects on the metabolism that produce the interruption of the circadian clock. In addition, people whose work involves irregular hours and forced exposure to bright light at night (night workers/shifts) show significant disruptions in sleep and higher prevalence of MS. These lines of evidence indicate that the system does not adequately adjust to environmental and/or stressful changes that disrupt general metabolic homeostasis.

Melatonin may provide an innovative strategy in MS by combining its chronobiotic effect on the circadian rhythm with its cytoprotective properties. Melatonin protects against several comorbidities of MS, such as diabetes and damage mediated by concomitant radiation, inflammation, microvascular disease, and atherothrombotic risk. Melatonin can have a place from the early stages of MS treatment. It has a high safety profile and shows reduced toxicity, which is why it differs from most pharmaceutical agents used in patients with MS.

Melatonin exhibits both hypnotic and chronobiotic properties and has been used therapeutically for the treatment of insomnia related to age, as well as other primary and secondary insomnia [230, 231]. Several meta-analyses support this

role [232–234]. A consensus of the British Association for Psychopharmacology on the evidence-based treatment of insomnia, parasomnia, and sleep disorders in the circadian rhythm concluded that melatonin is the first-choice treatment when a hypnotic is indicated in patients older than 55 years [235].

As discussed in this article, studies using 2–5 mg of melatonin/day are not adequate to provide an adequate comparison with data on the protection of MS derived from animal studies. Melatonin is remarkably nontoxic, and its safety is very high. The lethal dose 50 for the intraperitoneal injection of melatonin was determined for rats (1168 mg/kg) and mice (1131 mg/kg), but the oral administration of melatonin (tested up to 3200 mg/kg in rats) could not be determined and for melatonin subcutaneous injection (tested up to 1600 mg/kg in rats and mice) [236]. In humans, melatonin has a high safety profile and, in general, is very well tolerated (Table 22.4). At present, the only option for the attending physician interested in the use of melatonin as a cytoprotective is the off-label indication of the drug.

Medicines not included in the label are defined as uses of medicines that are not included in the indications or dosing regimens listed by the administrative body that registers, controls, and authorizes medications, for example, the US Food and Drug Administration [248]. The off-label use of medications is common in many clinical areas, such as psychiatry, pediatrics, oncology, and intensive care unit [249–252]. In general, law does not prohibit the unauthorized use of medicines, and the prescription of unauthorized medicines is legally accepted in most legislations [253]. The prescription of medications by physicians is limited only by the common requirement of physicians' duty to act and drive with care and attention.

Table 22.4 Safety for off-label prescription of melatonin

Clinical condition	Melatonin dose	Ref.
Dermal hyperpigmentation	1 g/day p.o. for 1 month	[237]
Parkinson's disease	0.25 and 1.25 mg/kg i.v.	[238]
Amyotrophic lateral sclerosis	60 mg/day p.o. for 13 months	[239]
Amyotrophic lateral sclerosis	300 mg/day, rectal for 2 years	[240]
Muscular dystrophy	70 mg/day for 9 months	[241]
Multiple sclerosis	50–300 mg/day p.o. for 4 years	[242]
Liver surgery	50 mg/kg	[243]
Healthy individuals	80 mg/hr. for 4 h	[244]
Healthy women	300 mg/day for 4 months	[245]
Dose escalation in healthy individuals	10–100 mg p.o.	[246]
Dose escalation in healthy individuals	10–100 mg p.o.	[247]

In Argentina, the National Administration of Medicines, Food, and Medical Technology (ANMAT) approved melatonin (3 mg capsules or tablets) as an over-the-counter drug in 1995. In 2017, ANMAT authorized a prolonged-release preparation of 2 mg of melatonin (Circadin^{R}) as a prescription medication. Although ANMAT cannot authorize the use of a drug for an indication that is not listed in the package leaflet, it does not mean that the indication of a drug for other clinical situations is prohibited. According to ANMAT, the unauthorized prescriptions are "the sole responsibility of the attending physician, who performs them in the full exercise of their professional activity, based on their experience and available scientific knowledge, motivated by the need to provide an answer to health problems for which there are no treatment standards or, if they exist, they are very difficult to access."

In many countries, melatonin is widely used as a dietary supplement or dietary products. The European Food Safety Authority (EFSA) has admitted that melatonin reduces sleep-onset latency. This allows the introduction of melatonin as a food to improve the "regulation of the sleep-wake cycle," the "relaxation," and the "sleep patterns." Melatonin, melatonin-rich foods, and their bio-extracts are now being developed to serve as nutritional supplements, dietary products, and medications. The target group was defined by the EFSA as the general population, and, as such, these extracts can be marketed in all EU countries.

Different studies indicate that, as in animal tissues, melatonin reduces oxidative stress in plants. In fact, its discovery in plants two decades ago has

opened an emerging field of research that has made substantial progress in understanding the actions of melatonin that contribute to the ecological success of the plan. Overexpression of melatonin in plants facilitates the germination of seeds and improves the development and maturation of the roots, protecting plants from biotic and abiotic stress [254–256]. Therefore, the presence of melatonin in plants has implications not only for plant growth and crop yield but also in terms of human and animal nutrition. When plant products containing melatonin are consumed, the compound is easily absorbed and exerts its functions at the cellular level. Therefore, in animals and plants, melatonin is a highly useful molecule that neutralizes the physiopathological processes that compromise a healthy lifestyle. The enrichment of melatonin in foods seems to be necessary to achieve the amounts that provide effective protection. Therefore, an area of interest is the development of functional foods with high levels of melatonin. In parallel, the toxicity of long-term use of melatonin should be evaluated.

In conclusion, from studies in animals, several potentially useful effects of melatonin, such as those in MS, require high doses of melatonin to be evident. If melatonin is expected to be effective in improving health, especially in the elderly, it is likely that the low doses of melatonin commonly used (less than 10 mg/day) are not very beneficial so far [257].

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Neural Network Simulations of a Possible Role of the Hippocampus in Pavlovian Conditioning

José E. Burgos and Juan M. Galeazzi

Neural Network Simulations of a Possible Role of the Hippocampus in Pavlovian Conditioning

In this paper, we take up the following suggestion [1]:

> … it may be more productive to evaluate whether the proposed mechanisms function as they do in nature … One approach would be to see if "damage" to UTR neural networks has the same behavioral effects as brain damage in living organisms. For example, the hippocampal learning rule could be disabled or impaired and the resulting network's behavior could be compared to that of animals with hippocampal lesions. (p. 60)

The suggestion refers to the neural network interpretation of a unified theory of reinforcement (UTR)[2] and was part of a criticism of this interpretation. Although the criticism was successfully replied to [3], the suggestion provides an opportunity to test such interpretation. In this paper we take the opportunity to begin exploring the model's potential to theorize about the role of the hippocampus in Pavlovian conditioning. As a starting point, we focus on a few Pavlovian conditioning phenomena that have been investigated to determine this role.

The study of the hippocampus, named after its characteristic resemblance to the seahorse, has received much attention in neuropsychology and neuropsychiatry. It all began with reports of certain cognitive deficits resulting from bilateral temporal lobectomies to relieve severe epilepsy symptoms (e.g., patient R.M [4]) or cardiovascular accidents (e.g., patient R.B [5]). One of the deficits is known as anterograde amnesia, the inability to form new memories after the lesion. These reports started an extensive field of research about the possible roles of the hippocampus in memory.

Part of this field has been the experimental study of the role of the hippocampal formation (henceforth "hippocampus," for short) in Pavlovian conditioning, using a variety of procedures. Some studies have used delay and trace procedures. In a delay procedure, the unconditioned stimulus (US) begins some time after the conditioned stimulus (CS) onset but before the CS offset. This time is known as the interstimulus interval (ISI). In trace procedures, the US begins some time after the CS offset. This time is known as the trace interval (TI). Normally, Pavlovian conditioning with these paradigms depends on the respective intervals, being stronger with short than long intervals, although too short an ISI also disrupts delay conditioning. In particular,

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short-trace conditioning tends to be weaker than short-delay conditioning.

Uses of these procedures to study the role of the hippocampus in Pavlovian conditioning have largely focused on differential effects of hippocampal lesions [6–14]. Some of the evidence indicates that hippocampal lesions disrupt acquisition of trace more than delay conditioning. This evidence led to the view that the hippocampus in Pavlovian conditioning was relevant only to trace procedures, perhaps by "filling-in" the "empty," TI. Since there was no such empty time in delay procedures, the hippocampus was viewed as irrelevant to them. This view inspired some neural network modeling of the role of the hippocampus in Pavlovian conditioning [15–17].

However, some of the evidence [8] has challenged this view, suggesting that hippocampal lesions also disrupted Pavlovian conditioning with long-delay more than short-trace procedures. Models that restrict the role of the hippocampus in Pavlovian conditioning to trace procedures cannot account for this evidence. It thus appears that the role of the hippocampus in Pavlovian conditioning is not restricted to "filling" the TI. Contrary to those models, the hippocampus also seems to be relevant for long-delay conditioning.

Two neural network models have answered the challenge and rejected that restriction, in different ways. There are other models, but we focus on these as representative of models about the role of the hippocampus in Pavlovian conditioning guided by such rejection. In one model [18], the authors stated that "it is the combination of variables used in the experimental design (e.g., the combination of CS duration and ISI), rather than the type of paradigm (e.g., delay vs. trace conditioning), that serves as a predictor for many experimental outcomes" (pp. 90, 92). This statement rejects the traditional restriction of the role of the hippocampus in Pavlovian conditioning to trace procedures in favor of a more central role for delay procedures.

In another model [19], the authors also reject the traditional restriction of the role of the hippocampus in Pavlovian conditioning to trace procedures, but more inclusively: "while the idea that trace conditioning is hippocampal-dependent whereas delay conditioning is hippocampal-independent provides a useful rule of thumb, it is not sufficient to adequately address the full range of existing data" (p. 49). However, they conclude that "a conceptualization of eyeblink conditioning in which the length of the ISI, in addition to the presence of a TI, determines hippocampal dependence would appear to help integrate our understanding of the eyeblink conditioning literature with that of other preparations" (p. 50). In contrast to the other model [18], then, this one seems to be more inclusive in the role of the hippocampus in Pavlovian conditioning, giving equal importance to a possible role in trace and delay conditioning.

This second model [18] is a real-time extension of a trial-level model [20] and conceives the hippocampus as a "predictive autoencoder which learns to predict the next state of the world given current inputs," to monitor "environmental regularities … using prior experience and current inputs to predict what (out of all possible events) is likely to happen next" (p. 51). This model simulates CS trials (in a context) as input activations in multiple successive moments. The hippocampus is simulated as a network of real-valued activation units where connection weights change via error correction to predict input activations for the next moment. This network includes feedforward and recurrent connections in a 10-node hidden layer and an output layer of 20 nodes. The recurrent connections allow the network to recall past occurrences of the CS and contextual cues.

The model uses a least mean squares error correction learning rule, where the error signal is the difference between the UR and CR. The hippocampal network connects to a motor network consisting of a single output node that simulates responding. A hippocampal lesion was simulated by preventing weight changes in the hippocampal network. In this way, this model can simulate all the aforementioned differential effects of hippocampectomy on long- and short-delay and trace conditioning.

In the present paper, we use an alternative existing model that propounds a different, perhaps simpler, role for the hippocampus in

Pavlovian conditioning that accounts for all the effects of hippocampal lesion on delay and trace conditioning, long as well as short. Intuitively (we articulate it in more technical detail in the first section), we hypothesize that the hippocampus lessens the detrimental effects of (1) momentary nonreinforcement (moments without the US) and, perhaps more substantially, (2) weaker cues on Pavlovian conditioning. Networks in this model simulate both roles by having special units (H) that are intended to simulate some hippocampal structure (e.g., CA1). The function of these units is to send a discrepancy (temporal difference) signal ($d_{H,t}$) that, if sufficiently high, promotes weight gain by connections from input units (S', intended to simulate primary sensory areas activated by the CS and contextual cues) to hidden units intended to simulate polysensory areas (S'').

Simulations of Pavlovian conditioning with these networks depend crucially on those weights. Normally, nonreinforcement and weaker cues have a detrimental effect on Pavlovian conditioning in these networks (in agreement with the evidence). However, the removal of the H units from a network, the way the model simulates hippocampal lesions, results in the absence of this signal (i.e., $d_{H,t} = 0.0$). This removal thus has an even more detrimental effect of nonreinforcement and weaker cues on those weights and, to this extent, on Pavlovian conditioning. We hypothesize that an analogous process might occur in animals. In Simulation 1, we sought to determine if this process can account theoretically for the differential effects of hippocampal lesions on short-delay and short-trace versus long-delay and long-trace conditioning, which have also been simulated by another model [19].

We also sought to assess whether the present model can account for two other differential effects of hippocampal lesions on Pavlovian conditioning not examined with other models. One is the more deleterious effect of hippocampal lesions on backward [21] than contiguous-trace conditioning (CTC), where the CS is reinstated to occur simultaneously with the US after a long TI [22]. We studied this effect in Simulation 2. We summarize the model in the first section, describe the simulations in the second section, and end with a general discussion about some implications, limitations, and future directions.

The Model: Brief Intuitive Description

The model was originally intended as a unified neural network account of Pavlovian and operant conditioning [2, 23], but here we focus on Pavlovian conditioning. The model is a relatively high-level, neural systems model guided by a few general principles of gross vertebrate neuroanatomy that allow theorizing about the hippocampus in a wider framework, taking into account its relations to synapses from primary sensory to polysensory areas, and how it is affected by dopaminergic structures (e.g., the ventral tegmental area).

The model has been shown to simulate a reasonably wide range of Pavlovian conditioning phenomena: acquisition and extinction with resistance [2]; faster reacquisition [2]; blocking and overshadowing [2, 24]; ISI function and dependence of optimal ISIs on network size [25, 26]; discrimination and generalization [2]; simultaneous conditioning [27]; reinforcement revaluation [28]; latent inhibition [29]; effect of the C/T ratio [30]; autoshaping and automaintenance [31]; second-order conditioning [32]; context specificity and renewal [33]; autoshaped choice [34]; and misbehavior [35]. Many other phenomena remain to be simulated, and some are currently being investigated with the model (e.g., conditioned inhibition, spontaneous recovery, cue-to-consequence effects, impulsivity, resurgence, redundancy, etc.). The present study is thus a continuation of research with the model, but the first one explores the model's predictions about the role of the hippocampus in Pavlovian conditioning.

The model has a computational component and a network component. The former specifies an activation rule to compute the state of a computational unit or node and a learning rule to compute changes in connection weights at every moment or timestep (ts) of indefinite duration.

We describe this computational component in the Appendix. The network component specifies the types of units that can constitute a network in the model and some guidelines on how to connect them, inspired by a few principles of vertebrate neuroanatomical organization. Next, we describe this network component and include the basic intuition behind how the model implements our proposed hypothesis about the possible role of the hippocampus in Pavlovian conditioning.

The Networks

The network component is best described through examples. Figure 23.1 shows the two network architectures used in the simulations described later, labeled as H (top panel, with a hippocampal, *H* unit) and ~H (bottom panel, without any *H* unit). Indefinitely many other architectures are possible, but we used these to get a first sense of the model's prediction with relatively simple architectures. As in any neural network model, networks in this model are intended as minimal abstract, simplified theoretical structures roughly interpreted in terms of certain neuroscientific concepts and used to explain behavioral phenomena of interest.

The gross neuroanatomical principle underlying all networks in this model is that vertebrate brains are organized according to the minimal basic structure $S' \rightarrow S'' \begin{smallmatrix} \searrow H \\ \rightarrow M'' \end{smallmatrix} \begin{smallmatrix} \searrow D \\ \rightarrow M' \end{smallmatrix}$. Primary sensory areas (S') are simulated as the input layer of an artificial neural network. Units in this layer connect to (and to this extent can activate) units in a first hidden layer that simulates polysensory areas (S''). S'' units, in turn, connect to hippocampal (H) units and units in a second hidden layer that simulates prefrontal/premotor areas (M''), which connect to a dopaminergic (D) unit and an output unit that simulates primary motor areas (M'). All connections are one directional, meaning that a unit can activate another if the former is connected or projects or connects to the latter, and the strength of the connection is sufficiently high. Of course, this general structure admits indefinitely many variations that can function differently under the same conditions, but then again, here we follow conventional wisdom in modeling and start the simplest networks possible in this model (Fig. 23.1).

Both architectures have a feedforward, left-to-right organization that follows the same basic structure $S' \rightarrow S'' \begin{smallmatrix} \searrow H \\ \rightarrow M'' \end{smallmatrix} \begin{smallmatrix} \searrow D \\ \rightarrow M' \end{smallmatrix}$, consisting of four layers: one input (S'), two hidden (S''-H, M''-D), and one output (M'). In most cases (except for H and D), a unit in one layer connects to the unit in the immediately adjacent unit to the right. In these architectures, the input (leftmost) layer consists of three units (squares labeled as S'_1 and S'_2 and hexagon labeled as S*). Their activations simulate primary sensory effects of the kinds of exteroceptive stimuli that function as cues in Pavlovian conditioning, including the CS (e.g., lights, tones), contextual cues (Ctx; e.g., the walls, sounds, and smells of the experimental chamber), and unconditioned stimuli (US or S*, e.g., food, water, electrical shock). S' activations simulate primary sensory effects of these stimuli.

Both S' units connect to the first hidden unit (S'', for "polysensory"), which, in turn, connects to the H unit and the second hidden unit (M'', for "prefrontal/premotor"). M'' connects to the D (for "dopaminergic") and the unit output unit (M', for "primary motor"). All these connections (thin lines with button endings) represent variable initially weak (0.1 our out of a maximum possible of 1.0) connections, and their strength or "weight" changes according to the learning rule (see Eq. 23.2 in the Appendix). Thick lines from S* to D and the output unit (M') depict fixed maximally strong connections. M' activations simulate primary motor precursors of responding (R*).

The only difference between the two architectures was ~H's lack of the H unit, intended to simulate a hippocampal lesion of some kind (e.g., excitotoxicity, hippocampectomy, etc.). Everything else was identical (free parameters included; see Appendix). The shaded rectangles from the H (H architecture only) and D units depict the hippocampal discrepancy ($d_{H,t}$) and the dopaminergic discrepancy ($d_{D,t}$), respectively, at

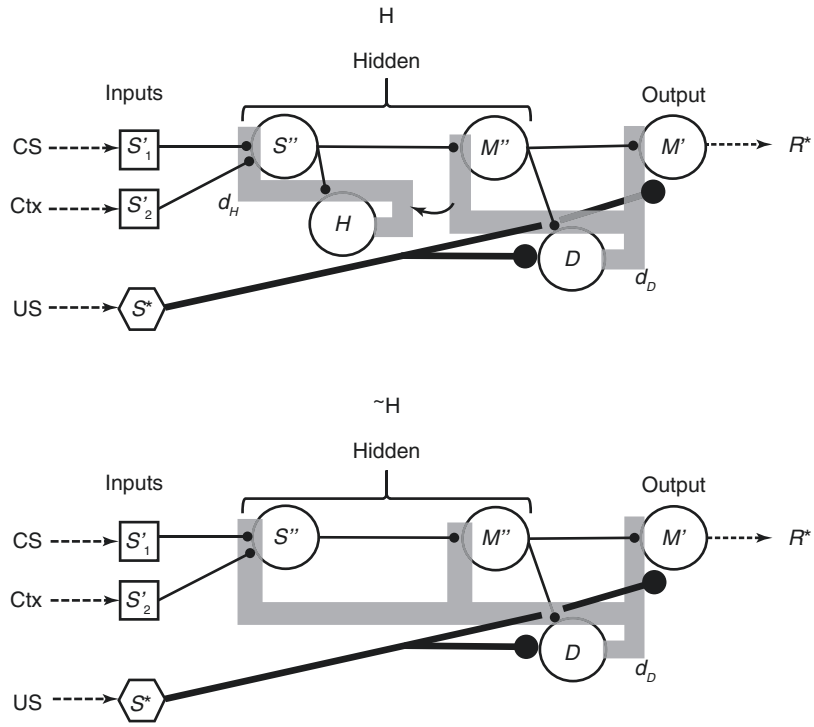

Fig. 23.1 Network architectures used in the simulations described later. Top panel: H architecture, with a hippocampal (*H*) unit. Bottom panel: ~H architecture, without the *H* unit, which simulates some damage to hippocampal structure. *S′*: input units activated by the conditioned stimulus (CS, S'_1) and contextual cues (Ctx, S'_2). *S**: input unit activated by the unconditioned stimulus (US). Thin lines: variable initially weak connections with weights that change according to the learning rule (see Appendix). All initial variable weights were set to 0.1, out of the maximum possible of 1.0. Thick lines: fixed maximally strong connections. *S″*: First hidden (sensory association, polysensory) unit (which receives variable, initially weak connections from S'_1 and S'_2). *H* (in architecture H): hippocampal-like unit, source of d_H, diffuse discrepancy signal that modulates changes in the *S′*-*S″* and *S″*-*H* connections. For ~H, $d_H = 0.0$ at all times. Shaded rectangles: signals that modulate weight changes. *M″*: motor association unit. *D*: dopaminergic-like unit, source of d_D, the signal that modulates changes in the weights of the *S″*-*M″*, *M″*-*D*, and *M″*-*M′* connections and amplifies d_H (curved arrow in H). *M′*: output (primary motor) unit. *R**: either a conditioned response (if activated by *S′* via *M″* and *M′*, after connections get gain weight) or an unconditioned response (if activated directly by *S**, which occurs whenever *S**'s activation is greater than 0.0)

t. $d_{H,t}$ modulates changes in the weights of the *S′*-*S″* and *S″*-*H* (H architecture only) connections, whereas $d_{D,t}$ modulates changes in the weights of the *S″*-*M″*, *M″*-*D*, and *M″*-*M′* connections.

In the model's initial formulation, and in most previous simulation research with it, the CA1 area was hypothesized as a good candidate for a specific neurobiological interpretation of the *H* units. Not only has the CA1 area been widely investigated in relation to the phenomenon known as "long-term potentiation." Some evidence also suggests that CA1 might be more critical to Pavlovian conditioning than other areas of the hippocampus [36–38]. Of course, this is not to say that other parts of the hippocampus (or the brain: e.g., amygdala, cerebellum, etc.) do not play a role in Pavlovian conditioning. Our present focus on the hippocampus, rather, is just a theoretical abstraction as a strategic simplification. Nor do we mean to say that the hippocampus plays a role only in Pavlovian conditioning. The hippocampus plays other roles (spatial learning) that may or may not relate to Pavlovian conditioning, but our present focus on Pavlovian conditioning phenomena is yet another strategic simplification.

In H, according to the evidence [39–45], $d_{D,t}$ amplifies $d_{H,t}$ (curved solid arrow in Fig. 23.1). Because ~H lacks any H unit, $d_{H,t} = 0$ is always the case for this architecture, so changes in the S'-S'' weights are modulated only by $d_{D,t}$. Based on this, our working hypothesis is that efficacies of synapses from primary (S') to sensory association (polysensory, S'') areas can be modulated only by dopaminergic signals ($d_{D,t}$), without the hippocampus. Still, the absence of the hippocampus disrupts sensory learning by substantially reducing the net amount of discrepancy that contributes to weight gain by the S'-S'' connection.

The model's learning rule (see Eq. 23.2 in the Appendix) thus differs from others in that it has *two* related but separable "error" signals, hippocampal ($d_{H,t}$) *and* dopaminergic ($d_{D,t}$), for different sets of connections ($d_{H,t}$ for the S'-S'' and, in the H architecture, S''-H connections; $d_{D,t}$ for the rest of the variable connections). Both are defined not as synchronous discrepancies between the UR and the CR, as they are in other computational models of Pavlovian conditioning (H units in this model are not activated by the US), but as asynchronous discrepancies (temporal differences) in pairs of successive ts between the *actual* activations of H (for $d_{H,t}$) or D units (for $d_{D,t}$). Both signals are global or "diffuse" in that they synchronously affect all weight changes at t, but differentially, depending on factors such as the strength of the afferent signals, the amount of weight gained by the connection, the amount of weight available on the unit, and so on.

Signal $d_{H,t}$ is defined as the absolute value of the hippocampal temporal difference. This definition is guided by the assumption that the hippocampus broadcasts changes (whether incremental or decremental) in its own activity (caused by changes in polysensory activity, which in turn reflect changes in primary sensory areas), back to synapses from primary sensory to polysensory areas. In contrast, $d_{D,t}$ is not defined as the absolute temporal difference. Instead, $d_{D,t}$ negative values (whenever D activations decrease from $t - 1$ to t) are set to 0.0 (see Eq. 23.2 in Appendix). As a result, there tend to be more opportunities for $d_{H,t}$ than for $d_{D,t}$ to surpass the threshold that decides whether a connection gains or loses weight. Typically, then, weight loss by the S'-S'' and S''-H connections tends to be much slower than weight loss by the rest of the variable connections.

H and ~H are not abbreviations of the networks that were used in the simulations. They are exactly the network architectures that were actually used. Thus, H had exactly eight units (S'_1, S'_2, S*, S'', H, M'', D, and M'), six variable connections, and two fixed connections, whereas ~H had exactly seven units (the same as H's minus H) and five variable connections (plus the same fixed connections as H). Such simplicity contrasts with networks in previous models of the role of the hippocampus in Pavlovian conditioning, which have many more units and connections. For example, in the network used in another study [18], we counted 10 units, 3 variables, and 19 fixed connections. In the networks used in another model [19], we counted 20 input, 10 hidden, and 20 output units, for a total of 50 units, all fully connected in a feedforward manner (resulting in at least 400 feedforward connections), plus all the recurrent connections. The present networks are much simpler.

Two other features of H deserve mention. One is that the D signal ($d_{D,t}$) modulates $d_{H,t}$ (curved arrow in H). This modulation is intended to simulate a dopaminergic influence (e.g., from the ventral tegmental area) on the hippocampus, which has been well documented [40, 43, 45]. Again, in the ~H architecture, $d_{H,t} = 0.0$, for which weight changes in the S'-S'' connections in this architecture are modulated only by $d_{D,t}$, which will always be weaker than a greater-than-zero $d_{H,t}$ amplified by $d_{D,t}$.

Another feature is that H is also intended to simulate relations between the hippocampus and polysensory areas, in two ways. In the first way, the H unit in the H architecture received a connection from S'', intended to simulate projections from polysensory areas to the hippocampus (via the entorhinal cortex), which have been documented as well [46–49]. In the second way, $d_{H,t}$ modulates weight changes in the S'-S'' and S''-H connections. This second way is more conjectural. Although the hippocampus is known to send projections back to polysensory areas [50],

their precise synaptic structure and function remain unknown. The working hypothesis in the present model is that such projections modulate changes in the efficacies of synapses from primary sensory to polysensory neurons via modulatory axo-axonic synapses. In contrast to another model [19], and despite evidence to the contrary [51], the present model does not include projections from the hippocampus to motor association (M'', viz., prefrontal and premotor) areas. Nor does the present model simulate hippocampal projections to dopaminergic areas either, such as the ventral tegmental area, despite evidence to the contrary [52]. All these omissions, however, are only strategic simplifications for theorizing purposes, not factual claims about the real neuroanatomy of vertebrate brains.

The output unit (M') can be activated either by M'', if the activation of S^* is 0.0 (which simulates the absence of the US), or by S^*, if this activation is greater than 0.0 (which simulates the occurrence of the US). The two modes of activation are mutually exclusive. The level of activations of M' by M'' will depend on the weight of the M''-M' connection, everything else being equal.

H and ~H are the "templates" of the networks we used in the simulations. That is to say, they are generic *types* or *classes* of networks. For the simulations, different tokens (instances or copies) of each template were used. To label network tokens in the simulations, we used a code that began with the type name (H or ~H), followed by an abbreviation of the treatment (e.g., "d" for "short delay," "D" for "long delay," "BCK" for "backward," etc.). We used an individual network for each condition of a simulation experiment.

Simulations

We ran two simulations using different tokens of the architectures shown in Fig. 23.1, with a simulator coded by the first author with Delphi 7© for Windows©. The relevant source code, instructions and files necessary to repeat the simulations, output files of the simulations described here, and a description of the data folder structure are available at https://www.dropbox.com/sh/g62dxo92q-9i9q4e/AADyECFcuolC-dY1CXYeySJNa?dl=0. All results are intended as comparative, ordinal approximations, rather than quantitatively precise emulations, of the animal evidence. Only output activations will be shown. Due to the stochastic component of the model (a Gaussian threshold in the activation rule) and the update algorithm (asynchronous random), simulations were run with different random seeds to check for reliability. There were differences (some networks did not show the effects of interest) but also reasonably reliable overall trends to simulate the effects of interest. Individual networks visually judged as representative of the effects were chosen for the results. As a convenient starting point, the study was descriptive, without any statistical analysis.

Simulation 1: Role of the Hippocampus in Delay and Trace Conditioning

Four tokens of the H architecture (labeled as Hd, Ht, HD, and HT) and four tokens of the ~H architecture (labeled as ~Hd, ~Ht, ~HD, and ~HT) received a training protocol intended to simulate either a short-delay (d), short-trace (t), long-delay (D), or long-trace (T) procedure. Figure 23.2 shows diagrams for each protocol.

The CS was defined as a maximal activation (1.0) of input unit S'_1 (see Fig. 23.1) for 3 ts in all procedures except for the long-delay procedure (D), where the CS lasted 7 ts. The training context (Ctx, see Fig. 23.1) was defined as a lower activation of unit S'_2 (0.8) concurrent with the CS and the TI (i.e., Ctx "filled" the TI) in the trace procedures. This lower activation simulated a less salient context [53]. The US was defined as the maximal activation of input unit S^* for 1 ts at CS's last ts in the delay procedures (for an ISI of 2 and 6 ts in the short- and long-delay procedures, respectively) or immediately after the last ts of the TI (for a TI of 1 or 3 ts in the short- and long-trace procedures, respectively) (Fig. 23.2).

Each network received 100 trials of its assigned protocol. For simplicity, the intertrial interval was not explicitly simulated, because it

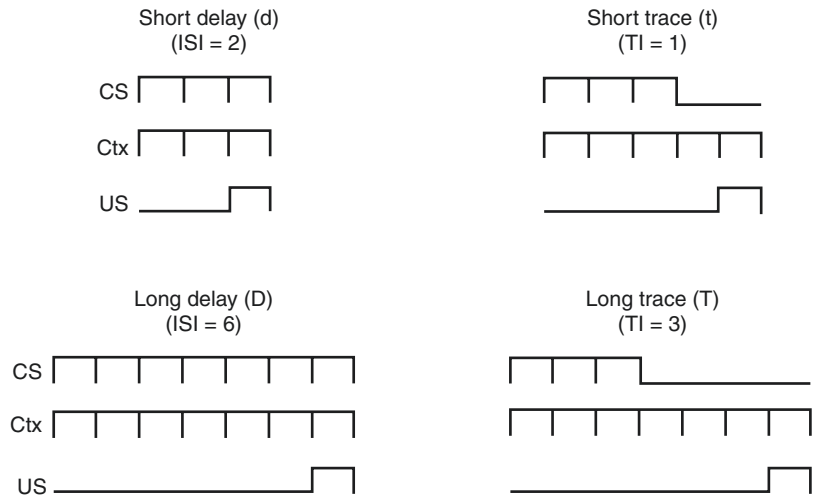

Fig. 23.2 Training protocols in Simulation 1. ISI: interstimulus interval. TI: trace interval. CS: conditioned stimulus, defined as the maximal activation (1.0) of S'_1 (see Fig. 23.1) for a number of ts (either 3 or 7). Ctx: training context, defined as an S'_2 activation of 0.8 throughout the ISI and TI. US: unconditioned stimulus, defined as a maximal activation of S^* for 1 ts. Each segment represents 1 ts of indefinite but equal duration. d short delay (ISI = 2 ts), t short trace (TI = 1 ts), D long delay (ISI = 6 ts), T long trace (TI = 3 ts)

complicates the simulations beyond our sought-after simplicity as a starting point [33]. Instead, we assumed the intertrial interval to be sufficiently long to allow for all activations to decrease to near-zero values (the logistic function with $x = 0$ and $\sigma = 0.1$, approximately 0.007) at the moment immediately before the beginning of each trial (at $t = 0$). Initial weights for all connections and networks were set to 0.1. All this, as well as all free parameters (see Appendix), were the same for all networks and simulations.

Figure 23.3 shows the results in terms of $a(M')$ (output activation) at the second-to-last CS moment across all training trials for an individual network representative of each condition. Ht and HT show for the first time that this model can simulate trace conditioning with short and (more weakly) long TIs, respectively. Also consistent with the animal evidence is that the absence of H units disrupted short-trace (~Ht) more than short-delay conditioning (~Hd). This result is consistent with animal evidence that, as we said, led to the premature conclusion that the role of the hippocampus in Pavlovian conditioning was just to "fill the A-US gap" in trace conditioning and played little if any role in delay conditioning. However, also consistent with the evidence and another model [19], but against that premature conclusion, the absence of H units disrupted long-delay (~HD) more severely than short-trace conditioning (~Ht).

This disruption was much more severe (near-zero output activations after 100 conditioning trials) than that simulated with another model [19] and observed in animals, although this model simulated less disruption (about 45% mean output activation; see Fig. 23.5 of that paper [19]) than that observed in animals (about 35% conditioned response; see Fig. 23.4 of the other paper [8]). Despite this, our results are consistent with the revised notion that the role of the hippocampus in Pavlovian conditioning is not restricted to trace but also includes delay conditioning. Both, the ISI in delay conditioning and the TI in trace conditioning, are equally relevant to such role. The results of trace conditioning are also consistent with predictions of models that do not postulate a role of the hippocampus for delay conditioning [16, 17] (Fig. 23.3).

These results are due to how the model's learning rule for changing connection weights works. The technical details can be seen in the learning rule in the Appendix, but, intuitively, two factors in this model promote weight loss

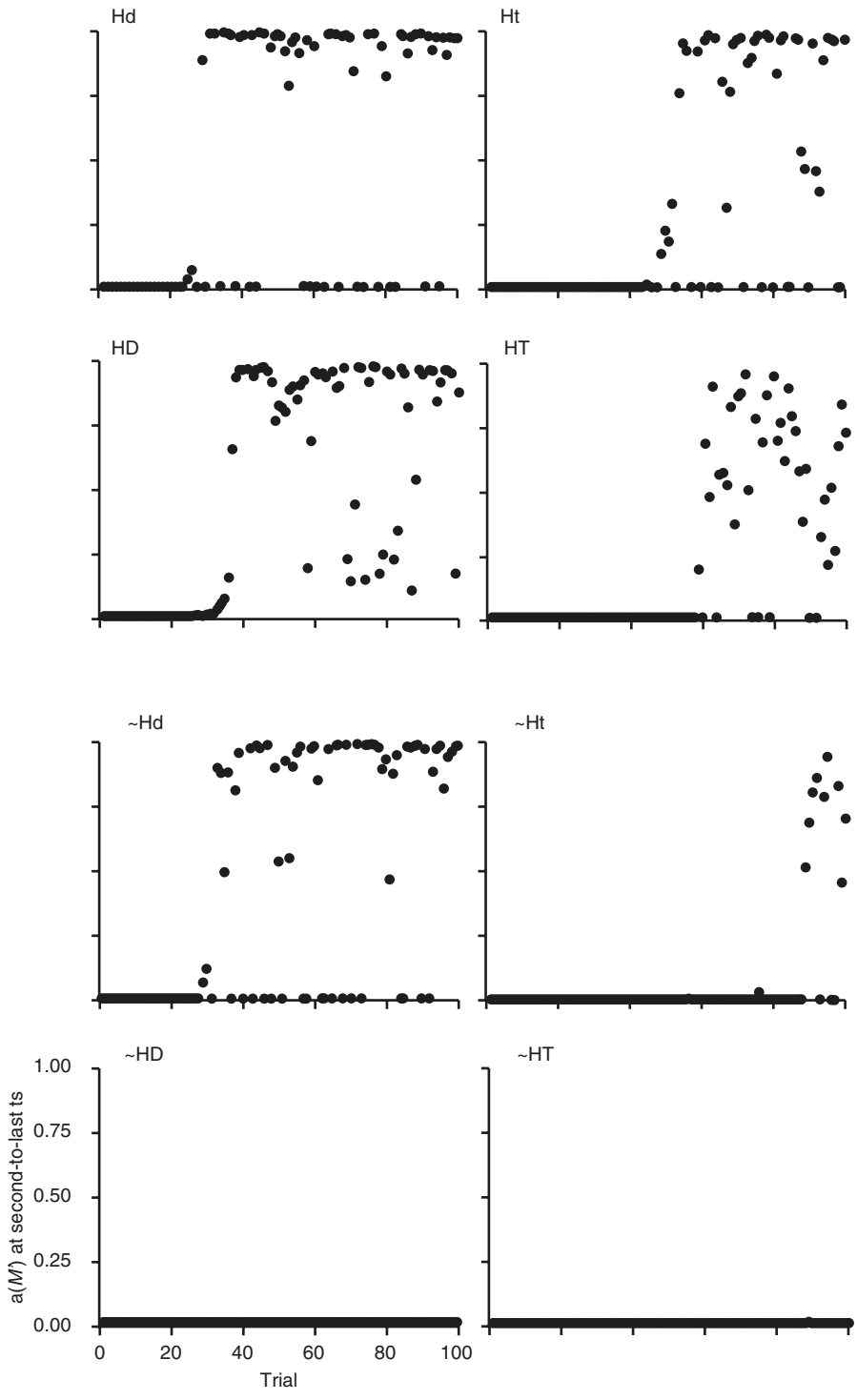

Fig. 23.3 Changes in a(M′) (output activation) at CS's second-to-last ts across training trials, for an individual network representative of each condition in Simulation 1. Different tokens of architectures H (with an H unit; see Fig. 23.1, top panel) and ~H (without any H unit; see Fig. 23.1, bottom panel) received different training protocols (see Fig. 23.2). Four copies of each architecture were thus used, and each copy received one protocol. d short delay (ISI = 2 ts), t short trace (TI = 1 ts), D long delay (ISI = 6 ts), T long trace (TI = 3 ts)

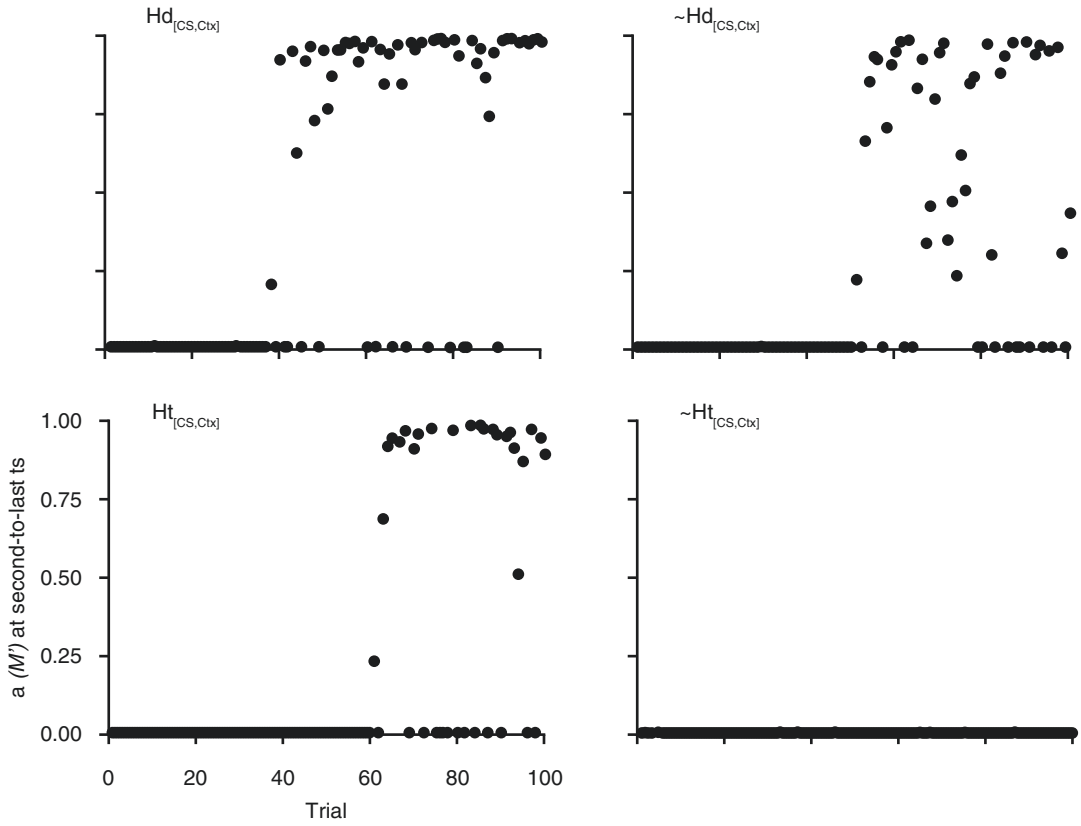

Fig. 23.4 Changes in output (M′) activations at the second-to-last ts of each CS across all training trials for a replication (one individual network per condition) of the simulation of short-delay (d) conditioning (upper panels) and short-trace (t) conditioning (lower panels) with a weaker CS-Ctx compound ([CS,Ctx] subscript), for H (left panels) and ~H networks (right panels)

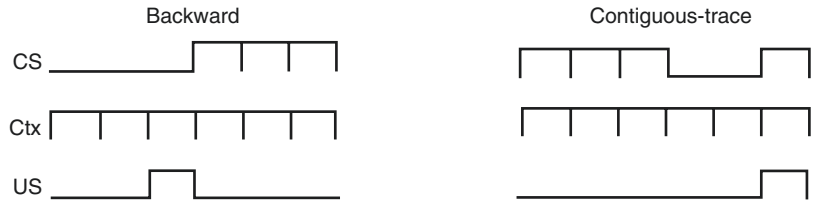

Fig. 23.5 Training protocols used in Simulation 2. CS, Ctx, and US were defined as before. In the backward procedure, US occurred one moment before CS in the presence of Ctx alone. In the contiguous-trace procedure, US occurred concurrently with CS for one moment, after a TI of two moments after CS without the US

(and thus the extent to which the input units can activate the output unit via the hidden units). Both factors have been widely shown to weaken Pavlovian conditioning and are among the basic principles of this kind of learning.

The one is weaker exteroceptive, sensory cues, as determined by cue intensity (or perhaps by limitations of the animal's sensory system; the model makes no essential distinction in this regard). In the model, weaker cues are simulated by lower sensory input activations (of S'_1 and S'_2 in Fig. 23.1). The other factor that has been shown to weaken Pavlovian conditioning is nonreinforcement, the absence of the biologi-

cally significant stimulus used as the US, simulated in this model as an S^* activation of 0.0. The negative effect of such absence worsens over time, so that the longer the time (in the present model, the greater the number of moments) without reinforcement, the more harmful the effect will be on conditioning (in these networks, the slower the weight gain and, hence, the less effective the activations of the output by the sensory input units).

Each factor can act individually, which is less detrimental, or in combination, which is more detrimental to learning in these networks. Both factors promote weight loss by the relevant connections, which reduces the efficacy of the cue inputs (S'_1 and S'_2) in activating the output unit (M') via the hidden units (S'' and M''). Nonreinforcement promotes significant weight loss by generating a dopaminergic discrepancy signal ($d_{D,t}$) below a certain threshold (see Eq. 23.2 in Appendix). In the present simulations, the long-delay (D) procedure had the most moments without the US (eight vs. two in the short-delay, four in the short-trace, and six in the long-trace procedure). Thus, this was the procedure where nonreinforcement had the most disruptive effect on learning. The effect can be seen in HD, which showed a noticeably slower acquisition and less stable maintenance, compared to Hd. The presence of H units in HD, however, prevented a more severe disruption of long-delay conditioning, by providing a greater-than-zero hippocampal discrepancy signal ($d_{H,t} > 0.0$; see Eq. 23.1 in Appendix). The absence of the H units in ~HD, in contrast, resulted in $d_{H,t} = 0$, causing a much more severe disruption of long-delay conditioning by promoting more weight loss.

The long-trace (T) procedure was the second most disruptive, for similar reasons: too many ts without the US, which promoted more substantial weight loss and, thus, acquisition retardation and unstable maintenance. This effect is observed in HT and was comparable to that observed in HD, but like HD, the presence of H units protected HT from it. The lack of H unit in ~HD resulted in a more disruptive effect of the T, although this effect was comparable to that of D.

The trace procedures tended to be more disruptive also because, locally, the US occurred in the presence of Ctx alone, a much weaker cue than the compound [CS,Ctx]. In this model, then, and in agreement with the evidence [54], trace conditioning depends more heavily on contextual learning (to different degrees, depending on the TI) than delay conditioning. Such dependence was more notorious in T than t in the H networks but exacerbated by the lack of H in the ~H networks. The presence of H in the H networks thus lessened the deleterious effect of the context alone as a weaker input signal. According to the model, then, not only is trace conditioning *largely* context conditioning (depending on the TI), but also its deleterious effect is due to the context alone as a weaker cue.

The effects of these differences on learning are better appreciated in the final weights (all initial weights were set to 0.1). Most directly relevant to our present purposes, the final weights of the S'_1-S''_1 connection (S'_1 was activated by the CS), rounded up, were 0.52 for HD and 0.22 for ~HD. This connection thus gained much less weight in ~HD, due to a long delay (too many moments without reinforcement) as a result of lacking the H unit, which made CS less effective in activating the output unit (M') through the hidden units (S'' and M'') than the corresponding connection in HD. The final weights of the same corresponding connection were 0.15 for HT versus 0.00063 in ~HT. T thus actually caused substantial weight loss in ~HT, having a far more detrimental effect on learning, in the form of the weight loss mechanism that simulates extinction and latent inhibition in this model. This effect was due to reinforcement of Ctx alone, a weaker cue than the compound [CS,Ctx] which occurred in D), in the absence of H units.

Simulation 1b: Effects on short-delay conditioning with weaker cues. All this implies that hippocampal lesions should be more detrimental with weaker CS and Ctx cues. To determine whether the model makes this prediction, we repeated the short-delay (d) and short-trace (t) simulations with new tokens of the H architecture (see Fig. 23.1), labeled as $Hd_{[CS,Ctx]}$ and $Ht_{[CS,Ctx]}$, and new tokens of the ~H architecture labeled as

~$Hd_{[CS,Ctx]}$ and ~$Ht_{[CS,Ctx]}$, where the subscripts indicate weaker cues. $Hd_{[CS,Ctx]}$ and $Ht_{[CS,Ctx]}$ received, respectively, the same short-delay (d) and short-trace (t) procedures as before (see Fig. 23.2) but with a weaker CS-Ctx compound where both input activations were set to 0.7. The results in Fig. 23.4 show that the model indeed makes the prediction.

As expected from the animal evidence, weaker CS and Ctx resulted in weaker, or at least slower, conditioning of $Hd_{[CS,Ctx]}$ (compare the upper left panel of this figure to the Hd panel of Fig. 23.3). This result is consistent with observations that generally weaker cues promote slower conditioning. However, ~$Hd_{[CS,Ctx]}$'s conditioning (upper right panel) was even slower, with the very same short delay (compared to the ~Hd panel of Fig. 23.3). This result is also consistent with the animal evidence [54]. Similarly, for the short-trace procedure (t): Weaker cues severely disrupted conditioning in this procedure for the ~$H_{[CS,Ctx]}$ network (compared to the ~Ht panel in Fig. 23.3). We are not aware of any animal evidence to that directly corroborates or falsifies this second result (but see [55]), so we submit it as a novel prediction to be tested in animals. The concrete prediction is that hippocampal lesions severely disrupt short-trace conditioning with weaker cues (Fig. 23.4).

Overall, these results fit our hypothesis that a possible role of the hippocampus could be to lessen the detrimental effects of weaker cues in Pavlovian conditioning. The effects on learning are more directly appreciated in some of the final weights. As before, focusing on the S'-S'' connection as the most critically affected by the absence of H, the final weights for this connection (rounded up) are shown in Table 23.1.

Table 23.1 Rounded-up final weights for the S'-S'' connection of the four individual networks used in Simulation 1b, where the short-delay (d) and short-trace (t) procedures as before (see Fig. 23.3) were repeated but with weaker cues, indicated by the subscripts

$Hd_{[CS,Ctx]}$	0.5
~$Hd_{[CS,Ctx]}$	0.4
$Ht_{[CS,Ctx]}$	0.09
~$Ht_{[CS,Ctx]}$	0.001

The results suggest, vis-á-vis our main hypothesis, that the hippocampus might play a more critical role in lessening the detrimental effects of reinforcement of weaker cues than momentary nonreinforcement on synaptic efficacies in polysensory areas. We are not aware of any attempt to simulate this particular phenomenon with other neural network models of conditioning. Nor do we know if the other models we have cited can actually simulate it. It remains to be seen if they can. In the meantime, we submit the result as the first time a neural network model has been shown to be able to simulate this particular phenomenon.

Discussion. The present model's hypothesis that a possible role of the hippocampus in Pavlovian conditioning is to lessen the detrimental effects of nonreinforcement (moments without the US) and reinforcement of weaker cues on polysensory learning (increases of efficacies of polysensory synapses) explains the animal evidence of the effects of hippocampal lesions on delay and trace conditioning. This role seems more parsimonious and precise than the one hypothesized in other models. There are several models available and a detailed point-by-point comparison would take too long. Here, we will just discuss a few relevant comparisons.

For example, another model [19] proposes that the hippocampus is "a predictive autoencoder, which learns to predict the next state of the world given current inputs" and "forms new stimulus representations in its internal layer;" "the essential function of the hippocampus is monitoring environmental regularities … using prior experience and current inputs to predict what (out of all possible events) is likely to happen next" (p. 51). (Table 23.1) We are not saying that this is wrong. We are only saying that it covers a very large class of neural networks and, hence, says very little, if anything, that is unique to the hippocampus. We are also saying that our hypothesis, as implemented in the model, is propounded as a unique feature of the hippocampus. In the networks we have used here, only the H units play the hypothesized roles. No other type of unit does what the H units do in these networks.

Also in contrast to that other model [19], ours is more neuroanatomically explicit (without enlarging the network architecture) in specifying other kinds of brain structures that participate in the phenomenon with the hippocampus. In particular, that model does not specify a source of the sensory inputs to the hippocampus, whereas we hypothesize (in agreement with the evidence) that such inputs are specifically projections *at least* from polysensory areas (which in turn receive projections from primary sensory areas affected by the CS and contextual cues).

The present model also makes the prefrontal/premotor versus primary motor distinction and hypothesizes a role for dopaminergic (*D* in Fig. 23.1) structures (e.g., the ventral tegmental area) not only in changes of efficacies (weights) of synapses (connections) from polysensory to prefrontal/premotor areas but also, more importantly, in modulating the role of the hippocampus. As we admitted, our model does not include other structures that other models do, like the cerebellum and amygdala. Such exclusions are important limitations of the present model compared to others.

Similar considerations apply to other attempts to model the role of the hippocampus in Pavlovian conditioning. Another model [18] used attentional theory of embedding fields [56], published well before animal evidence on the differential roles of the hippocampus in delay and trace conditioning was available. Therefore, this theory was not intended to account for such differential roles, even if it included a simulated hippocampal structure. Schmajuk and DiCarlo (Fig. 23.2) [18] adapted the theory, mapping it into a circuit that included a structure intended to simulate the hippocampus (and others, e.g., the cerebellum). In this adaptation, most of the connections are fixed [17], and only three are variable. Therefore, the network's functioning depends relatively little on learning (if defined as weight changes in variable connections and if the number of variable connections determines how much a network's functioning depends on learning). In contrast, only three connections in our networks (thick lines in Fig. 23.1) are fixed; the rest (six in H, five in ~H) are variable (thin lines in Fig. 23.1). Our networks' functioning thus depends much more heavily on learning than the one used by that other model [18]. It remains to be seen whether this is also the case in animals.

Another important difference is that the way hippocampal lesions are simulated seems less clear in the approach described by that other model [18]. It is unclear if the hippocampus box was removed from the diagram in the Fig. 23.2 or did it in a more mathematical way (see their Appendix B). In the present model, in contrast, how we simulated hippocampal lesions is clearer: by removing the *H* unit from H to obtain ~H. The authors of that other model [18] said this: "Hippocampal lesions eliminate incentive motivation, competition, and self-excitation among sensory representations" (p. 89). In our simulations, hippocampal lesions (simulated by the removal of the *H* units) did none of that: They eliminated neither incentive motivation (did not affect activations of the *D* unit), nor competition (competition occurred only between S'_1 and S'_2 for the limited amount of weight available on S'', but was unaffected by the absence of *H* in ~H), nor self-excitation among sensory representations (input units were activated equally in the H and ~H networks). In our simulations, the simulated hippocampal lesions in ~H only exacerbated the detrimental effects of momentary nonreinforcement (long delays and traces) and weaker cues (the context alone in trace conditioning is a weaker signal than the context in compound to the CS).

The same authors used a different model [57] to simulate many of the same phenomena they simulated in their previous paper [18], including some simulated here, although we included short and long delays, as well as short and long traces, whereas neither of their two papers did. But what should we make of the use of different models by the same authors to simulate the same phenomena? Are both models valid? If not, which one is more valid and why? No obvious answers present themselves, but seeking them here would require a different paper. In the present study, in contrast, we have used exactly the same model used over the past 20+ years to simulate a wide variety of other conditioning phenomena.

With all these comparisons, we do not mean to say that the present model is "better" than others. Making this determination is exceedingly difficult, due to the great complexity of the phenomena involved, the massive amount of evidence to be accounted for, and the various features of models that influence their assessment (explanatory power, predictive power, parsimony, generality, etc.). We only mean to say that our model could contribute to the theorization of the role to the hippocampus in Pavlovian conditioning. As such, we present the model as just another piece to the theoretical puzzle, perhaps a step toward understanding the role of the hippocampus in Pavlovian conditioning. In the ensuing simulations, we show that this simpler account works reasonably well with other Pavlovian conditioning phenomena that have also been studied in relation to the hippocampus.

Simulation 2: Role of the Hippocampus in Backward and Contiguous-Trace Conditioning

Our hypothesis prompts further simulations of differential effects of hippocampal lesions on Pavlovian conditioning correlated with differences in the intensities of the cues involved. The hypothesis is also consistent with another differential hippocampal dependence of Pavlovian conditioning in two procedures that have not yet been simulated by any model thus far: backward and contiguous-trace conditioning. Figure 23.5 shows a diagram of both procedures, as defined for the simulation. Again, the evidence shows hippocampal dependence of maintenance of backward [21] but not contiguous-trace conditioning [22].

The hypothesis that the hippocampus may play a key role in lessening the deleterious effect of reinforcement of weaker cues, than of nonreinforcement, is consistent with this evidence. The specific prediction here is that hippocampal lesions should be more deleterious to backward than to contiguous-trace conditioning. According to our hypothesis, the reason is that in backward conditioning the US occurs in the presence of the context (Ctx) alone, whereas in contiguous-trace conditioning the US occurs in the presence of the [CS,Ctx] compound, a stronger cue. Therefore, hippocampal lesions should be more deleterious of backward than contiguous-trace conditioning, as indeed has been observed in animals.

To determine whether the model makes this prediction, two new tokens of the H architecture, labeled as H-BCK and H-CTC, and two new tokens of the ~H architecture, labeled as ~H-BCK and ~H-CTC, received simulated versions of either a backward (BCK) or a contiguous-trace conditioning (CTC) procedure, as depicted in Fig. 23.5. CS, Ctx, and US were defined as before, but the US occurred at the ts immediately before the CS onset in BCK (in the presence of Ctx alone) and concurrently with the last 1-ts reoccurrence of the CS in CTC. Each network received 100 trials of its assigned protocol, followed by 20 test trials of the CS alone (in compound with Ctx), during which the learning rule was disabled to freeze the connection weights and assess the differential effects of the treatments on the activations only, without any learning (a common practice in neural network modeling).

The results are shown in Fig. 23.6 in terms of the mean $a(M')$ (output activation) across all test trials (after conditioning) for an individual net-

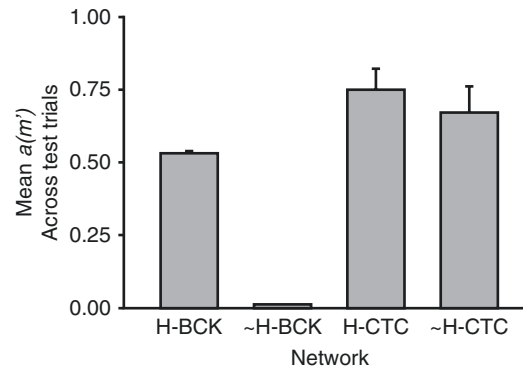

Fig. 23.6 Results of Simulation 2 in terms of mean $a(M')$ (output activation) across test trials (with the learning rule disabled) after training, for an individual network representative of each condition. H: networks with the *H* unit. ~H: networks without any *H* units. BCK backward procedure, CTC contiguous-trace conditioning procedure (see Fig. 23.5)

work representative of each condition. These results show for the first time that this model can simulate backward conditioning. More directly relevant to our present focus on the role of the hippocampus in Pavlovian conditioning, the ~H network also simulated a more disruptive effect of hippocampal lesion on backward conditioning than CTC, in agreement with the animal evidence [21, 22].

These results are explained by the same mechanism described before. Again, in this model, ts without the US promote weight loss, and weaker input activations decelerate weight gain. In BCK, the US occurred only in the presence of the context, which provided a weaker input signal than the context in compound with the CS. This promoted weaker conditioning in H-BCK than H-CTC (which received the US with the context in compound with the CS). On this model, then, and in agreement with the evidence [57], backward conditioning depends heavily on contextual learning. The detrimental effect of BCK, which involved the occurrence of the US in the presence of C1 alone, a weaker cue, was exacerbated in ~H-BCK. The *H* units protected H-BCK from this weakening effect.

CTC, however, had the same number of ts without the US as BCK. Despite this, the absence of *H* was much less detrimental to ~H-CTC than to ~H-BCK. The reason is that the US occurred in the presence of the [CS,Ctx] compound, a stronger cue. Thus, *H* in this simulation played a more substantial role in lessening the detrimental effects of reinforcement of a weaker cue than nonreinforcement. This result also suggests that the hippocampus might be more critical in lessening the effects of reinforcement of weaker cues.

The final weights explain these differential effects on learning. Table 23.2 shows these weights for the S'_1-S'' (affected by the CS) and S'_2-S'' (affected by Ctx) connections for all the networks. BCK was mostly Ctx conditioning, for which S'_2-S'' gained most of the weight in both BCK networks, but much more so for H-BCK than ~H-BCK. Context conditioning was thus much stronger in H-BCK than ~H-BCK. CTC, in contrast, was mostly CS learning, although there also was some Ctx learning, but the final S'_1-S'' weights were very similar in both CTC networks, indicating that the lack of *H* in ~H-CTC had not deleterious effect on CTC. The more substantial difference was gained between the two S'_2-S'' final weights: H-CTC's was substantially greater that ~H-CTC's, indicating that the lack of *H* in ~H-CTC had a substantial deleterious effect on contextual conditioning during CTC.

Table 23.2 Rounded-up final weights of the S'_1-S'' and S'_2-S'' connections (see Fig. 23.2) for all networks in Simulation 2

	S_1'-S''	S_2'-S''
H-BCK	0.001	0.98
~H-BCK	0.0001	0.6
H-CTC	0.677	0.32
~H-CTC	0.728	0.17

H architecture with *H* units (see Fig. 23.1, top panel). *~H* architecture without any *H* units (see Fig. 23.1, bottom panel)
BCK backward procedure, CTC contiguous-trace conditioning procedure

Discussion. The results of this second simulation are consistent with the hypothesis derived from the model that a possible role of the hippocampus in Pavlovian conditioning is to lessen the detrimental effects of weak cues. The [CS,Ctx] compound in CTC provides a much stronger cue at the moment of the US than Ctx alone in BCK. We are unaware of other attempts to model the present results. It remains to be seen whether other models make these predictions (we did not make this determination here because it would make the paper too long, perhaps the reason why it is not common practice to do it). In the meantime, we submit the present results as the first successful simulation of this particular differential effect of hippocampal lesions with a neural network model.

According to conventional wisdom, backward conditioning does not exist, which is inconsistent with the relatively high level of backward conditioning in H-BCK. However, this conventional wisdom has been questioned by evidence that shows that backward conditioning is more substantial than learning psychologists initially thought [58–61]. Certainly, backward conditioning

is not as strong as forward-delay conditioning with an optimal ISI but still is sufficiently strong to often make a statistically significant difference from control conditions.

More problematic for the model is that it accounts BCK as a case of context conditioning: There was virtually no learning to the CS during BCK in either network. In fact, there was a considerable weight loss by the S'_1-S'' and connections during BCK in both networks, albeit far more so in ~H-BCK than H-BCK. This result goes against conventional wisdom according to which all conditioning, even BCK, involves some CS learning, however weak. This model, however, predicts that BCK is just context conditioning. There might be ways to handle this, but this is not the proper place to discuss them. At this point, then, we submit this particular result as a limitation of the model.

General Discussion

This study is a first step to determine what an existent neural network model of conditioning predicts about the possible role of the hippocampus in Pavlovian conditioning. Our aim was only to show the model's potential in this regard. The simulation results are reasonably consistent with some of the relevant animal evidence on the role of the hippocampus in Pavlovian conditioning with delay, trace, BCK, and CTC procedures. The model hypothesizes that this role could crucially be to lessen the detrimental effects of momentary nonreinforcement and weaker cues, both of which have been widely documented. If indeed they obtain in animals, we would expect these two roles to interact in significant ways. We gave two theoretical examples of how they could interact. Simulation 1b showed that the model predicts hippocampal lesions to cause a significant deterioration of short-delay conditioning with a weaker CS.

It remains to be seen whether and how the two roles we have hypothesized relate to those postulated in other models and whether the different hypothesized roles make differential empirically testable predictions that allow for a more precise decision on which hypothesis is closer to the truth. If they do not, they are empirically underdetermined and will have to be judged on more analytic, conceptual grounds, such as clarity and parsimony, if that they are mutually exclusive. Perhaps they are not: Unless they are proven beyond reasonable doubt to be contradictory, it is entirely possible that all of them coexist in the hippocampus. It would be redundant, but natural brains are that way.

We do not mean to say that all the parts of the hippocampus play the two roles we have hypothesized or even either role. Certain parts of the hippocampus might play neither role, just either role, or perhaps both, and several parts might play both roles (there may well be considerable redundancy in this regard). A key limitation of the model in this regard is that the hippocampus is modeled very abstractly, as an input-output black box the internal structure and function of which remain unspecified. Unfortunately, then, the model gives no clues as to exactly which part(s), if any, might play which role(s), to what extent, and how. The model only hypothesizes specifically, in agreement with present knowledge, that among the inputs to the hippocampus (via the entorhinal cortex) are projections from polysensory neurons (of course, the hippocampus receives inputs from many other parts of the brain, but the model excludes them for theoretical abstraction purposes) and sends a diffuse discrepancy signal (through the CA1 area) back to synapses from primary sensory to polysensory neurons. Fortunately, the anatomy of the hippocampus is sufficiently well known to guide neurobiological research to determine how the hippocampus generates the hypothesized modulating signal, if indeed it does, and specifically how it exerts its effects on polysensory synapses, especially in Pavlovian conditioning.

The model is simplifying also in excluding other structures that have been shown to be involved in Pavlovian conditioning (e.g., cerebellum, amygdala, hippocampus-prefrontal projections, etc.). But then again, such exclusion should not be interpreted as the theoretical assertion that these structures are irrelevant but only as convenient, strategic simplifications that only seek to

determine the possible roles of different brain structures counterfactually, in theoretical isolation from other structures.

Another limitation is that we did not try to simulate several other phenomena that have been studied to determine the role of the hippocampus in Pavlovian conditioning, such as latent inhibition [62–63], conditioned inhibition [64], differential conditioning [7], discrimination reversal [7], overshadowing, and blocking [64–69]. Although most of this evidence is mixed, precluding a clear conclusion, it remains to be seen what the present hypothesis predicts about the effects of hippocampal lesions in all these phenomena. We did not do these additional simulations because, again, they would have made the paper too long. We thus decided to leave them for further research.

It also is very likely that the simulation results reported here will vary with manipulations of the parameters of the training protocols we have used, such as the number of training trials, the salience of the context, and the magnitude of the US. We did not simulate the intertrial interval explicitly either (again, as a strategic simplification). All of these variables can change the results, so a more parametric set of simulations would be needed to determine how the results vary with all these variables, which could be a rich source of novel predictions. As far as we know, there is little research on how the role of the hippocampus in Pavlovian conditioning is affected by all such parameters, especially intertrial interval. One variable that has been shown to be relevant in Pavlovian conditioning is the C/T ratio, where C denotes the intertrial (or, more precisely, interreinforcement) interval and T the trial duration. We are not aware of evidence showing interaction effects between hippocampal and C/T manipulations.

We also simulated the context in a very simplified way, as just another CS that accompanied other CSs. The model allows for more realistic ways to simulate the context [33], but for the present study we preferred to simulate the context more simply, as a convenient starting point. Future simulations where the intertrial interval is explicitly simulated will allow more realistic, although this raises the issue of how Ctx could be activated throughout this interval. We simulated Ctx as a constant relatively less salient cue than CS, but it is unclear if such strict constancy obtains throughout the intertrial interval.

In contrast to other models [18, 57], the present one explains the effects of hippocampal lesions on Pavlovian conditioning only in terms of learning deficits during acquisition, without appealing to any attentional process. The only aspect of the model that relates most closely to attentional factors is the input activation levels. A low input activation could be interpreted as less attention, but it could also be interpreted as a low cue intensity (which is how we have interpreted here). However, unlike other models, the present one does not include a mechanism for simulating changes in such levels (they take place only by being assigned as part of the design of training protocols).

Of course, we do not mean to say that the hippocampus plays no role in attentional processes. Rather, the present hypothesis is that the role of the hippocampus in Pavlovian conditioning is not purely attentional but also associative and thus plays a key role during *acquisition*. The model just allows to investigate this associative role as a counterfactual simplification, in isolation from attentional processes (at least as modeled by other models), to determine how much of the evidence can be accounted for if the role of the hippocampus in Pavlovian conditioning *were* only associative. The present results suggest that a good portion of the evidence can be accounted thusly.

Another issue arises from the kinds of surgical controls typically used in studies of the role of the hippocampus in Pavlovian conditioning. A common control is the so-called cortical, which involves cortical instead of hippocampal damage. Although uses of this control do not always specify the exact cortical areas damaged, some of them may correspond to those hypothesized in the present model (primary sensory, polysensory, prefrontal, premotor, and primary motor areas). Clearly, removing all these units from the networks used here would result essentially in no network and, hence, neither learning nor behavior.

To determine whether removing such units from networks in this model affect their performance in Pavlovian conditioning, much larger networks would be needed. It thus remains to be seen how "graceful" performance degradation is in larger networks with progressive removal of those other kinds of units. The gracefulness of performance degradation in an artificial neural network depends on its size. If five units (other than the *H* units) are removed from the networks used here, their performance will degrade sharply, compared to the removal of the same number of units from a 100-unit network. In any case, we assume that the brains of the animals used in studies of the effects of hippocampal lesions on Pavlovian conditioning are sufficiently large to expect control cortical lesions to leave sufficient brain matter in those other areas as to allow animals without hippocampal lesions to still perform normally in Pavlovian conditioning tasks.

As yet another simplification, we did not include any inhibitory processes in the present study. Networks in this model can have inhibitory units, but thus far they have been restricted to S'' and M'' units. Hence, some S'' units could inhibit hippocampal units (and M'' units, and some of these could inhibit *D* and output units). However, hippocampal units per se have played no inhibitory role thus far, although it would be relatively easy to add this feature (and others, such as hippocampal effects on M'' units). Additionally, as we said, the present model does not include any influence of the hippocampus on the modulation of synaptic efficacies of projections from polysensory to premotor areas and from these to primary motor areas.

Finally, there is the issue of whether and how the role of the hippocampus in Pavlovian conditioning relates to the role in other, seemingly different, tasks, in particular, those used to study spatial learning in navigational tasks (e.g., water mazes). Whether there is a relation, and its nature, will depend on whether and how Pavlovian conditioning, which is not considered as a spatial learning task, plays any role in spatial learning. As is well known, hippocampal lesions severely disrupt spatial learning in these tasks (e.g., lesioned animals have a much greater difficulty to find the submerged platform in a water maze). If spatial learning is interpreted as involving Pavlovian conditioning where the contextual surrounding cues function as CSs, and correctly resolving the task (e.g., reaching the submerged platform in a water maze) is the reinforcer, then the present model predicts that a sufficient increase in the salience of certain key contextual cues should improve performance in these tasks by animals with hippocampal lesions.

Reinforcement frequency might be more difficult to manipulate in water mazes, but in other kinds of mazes (e.g., radial mazes), it could be manipulated by changing the amount of reinforcer (food) available at each arm. It is also well known that animals with hippocampal lesions perform poorly in these mazes (they have difficulties learning to avoid arms already visited). According to this model, perhaps a sufficiently greater amount of reinforcement (and more intense cues) in the arm might improve performance in these mazes in animals with hippocampal lesions. In fact, there are attempts to design mazes that allow for Pavlovian conditioning procedures and the manipulation of the ISI and cue intensity [70, 71]. These kinds of studies allow for a closer relation between Pavlovian conditioning and spatial learning.

In sum, the present account is quite conjectural, so we intend it only as a suggestive step toward a better theoretical understanding of the role of the hippocampus in Pavlovian conditioning. The model certainly has the abstract, simplifying character of other computational models, and has limitations, as all models do. The degree of simplification may be too high to some, perhaps to the point of making this a "toy" model, but this is not the only model guilty of this. Fortunately, some [72] still believe that "mathematical toy models will continue to play a major role in guiding the way we think about neuroscience" (p. 64). Certainly, such models are not enough, but to dismiss them because they are abstract or limited goes against the spirit and raison d'être and thus the whole institution of scientific theorizing: Abstraction and simplification are the means by which models become analytically tractable and, to this extent, can give clear

and precise explanations and make clear and precise empirically testable predictions.

Compliance with Ethical Standards This study was funded by no grant.

Ethical Approval No animals were used or participated in this article, excepting the authors, who were not mistreated in anyway; nor does it cite any studies with animals performed by any of the authors. Whether or not the networks used were mistreated requires an expanded code of ethics that includes the possibility and nature of suffering in artificial systems. Unfortunately, no so such code is yet available, and the issue of whether silicon computer quantum states are capable of suffering remains controversial.

Appendix

Activation Rule

The model's two main equations are the activation rule and the learning rule. The activation rule is used to compute the momentary level of activation of a neural processing unit *j* at moment *t*. The learning rule is used to compute the change in the weight of a connection from afferent (preconnection, presynaptic) unit *i* to target (postconnection, postsynaptic) unit *j*. The activation rule is defined as follows:

$$a_{j,t} = \begin{cases} a_{S^*,t}, \text{ if } a_{S^*,t} > 0 \text{ and } j \text{ is } V_D \text{ or } R^* \text{ (unconditional activation); otherwise,} \\ L\left(exc_{j,t}\right) + \tau_j L\left(exc_{j,t-1}\right)\left[1 - L\left(exc_{j,t}\right)\right] - L\left(inh_{j,t}\right), \\ \quad \text{if } L\left(exc_{j,t}\right) > L\left(inh_{j,t}\right) \text{and } L\left(exc_{j,t}\right) \geq \theta_t \text{ (reactivation)} \\ a_{j,t-1} - \kappa_j a_{j,t-1}\left(1 - a_{j,t-1}\right) - L\left(inh_{j,t}\right), \\ \quad \text{if } L\left(exc_{j,t}\right) > L\left(inj_{j,t}\right) \text{and } L\left(exc_{j,t}\right) < \theta_{j,t} \text{ (decay)} \\ 0, \text{ if } L\left(exc_{j,t}\right) \leq L\left(inh_{j,t}\right) \text{(deactivation)} \end{cases} \tag{23.1}$$

where *j* is a neural processing (hidden, output, *H*, or V_D) unit, *t* is a moment in time, and

$$L(x) = \frac{1}{1 + e^{\frac{-x+\mu}{\sigma}}}$$

is the logistic function with constant mean $\mu = 0.5$ and standard deviation $\sigma = 0.1$ (a spontaneous activation free parameter). In this function

$$x = \sum_{i=1}^{m} a_{i,t}{}^{+} w_{i,j,t}{}^{+}$$

for $exc_{j,t}$, and

$$x = \sum_{i=1}^{n} a_{i,t}{}^{-} w_{i,j,t}{}^{-}$$

for $inh_{j,t}$, where *m* denotes the total number of excitatory units connected to *j* and *n* the total number of inhibitory units connected to *j*. No inhibitory units were used in this study, so the amount of inhibition was 0.0 for all units and networks in all simulations. Whether the rule is in reactivation or decay mode at *t* depends on a Gaussian threshold ($\theta_{j,t}$), a random number generated according to a Gaussian distribution with a mean of 0.2 and standard deviation of 0.15. $\theta_{j,t}$ is dynamical, as it is generated at every moment for every computational unit. The other two activation free parameters are temporal summation ($\tau_j = 0.1$) and decay ($\kappa_j = 0.1$). The same free parameters that were used here have been used in most previous simulation research with the model.

On input activations and stimulus traces. A network's inputs in this model are not activated via the activation rule but manually, by just setting their activations according to some training protocol that simulates a conditioning procedure of interest. The protocol includes simulations of sensory stimuli typically used as cues in conditioning studies (e.g., lights, tones, noises, etc.) and biologically significant stimuli typically used as USs or primary reinforcers (e.g., food, water, electrical shocks, etc.). In previous research with this model, we have assumed that these activations are real-time primary sensory effects of external stimuli. Hence, we do not intend input activations in this model to simulate "traces" qua

input activations in the absence of external stimuli but real-time effects of ongoing stimuli. Thus, a greater-than-zero input activation means in this model that a stimulus is effectively present, *roughly* at that moment.

Thus far in research with this model, we have had no need to conceive input activations as "stimulus traces." The notion of a stimulus trace in models of Pavlovian conditioning was introduced ad hoc to account for trace conditioning in a way that fits the conventional wisdom that the CS always acquires some associative strength, even if absent. In Simulation 1, however, we simulated trace conditioning (and in Simulation 2, backward conditioning) as *only* context conditioning. We know this to be extreme and against conventional wisdom, but it is consistent with the evidence (see Marlin, 1981).

Learning Rule

The learning rule is defined as follows:

$$\Delta w_{i,j,t} = \begin{cases} \alpha_j a_{j,t} d_t p_{i,t} r_{j,t}, \text{if } d_t \geq 0.001 \\ -\beta_j w_{i,j,t-1} a_{i,t} a_{j,t}, \text{otherwise} \end{cases} \quad (23.2)$$

where α (rate of weight increment) and β (the rate of weight decrement) denote the two free parameters of the rule ($\alpha = 0.5$ and $\beta = 0.1$ for all connections; the same parameters have been used in most previous simulation research with this model). Ideally, the relative values of various parameters would reflect independent, experimentally determined values. All initial weights were set to 0.1 (cf. Burgos 2007).

The other terms of the learning rule are:

$a_{i,t}$: activation of afferent unit (i), either excitatory or inhibitory

$a_{j,t}$: activation of target unit (j)

$d_t = d_{H,t} = |a_{H,t} - a_{H,t-1}| + d_{D,t}(1 - d_{H,t-1})$, if j is an S'' or H unit (see Fig. 23.1 for the different kinds of units and how they are connected)

$d_t = d_{D,t} = a_{D,t} - a_{D,t-1}$, if j is an M'', D, or M' unit; if $d_{D,t} < 0.0$, then $d_{D,t} = 0.0$

$$p_{i,t} = \frac{a_{i,t} w_{i,j,t-1}}{N}, \quad \text{where } N = exc_{j,t} \text{ or } N = inh_{j,t}$$

depending on whether i is excitatory or inhibitory, respectively

$$r_{j,t} = 1 - \sum_{i=1}^{n} w_{i,j,t}.$$

The key factor is d_t, a signal that modulates changes of all weights in the same moment (i.e., it is a diffuse signal), inspired by evidence on the roles of hippocampal (e.g., CA1, simulated by H units in the H architecture at the top of Fig. 23.1) and dopaminergic (e.g., ventral tegmental area, simulated by the D unit in Fig. 23.1) areas in conditioning. d_t also is a discrepancy signal in that it is defined as a temporal difference between the actual activations of H and D units in successive pairs of moments. The learning rule includes two such modulating signals: $d_{H,t}$, which depends on the activations of the H units, and $d_{D,t}$, which depends on the activations of the D units. As shown above, $d_{H,t}$ is amplified by $d_{D,t}$. However, in the ~H networks (bottom panel, Fig. 23.1), $d_{H,t} = 0.0$, for which $d_t = d_{D,t}$. Hence, d_t in the ~H networks tends to be weaker than in the H networks.

The $p_{i,t}$ and $r_{j,t}$ factors introduce a "rich get richer" sort of competition among connections for a limited amount of weight (1.0) on a common target unit. In the network architectures used in the simulations (see Fig. 23.1), this competition took place on units that received two connections (viz., all the S'' and M'' units, as well as the D unit). The $p_{i,t}$ factor, like some other models, includes a Hebbian component where connection weights partly depend on the co-activations of the connected (afferent and target) units.

In general, connections tend to gain weight (to a greater or lesser degree, depending on several factors) when S^* (see Fig. 23.1) is activated and lose weight when S^* is not activated. Successive timesteps with a zero S^* activation thus promote weight loss. The same learning rule was used to modify the connection weights across all times, connections, networks, units, and training protocols.

All activations and weights are updated at every moment t according to an asynchronous random procedure. In this procedure, a randomly ordered list of all units (or connections) is generated at t, and new activations (or weights) are computed in that order (according to Eq. 23.1 for activations or Eq. 23.2 for weights). The activations (or weights) from $t - 1$ are immediately replaced by the new activations at t. Hence, by chance, the activation of a unit at t could depend on the activations of its afferents (some or all) at $t - 1$. Therefore, the propagation of activations across the network, from input to hidden to output layers, is not strictly sequential and synchronous.

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Brassica Vegetables: Rich Sources of Neuroprotective Compounds

24

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Introduction

The most common types of psychiatric and neurological disorders include Alzheimer's disease, Parkinson's disease, dementia, anxiety, cerebrovascular impairment, seizures, stroke, and depression [1]. Due to the increase in the average life expectancy in the present era, incidence of neurodegenerative disorders has dramatically risen, due also to the fact that the risk increases dramatically with age. Concordantly, neurodegenerative diseases are predicted to be the second most common cause of death among the elderly by the 2040s.

Among the strategies of neuroprotection, traditionally synthetic therapeutic drugs have been employed, but there are several accompanying side effects. For instance, in Parkinson's disease pharmacological treatments are only successful for approximately ten years; long-term use leads to the accumulation of ROS (reactive oxygen species) and other toxic metabolites [2]. Besides, these drugs are unable to slow disease progression. Furthermore, synthetic neuroprotective agents are considered to have several side effects such as dry mouth, tiredness, drowsiness, sleepiness, and others. In this context, studying and characterizing natural neuroprotective compounds become crucial for preventing neurodegenerative diseases.

Phytochemicals are defined as the bioactive nonnutrient plant compounds in fruits, vegetables, grains, and other plant food [3]. Phytochemicals have proven to be effective in preventing several vascular and degenerative diseases including cardiovascular diseases, various types of cancers and neurodegenerative disorders. Additionally, they represent the advantage of having no side effects.

There are several classes of phytochemicals including sulfur, phenolics, alkaloids, steroids, terpenoids, saponins, flavonoids, etc. The Brassicaceae family contains more than 350 genera and 3000 species distributed worldwide represented mainly by broccoli, cauliflower, cabbage, radish, rocket, watercress, etc. These vegetables have been consumed for their distinctive flavor and also for their health-enhancing properties (Fig. 24.1). Foods that have disease-preventing potential are generally known as "functional foods." Since Brassicaceae members are rich sources of many beneficial compounds

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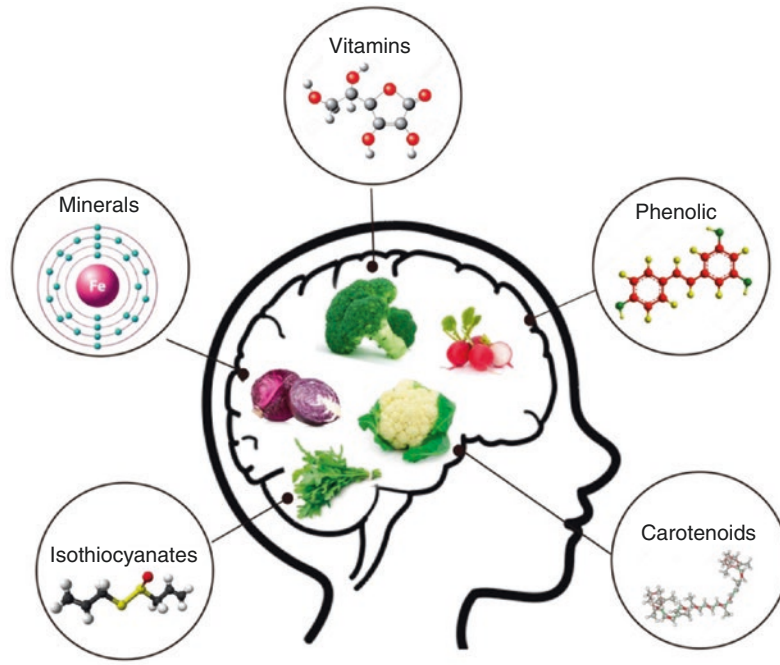

Fig. 24.1 Phytochemicals present in Brassica vegetables

such as isothiocyanates, phenolic, vitamins, several minerals, and carotenoids, they are considered functional crops. This chapter focuses in reviewing, for first time, cruciferous bioactive compounds that may contribute to improving neurological health.

Neuroprotective Compounds Found in Brassicaceae Family Members

Glucosinolates and Their Metabolic Derivatives

Glucosinolates are secondary plant metabolites that have attracted researchers' attention due to their multiple health-enhancing properties. More than 120 different glucosinolates (GSL) have been identified in plants, specifically in Brassicaceae family, in which GSL represents the dominant class of secondary metabolites, but also in a few other species such as *Capparis spinosa* (capers), *Carica papaya* (papaya), and *Moringa oleifera* (moringa) [4, 5]. Glucosinolates are secondary metabolites that consist of anionic thioglucosides with a diverse array of carbon skeletons [6].

GSL can be classified into three classes: aliphatic, indolic, and aromatic, according to their precursor amino acid (methionine, tryptophan or aromatic amino acid, tyrosine or phenylalanine), respectively [7]. The concentration and types of GSL present in food are highly variable depending on several factors, such as genetics, developmental stage, cultivation site, cultivation condition, cultivar, plant tissue, postharvest handling, and food processing methods [8].

When Brassica vegetables are processed and consumed, cells are disrupted. The glucosinolates – in vacuoles – are hydrolyzed by myrosinase (Fig. 24.2). The hydrolysis products include mainly isothiocyanates, nitriles, and thiocyanates. Besides, depending on the plant species studied, side-chain substitution and the reaction conditions, other compounds can be formed such as epithionitriles and oxazolidinethiones [9, 10].

Intact GSL does not have any known biological activity, and among GSL breakdown products isothiocyanates stand up as the major compounds for bioactivity. Isothiocyanates (ITCs) have been

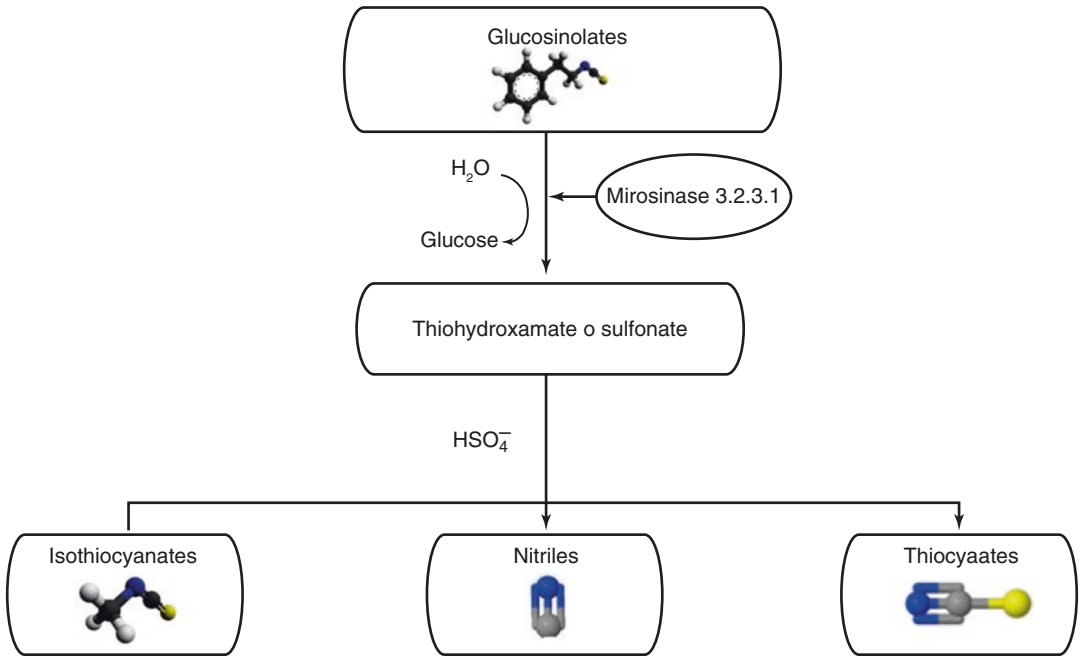

Fig. 24.2 Hydrolysis of glucosinolates by the endogenous enzyme myrosinase. Inspired by Keck [10]

shown to prevent the risk of carcinogenesis and certain cardiovascular acute and chronic diseases, such as neurodegenerative diseases. Moreover, the general molecular mechanism of action through ITC elicits their neuroprotective effect, as described by Giacoppo [11] and shown in Fig. 24.3.

The most researched ITC is sulforaphane, which is derived from glucoraphanin GSL and represents the main ITC in broccoli florets. Both in vivo and in vitro assays have been carried out for analyzing sulforaphane neuroprotective actions. Sulforaphane has been found to decrease disease progression in a Parkinson's disease rat model in vivo [12], stopping apoptosis in Parkinson's and Alzheimer's rat models and murine and human neuroblastoma cell lines [13–15], improving cognitive function [16] and ameliorating cognitive impairment [17] in Alzheimer's disease rat models. Sulforaphane also increases proteasome activity and proper folding in several different murine and human neuroblastoma cell lines [18, 19]. Several mechanisms of action have been proposed for these findings; the most reported being the modulation and enhancement of Nrf2 (nuclear factor erythroid 2-related factor) pathway. Other mechanisms proposed include modulation of Bax/Bcl2 (Bcl-2 associated X protein/B-cell lymphoma 2) pathways, ARE (antioxidant response element) pathways, phase II antioxidant enzymes, and modulation of pro-inflammatory and apoptotic pathway via activation of ERK1/2.

Other ITCs have also been studied as neuroprotectors such as phenetyl ITC, 6 methyl sulfinyl hexyl ITC, and erucin. The reported effects include: reducing, slowing down, or stopping inflammation in several multiple sclerosis mouse models [20]. Besides, in vivo as in vitro effects have been reported for these ITCs in different neurodegenerative diseases cell lines and animal models both [21, 22], improving cognitive function in cerebral ischemia/reperfusion rat model, delaying the onset of amyotrophic lateral sclerosis disease in rat models [23], avoiding neuronal death in a spinal cord injury in a rat model [24], alleviating severe pathological condition in a disease transgenic mice model [25], decreasing apoptosis, increasing cognitive function and improving behavior in a Parkinson's disease

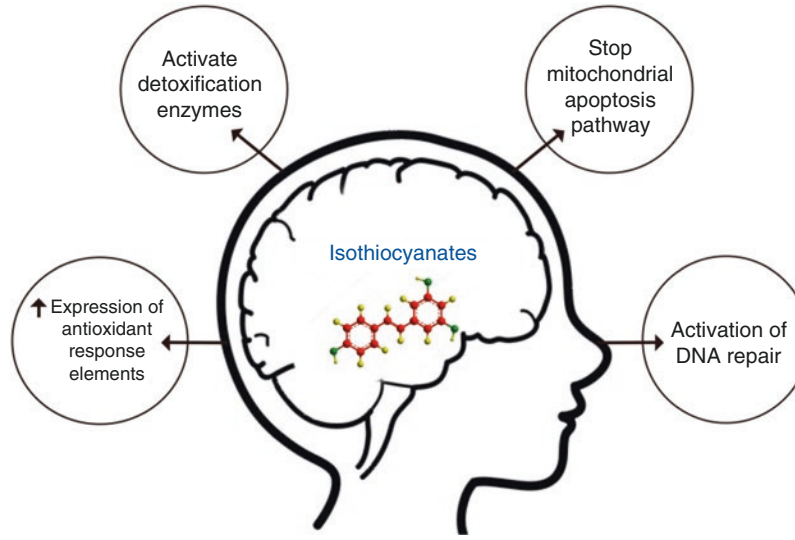

Fig. 24.3 Effects related to ITC mechanism for neuroprotection. Inspired by Giacoppo [11]

animal model [12], and slowing down apoptosis in neurological diseases cell lines model [15].

In a recent work, Burcul [26] analyzed the antioxidant, anti-inflammatory and cholinesterase properties inhibiting the activities of 11 ITCs in a model of Alzheimer's disease and found that aryl ITC (like phenyl, benzyl and phenethyl ITC) were more biologically active than the aliphatic ones, while all the ITCs analyzed showed antioxidant and anti-inflammatory effects to different degrees. Moreover, all ITCs tested show cholinesterase inhibiting activities reaching 61% (for 3-methoxyphenyl ITC) when compared with the traditional synthetic drug employed to block acetyl choline degradation. This activity was tested for ITC compounds for the first time and resulted in very promising molecules because actually there are no natural alternatives to the pharmacotherapy for increasing the levels of acetylcholine in the brain. Therefore, ITCs represent a great natural alternative for Alzheimer's and Parkinson's treatment for instance.

Despite the significant advances in ITCs research as neuroprotective, there are still several compounds whose role in the treatment of neurodegenerative diseases remains unknown. Among these compounds, we can include raphasatin and sulforaphene, which are the main ITCs found in fresh radish roots; Indol 3-carbinol, which is the main ITC found in cabbage but is also present in moderate concentrations in broccoli, radish, rocket, cauliflower, and watercress; and sativin, which is the main ITC found in fresh rocket leaves.

Phenolic Compounds

Phenolic compounds are a large group of secondary metabolites of plants. More than 8000 different phenolic compounds have been reported in the plant kingdom. In Brassicaceae vegetables, phenolic metabolites are mainly led by two groups: flavonols and hydroxycinnamic acids. Flavonoids are the largest group of polyphenols, and more than 2000 individual flavonoids are known [27]. The main flavonols found in Brassicaceae species are quercetin, kaempferol, and isorhamnetin. These compounds are generally presented as O-glycosides or acylated by different hydroxycinnamic acids. Besides these flavonoids, in some Brassicaceae species like red cabbage, red broccoli, red cauliflower, or radishes, anthocyanins are also found, mainly as cyaniding derivatives.

Among hydroxycinnamic acids, p-coumaric acid, sinapic acid, and ferulic acid are the most frequently found conjugated with sugars or with hydroxycinnamic acids [28, 29]. A recent study on 12 cruciferous species reported that among

the phenolic compounds, 70% were flavonols, represented mainly by quercetin and kaempferol derivatives, and phenolic acids represented 30% remain. Besides this, the phenolic profile of each species is different quantitatively, but not in a qualitative manner. Moreover, the phenolic profile varies also with the developmental stage of the vegetable and among the different organs of the plant.

Although many critical biological properties have been attributed to phenolic compounds including anticarcinogenic, anti-inflammatory, antimicrobial, antiallergenic, enzyme inhibition, etc., the antioxidant activity of phenolic compounds remains outstanding and the most reported [30, 31]. In this context, considering that oxidative stress caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the leading cause of neurodegeneration, potent natural antioxidant compounds such as phenolic ones have been extensively studied for the prevention and treatment of neurodegenerative disorders [1]. Two possible mechanisms through which the phenolic compounds exert their neuroprotective properties is by inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities, as well as preventing oxidative-stress-induced neurodegeneration [32, 33].

Several clinical and epidemiological studies have found significant inverse associations between flavonoids' intake and the incidence of dementia and Alzheimer's disease [34–38]. Moreover flavonoids have been reported to reduce the risk of Parkinson's disease [39] and improve the cognitive performance of Alzheimer's patients [40].

The most studied phenolic compounds as neuroprotective agents are quercetin, kaempferol, (−)-epigallocatechin-3-gallate (EGCG), phenolic acids, and t-resveratrol.

Quercetin is a major component of the flavonol subclass and represents 60–75% of the total flavonoid intake. Concordantly, it is the most widely studied flavonoid as neuroprotective agent. In vitro studies show that quercetin and its metabolites protect neuronal cell lines from cell toxicity induced by various oxidants (such as hydrogen peroxide and linoleic acid), several neurotoxic molecules, and from the amyloid beta peptide [41]. Furthermore, several in vivo studies show that quercetin can exert neuroprotection from neurotoxicity induced by several minerals, like mercury and tungsten, and also by organic compounds, like insecticides, and specific neurotoxins such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) [42–44]. Additionally, quercetin proved effective in diminishing inflammation and apoptosis in an intracerebral hemorrhage rat model [45] and promoted the functional recovery following acute spinal cord injury and spinal cord compression injury [46, 47].

Besides, Sabogal-Guáqueta [48], working with a transgenic Alzheimer's disease *mouse model* found that quercetin improved performance on learning and spatial memory tasks. The molecular mechanisms involved in quercetin neuroprotection are described in depth [49, 41] and include NRF-2 modulation, suppression of NF-κB signal transducer and activator of transcription-1(STAT-1), modulation of a broad number of kinase signaling cascades such as phosphoinositide 3- kinase (P13-kinase), AKT/PKB tyrosine kinase and protein kinase C (PKC), regulation of PON2 (paraoxonase family of genes). This gene plays a significant role in atherosclerosis [50], modulation of autophagy that allows maintenance of integrity of the neural system [51] and activation of sirtuins [52].

In summary, quercetin has been widely reported as an effective agent for reversing cognitive impairment, improving memory. It is considered as a protector of neurons and neuroglial cells from external damage, reducing the effect of ROS, modulating apoptotic pathways and activating the natural antioxidant mechanisms. Altogether, these effects allow considering quercetin as a powerful ally in the treatment of acute and chronic neurological disorders.

Resveratrol (trans-3,4′,5-trihydroxystilbene) is another intensely studied phytochemical. However, its presence in Brassicaceae vegetables has been recently reported by our group. Significant levels of this phenolic compound

were found, comparable with those reported in grapes [53].

Resveratrol has been reported to possess many biological and pharmacological activities including antimutagenic, antioxidant, anti-inflammatory and anticarcinogenic properties [54, 55]. Besides its comprehensively documented free radical scavenging and anti-inflammatory properties, numerous in vivo and in vitro studies have demonstrated that resveratrol inhibits beta-amyloid (Aβ) protein aggregation, a key feature of Alzheimer's disease and modulates intracellular effectors associated with oxidative stress involved in neuronal cell survival/death, neuronal energy homeostasis, programmed cell death or apoptosis, and longevity [56]. Figure 24.4 shows a summary of the different neuroprotective action mechanisms of resveratrol according to Bastianello [56].

Another phenolic compound investigated as neuroprotector is (−)-epigallocatechin-3-gallate (EGCG). EGCG protects dopaminergic neurons from neurotoxin damage in an animal model of Parkinson's disease [58]. In other reports [57, 59], EGCG proved to be a potent scavenger of singlet oxygen, superoxide anions, hydroxyl radicals, and peroxyl radicals. EGCG elicits profound effects on reduction of the amyloid β peptide generation, which could result in a deceleration of Alzheimer progression. The molecular mechanisms involved in the neuroprotective effect of EGCG are related to the regulation of the expression of apoptotic genes [60] and the modulation of protein kinase family enzymes that are known to have a fundamental role in the regulation of cell survival, programmed cell death, and neuronal differentiation [61–63]. In several in vivo and in vitro models, epicatechin

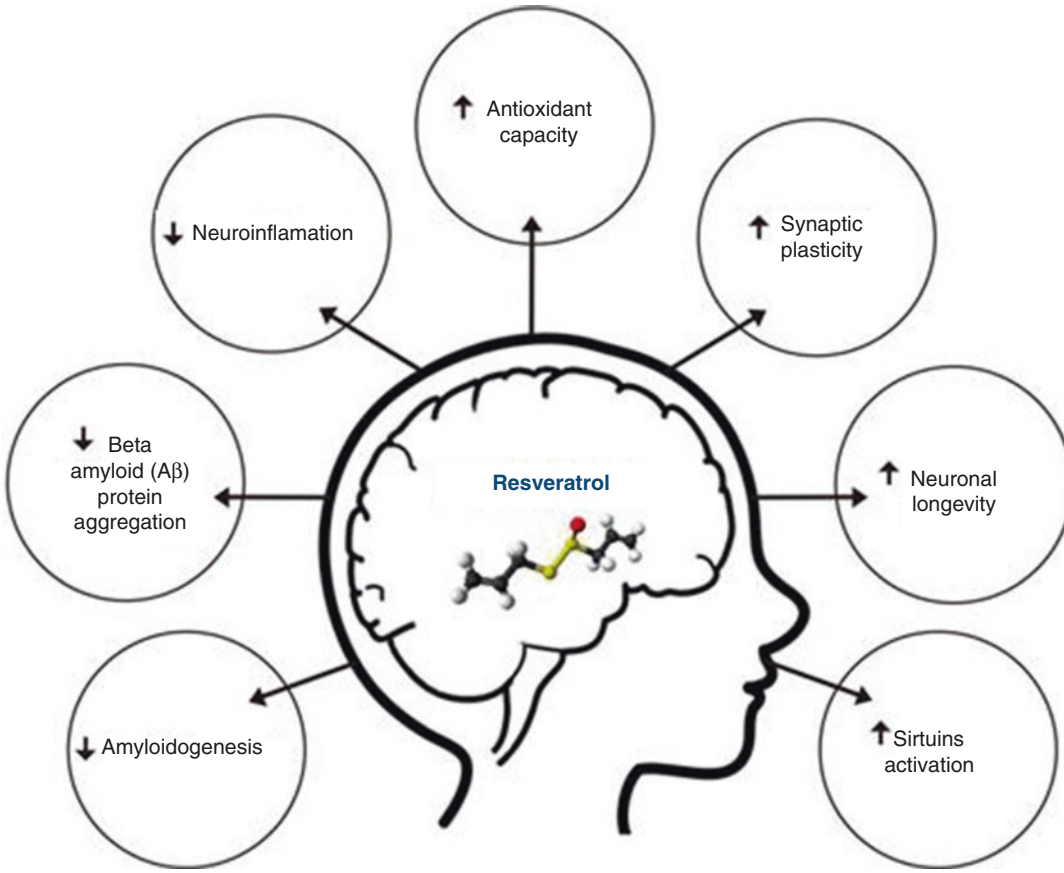

Fig. 24.4 Summary of the neuroprotective action of resveratrol. Inspired by Bastianetto [56]

resulted in protective effects against different oxidant and neurotoxic compounds [64, 65].

Another group of compounds intensely studied are phenolic acids, which are considered according to some epidemiologic reports the main phenolic compounds consumed in diet [66]. Among these compounds, ferulic acid has been the most reported as neuroprotective. Multiple neurological effects have been attributed to phenolic acid including reduction of neuroinflammation and apoptosis, amelioration of depression, memory impairment, and epilepsy, and neural protective effects against several types of insults in both in vivo and in vitro studies [62, 67–69].

Another phenolic compound present in Brassicaceae vegetables is isorhamnetin, which has also been studied as a neuroprotector, but to a lesser extent. In an in vivo report, this compound shows protective effects in mice brains against ischemic injury [70]. Besides, Kim [71] reported that isorhamnetin-3-O-galactoside showed anti-inflammatory activity in an in vivo mouse model. Beneficial properties of this compound are reviewed by Kandakumar and Manju [72].

Another widely studied compound is Kaempferol whose neuroprotective effects include prevention of ischemic brain injury and neuroinflammation [73]. Other reports have found that kaempferol rutinoside improves memory dysfunction and diminishes oxidative stress in a dementia model. It shows anti-inflammatory effects in a different edema model and it has been proposed as a novel neuroprotectant by restoring cerebral blood flow after a neurovascular cerebral injury [74].

In summary, phenolic compounds have proven to be potent phytochemicals in protecting the nervous system from oxidative stress, helping to restore post natural or produced injury, avoiding the onset and progression of neurodegenerative illness.

Carotenoids Compounds

Carotenoids are natural pigments produced by plants, fungi, and algae. More than 600 types of carotenoids have been described. There are two main groups of carotenoids according to their polarity: xanthophylls (polar carotenoids) and carotenes (nonpolar carotenoids). The main carotenoid compounds present in Brassicaceae are β-carotene, β-Cryptoxanthin, Zeaxanthin, and lutein [75, 76]. Kale, broccoli, red cabbage, white cabbage, and Brussels sprout are considered the most abundant sources of carotenoids among Brassicas [77].

The main bioactive properties of carotenoids have been recently reviewed by Cho [78] and include antioxidant, anti-inflammatory, and autophagy-modulatory activities. Several in vitro and in vivo reports have revealed carotenoids as potent anti-inflammatory agents in the nervous system, acting through a variety of mechanisms including ROS quenching, upregulation of antioxidant enzyme systems, hypocholesterolemic properties, anti-neuroinflammatory effects, anti-amyloid aggregation activity, and regulation of amyloid oligomer-induced signaling. Carotenoids may ameliorate mitochondrial dysfunction, oxidative stress, sustained neuroinflammation, impaired lipid metabolism, Aβ-aggregation, and Aβ-neurotoxicity, all of which are critically associated with the pathogenesis of Alzheimer's and Parkinson's diseases [79–81]. In Fig. 24.5, the molecular interaction between three carotenoid compounds and Aβ molecules is analyzed by molecular modeling; this interaction would further avoid Aβ-plaque formation by two proposed mechanisms: by preventing the formation of the fibril and through disruption of the Aβ aggregates [81].

Finally, numerous epidemiological correlation studies have found an inverse association between neurodegenerative diseases risk and carotenoid intake (measured in the blood) and have also suggested that carotenoids may inhibit the onset of neurodegenerative diseases [82–87].

Vitamins

Vitamin C is one of the 13 essential vitamins that are needed for normal functioning of mammalians' life and proper functioning of the nervous system. About 85% of vitamin C in the human diet is supplied by fruits and vegetables (and up

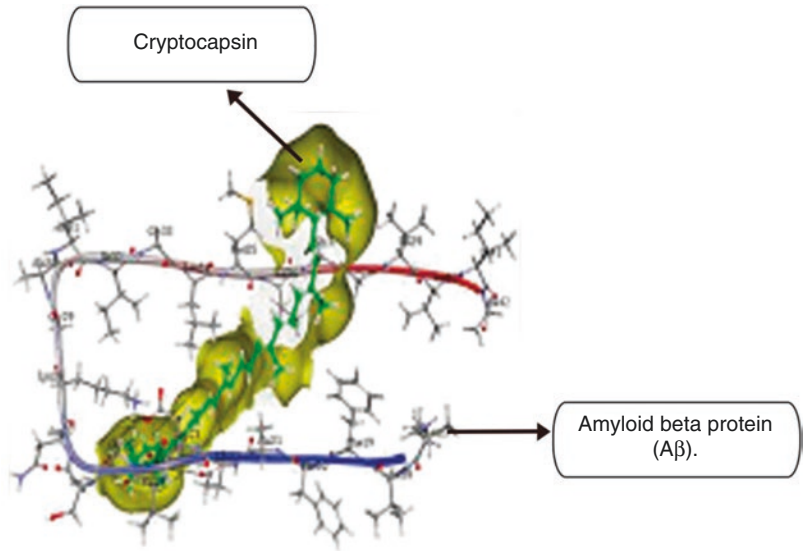

Fig. 24.5 Molecular modeling of interactions of carotenoids (in this case Cryptocapsin) and amyloid beta protein (Aβ). Inspired by Lakey [81]

to 50% specifically by Brassicaceae crops depending on consumer habits) [88].

Vitamin C works in the brain mainly as an antioxidant agent. Since antioxidant agents can stop the free radical's chain of damage, its absence or abnormal concentrations may lead to different neurodegenerative disorders. Moretti [89] reviewed clinical and nonclinical studies indicating that ascorbate (ASC) has neuroprotective effects mainly through reducing oxidative stress and formation of protein aggregates (Fig. 24.6), which may contribute to the reduction of cognitive decline or motor deficits in patients suffering neurological diseases.

There is wide evidence of the role of vitamin C in protecting the nervous system, avoiding neural damage, and diminishing multiple pathologies' incidence. In vivo, in vitro, and epidemiological reports support its use for the treatment of: seizures and epilepsy; stroke; schizophrenia; depressive, manic, and paranoid symptom complexes; Alzheimer's disease; parkinsonian movement disorder and attention deficit hyperactivity disorder; Parkinson's disease; Huntington's disease; multiple sclerosis; and amyotrophic sclerosis [90–94].

Vitamin E contributes to the first line of defense against oxidative stress because it quenches singlet oxygen [95]. Vitamin D has several neuroprotective effects including amelioration of cognitive impairment and reducing the incidence of depression and schizophrenia [96, 97].

Although the role of vitamin E in the central nervous system has not been fully elucidated, it is known that it can act as a protector in cell membranes from oxidative damage by neutralizing the effects of free radicals. Besides its antioxidant properties, vitamin E has been reported to act as a potent anti-inflammatory agent. Vitamin E neuroprotection properties have been reviewed by Ricciarelli [98] and include beneficial effects in Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and ataxia.

Minerals

Minerals exert their neuroprotective effect through serving as cofactors of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPx).

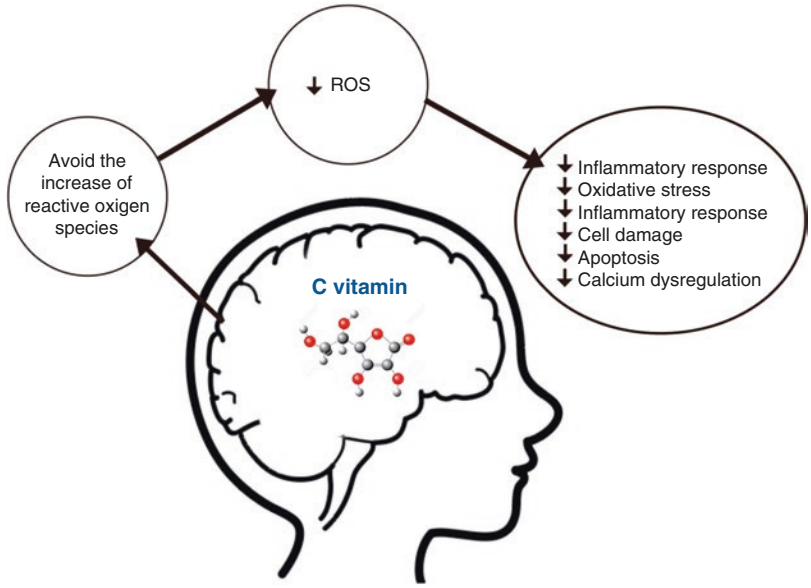

Fig. 24.6 Neurodegenerative diseases and the role of vitamin C in avoiding the increase of reactive oxygen species (ROS) in the brain. Inspired by Moretti [89]

Many reports indicate that some dietary minerals are associated with lower dementia risk [99, 100].

Brassicaceae crops also contain an appreciable amount of minerals highly beneficial for the maintenance of health and prevention of diseases, such as iron and zinc [101]. Metals are involved as components or cofactors of numerous enzymes antioxidants, and certain vitamins (ascorbic acid, α-tocopherol, and β-carotene, folic acid) act as a sequestrant of ROS [102].

Iron and zinc levels in Kale are in accordance with the daily intake recommended by the WHO (FDA WHO, 1988). This result is interesting since iron deficiency impaired performance in mental and motor test in children [103, 104]. On the other hand, iron has been associated with Alzheimer's disease since it has been found to co-localize with amyloid plaques [105] and has also been associated with cognitive function in elderly patients [106] and with Alzheimer neuropathology, progression, and cognitive impairment [107]. Ndayisaba [108] reported a detailed summary of iron accumulation effects on the brain as can be seen in Fig. 24.7. This indicates that iron levels play an important role in brain function, but excessive levels of this metal could also be detrimental to brain health.

On the other side, zinc represents the most abundant trace metal in the brain, acting as a signaling messenger molecule, participating in diverse biological reactions, and serving as a key component in hundreds of proteins [109]. Extensive evidence relates zinc levels with neural system pathologies. Zinc deficiency appears in subjects with Alzheimer's disease in controlled trials and observational studies, and dietary animal studies show that the impact of dietary Zn on cognitive performance depends on additional

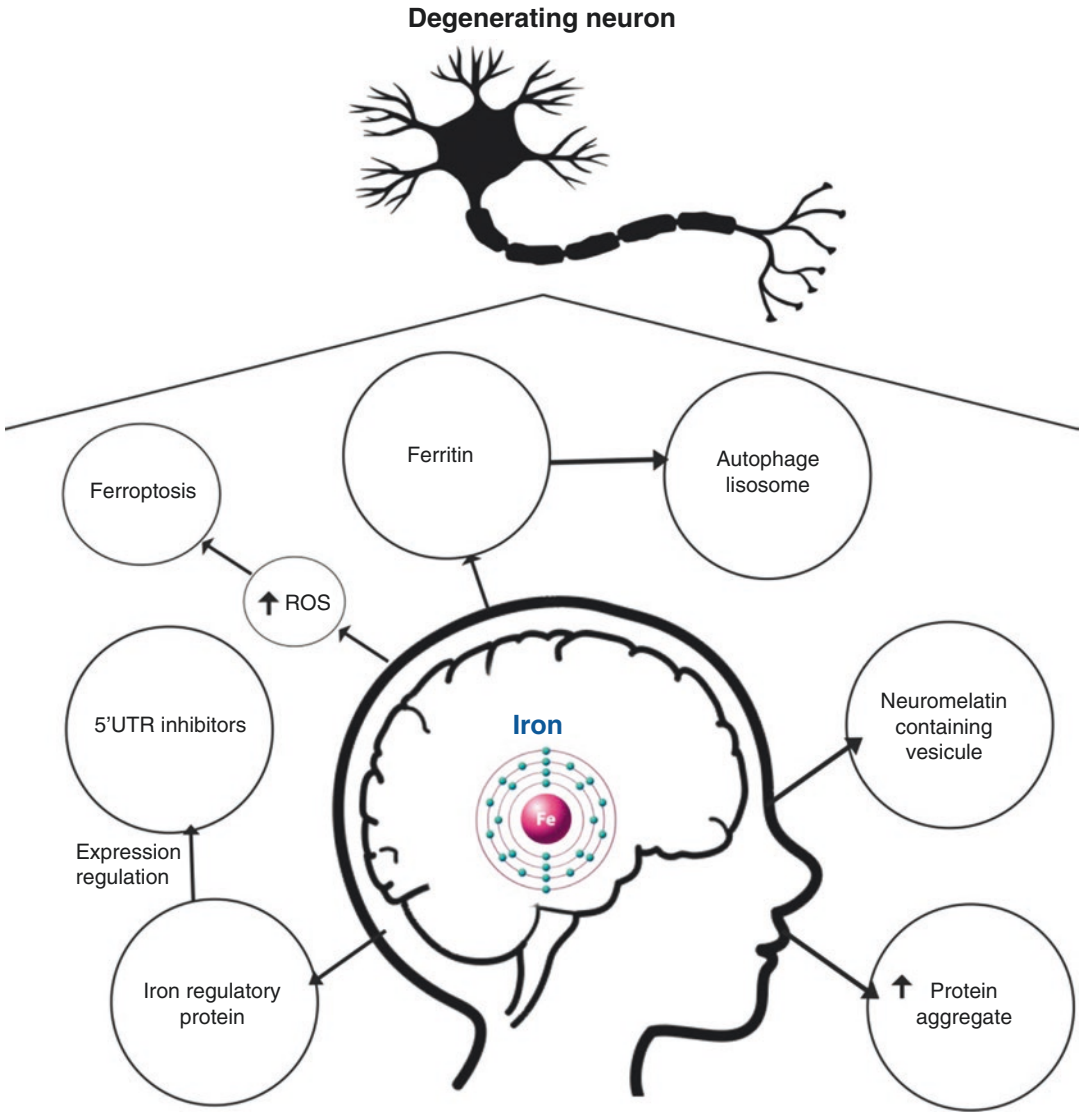

Fig. 24.7 Cellular and subcellular dysregulation associated with brain iron accumulation. Inspired by Ndayisaba [108]

nutrients [109]. Moreover, as Zn is a neuronal protective factor, it has been proposed that zinc deficiency could be partially causative of Alzheimer's disease [110].

Conclusion

Brassicaceae vegetables are promising sources of several neuroprotective compounds, both aqueous and liposoluble, which represent a natural and side-effect-free alternative therapy for the prevention and treatment of neurological disorders. The main mechanism of action includes quenching of free radicals, activation of antioxidant enzymes, inhibition of acetylcholinesterase and butyrylcholinesterase enzymes, activating defense metabolic pathways, avoiding β-plaque deposition, and avoiding neuronal apoptosis. Further studies analyzing the individual and combination effects of several phytochemicals in order to find synergisms are interesting. Studies

about bioavailability and cooked effects are of interest in order to elucidate the potential benefits of each brassica species. It is interesting to consider that besides individual compounds present in Brassicaceae members that have shown neuroprotective actions, studies regarding the neuroprotector effects of whole plant extracts are lacking. Future research addressing this topic is needed.

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Coloured Compounds in Fruits and Vegetables and Health

25

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Introduction

The health benefit of consuming fruits seems to be related to the presence of a wide range of compounds which belong to the so-called phytochemical group or bioactive substances. Their beneficial effects are associated with their role in preventing the development of different types of cancer, cerebrovascular and cardiovascular diseases (CVD), and even Alzheimer's disease. The action mechanisms of these compounds are still not clear, but their effect is the result of many interactions not only between the different food components but also with the organism itself. In this sense, it is not clear whether the same benefits could be obtained from the isolated components as those obtained from a properly balanced diet.

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Bioactive Substances or Fruits–Vegetables Phytochemicals

Plant-based foods (fruits, vegetables, cereals and their derivatives) are products of great interest since they provide macronutrients and micronutrients (carbohydrates, minerals, organic acids, carotenoids, phenolic compounds, vitamins and fibre). But also, a series of substances are present in them that do not have a classically defined nutritional function, or are not considered essential for human health, and have a significant impact on some diseases, crucial for human health in the long term. These bioactive substances or secondary metabolites of plant origin are also called phytochemicals or phytonutrients.

Compared with antioxidant vitamins such as vitamin E, fruits–vegetables (FV)-derived flavonoids were proved to be more efficient in antagonizing oxidative damage in vivo and in vitro [1]. In addition to the direct scavenging of free radicals, antioxidants in FV were able to protect against reactive oxygen species (ROS)-mediated oxidative damage by elevating cellular antioxidant capacity [2]. A series of dietary intervention studies carried out in humans and in animals also showed that a FV-rich diet can cause the elevation of peroxide-detoxifying enzymes activities including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) [3, 4]. Phase II antioxidant enzyme gene expression and activity were proved to be induc-

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ible by FV-derived antioxidants through the trans-activation of the Nuclear Factor Erythroid-Derived 2-like 2 (NFE2L2 or Nrf2) [5] and thus, the genes regulated by it through Nrf2 signaling pathway [6]. Additionally, in experimental animals the expression of antioxidant genes regulated by the Nrf2 signaling pathway was reported to play important roles in antagonizing the pathogenesis of oxidative-damage-related diseases [7]. Individuals who consumed an FV-rich diet appeared to have a lower risk of many chronic diseases [8]. However, the accurate mechanism by which an FV-rich diet decreases the risk of oxidative-damage-related diseases is still unclear. Previous studies carried out in experimental animals mainly focus on the effects of FV (or their juices) on the levels of antioxidant biomarkers [9]. However, a few studies have explored the profile of oxidative-damage-related biomarkers and antioxidant gene expression in different organs in animals.

Fruits and Vegetables as Elements of Adequate Nutrition

As mentioned, the benefits of fruit consumption for health seem to be related to the presence of this large group of bioactive substances called phytochemicals. Table 25.1 shows some of the phytochemicals present in different fruits and vegetables and their metabolic and beneficial effects for human health. It is very important to remember that the foods that contain them prevent but not necessarily cure by themselves diverse diseases. From this point of view, the classic concept of 'adequate nutrition' that provides sufficient nutrients (carbohydrates, proteins, fats, vitamins and minerals) to meet organic needs tends to be replaced by the 'optimal nutrition' concept which also includes the potential of food to promote health, improve well-being, and reduce the risk of developing diseases. In this area is where it naming of foods as func-

Table 25.1 Principal compounds, metabolic effect and fruits or vegetable examples

Compounds		Metabolic effect	Fruits or vegetable
Flavonoids	Anthocyanins	Antioxidants	Grape berry, cherry, blue berry, raspberry
	Flavonols (quercetin)	Antioxidants, decrease platelet aggregation. Decrease the oxidation of LDL, antimutagenic	Grape berry, cherry, apple
	Flavanones	Protect peroxidation, affect the permeability of vascular lipids	Citrus
	Flavonols (catequine, proanthocyanins)	Decrease platelet aggregation. Decrease the oxidation of LDL, antimutagenic	Grape berry, apple, cherry, pear
Carotenoids	Carotenes (β-carotenes, lycopene)	Substrate for vitamin A production Boosting the immune response. Anti-inflammatory	Tomatoes, papaya, mangoes, carrots. Watermelon
	Xanthophyll (lutein)	Essential function in vision. Age-related eye disease	Tomatoes, spinach. Carrots
Vitamins	Vitamin A	Age-related eye disease	Carrots, papilla, apple, apricot, banana, mango, plum, wáter melón, avocado, carrot
	Vitamin K		Papilla, green leafy vegetables, lettuce, kale, mustard greens, spinach, broccoli, Brussels sprouts, cauliflower
	Vitamin C	Strong antioxidant that may reduce the risk of chronic diseases. May help Battle high blood pressure, could reduce blood uric acid. Prevent Iron deficiencies by improving Iron absorption. Protect your memory and thinking as you age	Tomatoes, papilla, citrus, apricot, apple, banana, cherry, blackberry, kiwi, mangoes, pineapple

tional, since the last century. Phytochemicals and micronutrients are responsible for the intense biological activity after human consumption. These compounds contribute to the naming of foods as functional. Functional foods are foods which contain components that are important for their nutritional value and this would include the presence of certain vitamins, organic acids, fibre, minerals, phytochemicals and microorganisms in amounts that contribute to that effect. The additive and synergistic effects of phytochemicals present in fruits and vegetables are responsible for their potential antioxidant and anticancer activity. However, it has been shown that high-dose supplementation with antioxidant preparations can be counterproductive. Therefore, it is best to follow a varied and balanced diet in which there is no shortage of fruits in order to avoid, as far as possible, supplements and foods enriched with antioxidants. In this sense, the World Health Organization recommends the daily consumption of more than five servings of fruits and vegetables.

High fruit and vegetable intake is also linked to healthy skin and hair, increased energy, and lower weight.

Carotenes in Fruits and Vegetables

Fruits and vegetables with carotenes are coloured orange, red and yellow.

Carotenoids are a family of fat soluble pigments present in plants, photosynthetic microorganisms and some fungi [10] that participate in the light harvesting processes required for photosynthesis, which confer photoprotection and possess antioxidant activity [11].

Carotenoids are classified either by their chemical structure or by their functionality:

1. Chemical structure: based on the chemical structure, carotenoids that exist as pure hydrocarbons are referred to as carotenes (α-carotene, β-carotene and lycopene). Furthermore, carotenoids that contain oxygen as a functional group in its structure (β-cryptoxanthin, lutein and zeaxanthin) are referred to as xanthophylls. The presence of the polar group in the structure (e.g. epoxy, hydroxyl and keto) affects the polarity and biological function of the compounds.
2. Functionality: carotenoids can also be classified as primary or secondary carotenoids. Primary carotenoids are known as photosynthetic pigments, which play a key role in photosynthesis, transmitting light energy from the sunlight absorbed by chlorophyll. They are also known to act as antioxidants to the plant by absorbing energy from singlet oxygen formed during the photosynthesis process. On the other hand, the xanthophyll molecules are found abundantly in the leaves of plants absorbing the wavelength of sunlight, which is not absorbed by chlorophyll [12].

Approximately, 40 carotenoids are present in the human food supply [13]. The bioaccessibility, for example the incorporation of carotenoids into micelles, is indeed modulated by carotenoids' localization in the different plastids, such as chloroplasts and chromoplats, as well as by their physical state in theses plastids, such as crystals or associated with proteins.

As mentioned earlier, carotenoids are divided into two groups: carotenes and xanthophylls. The carotenes are hydrocarbons and the xanthophylls are their oxygenated derivatives. Carotenoids derive much of their diversity from the addition of a number of different functional groups, which are most commonly attached to the rings or the ends of the molecule, but rarely to the centre. Most of the tetraterpenoids (i.e. carotenoids with 40 carbon atoms) are named by adding prefixes to the name 'carotene'. These prefixes are Greek letters corresponding to the end groups. Carotenoids are orange because of the large light-absorbing chromophore in the centre of the molecule. This sequence of conjugated double bonds absorbs light at about 450 nm and dissipates the energy as heat. Higher wavelengths of light are reflected, giving them their characteristic orange colour. The extended chromophore of lycopene, which is one of the most important carotenes, absorbs more of the yellow–orange light and thus reflects red light (as in tomatoes). Carotenoids

vary in their absorption maxima, which is useful for identification.

Moreover, they are precursors for the biosynthesis of phytohormones (e.g. abscisic acid and strigolactones) that control abiotic stress acclimation, shoot and root branching as well as apocarotenoid signalling metabolites that mediate chloroplast to nucleus communications and root-mycorrhizal interactions [14–16]. Their biosynthesis and regulation occurs throughout the life cycle of a plant, with the composition and levels of carotenoids finely tuned to developmental stages and changes in the environment [17]. In order to attract the pollinators, the plants produce carotenoids with bright colours like yellow, orange and reddish-pink in flowers and fruits [18]. In animals, carotenoids provide a distinct coloration to flamingos, salmon and finches, in which they enhance ornament-based colour signalling and attraction thereby improving reproductive success [19, 20]. Carotenoids have essential functions in human body, for example vision (lutein and zeaxanthin protect against age-related macular degeneration of the eye), vitamin A biosynthesis (β-carotene is the substrate for vitamin A production), boosting the immune response (e.g. canthaxanthin, α- and β-carotene, lutein and astaxanthin), antioxidation (e.g. β-carotene), behavioural characteristics, reducing cancer (e.g. lycopene), as well as many other reported human health benefits [20–23].

Provitamin A carotenoids contain at least one unsubstituted β-ionone ring (e.g. β-carotene, α-carotene, β-cryptoxanthin) and can be enzymatically cleaved to produce vitamin A, an essential micronutrient for human beings [24]. The potential biological functions of non-provitamin A carotenoids and their metabolites in mammals continue to be investigated. In vitro and in vivo studies have suggested numerous activities for ingested carotenoids and/or their metabolites. These include antioxidant [25], anti-inflammatory and neuro-protective [26], anticancer [27], cardio-protective [28], vision protecting [29], lipid deposing and storage-modulating [30], photo-protective and immune-modulating [31] activities. In order to mediate such activities, these compounds must be delivered to target tissues following ingestion.

Important advances in recent years with several biotechnological and conventional breeding programmes have biofortified food and feed crops with promising achievements to enhance carotenoid supplementation to the food and livestock industry. For example, to combat vitamin A deficiency, 'GOLDEN' Rice and Bananas were engineered with enhanced levels of provitamin A, thereby making major food crops consumed around the world more nutritious [32]. Another breakthrough was the horizontal genome transfer of a synthetic operon for the biosynthesis of astaxanthin (which mostly accumulates in microorganisms such as microalgae, red yeast and marine bacteria) into plants, which provides a highly valued colour and micronutrient supplement for artificial feeds used in poultry and aquaculture [33]. In the last decades, the genes and enzymes of carotenoid metabolic pathway have been well characterized and thoroughly studied in many plants [18]. There are still gaps in our understanding of carotenoid regulation, sequestration and degradation [15, 17]. In general, all organisms that can perform the photosynthetic process naturally generate *cis*-carotenes between phytoene and lycopene. *Cis*-isomers of lycopene are found to be more soluble than their *trans*-configured isomers in organic solvents [34].

It is important to note that in addition, carotenes are also present in other fruits or green vegetables such as spinach and broccoli. Intracellular localization and the physique state of carotenoids in the foods are very different. In green vegetables as well as spinach and broccoli, the carotenoids are localized in the photosystems as proteins–pigments complex. Carotenoids in orange, red or yellow fruits and vegetables are located in the chromoplasts. The chromoplasts morphology is variable; they can be globular and tubular membranous. In tomatoes and carrots, the carotenoids are solid crystalline, but in papaya and mangoes the carotenoid are liquid crystalline.

Lycopene, Fruits and Vegetables

Nutrients in red fruits and vegetables include: lycopene, ellagic acid, quercetin, and hesperidin, to name a few. In general, these nutrients reduce the risk of prostate cancer, lower blood pressure, reduce tumor growth and LDL cholesterol levels, scavenge harmful free-radicals, and support joint tissue in cases of arthritis.

Fruits and vegetables that content lycopene are numerous. The more common ones are beets, blood oranges, cherries, cranberries, papaya, pink grapefruit, pink/red grapefruit, pomegranates, radicchio, raspberries, red apples, red bell peppers, red grapes, red onions, red pears, red peppers, red potatoes, strawberries, tomatoes and watermelon [35–37].

Lycopene is a lipophilic, biologically active, unsaturated, acyclic carotenoid with the chemical formula $C_{40}H_{56}$. In human nutrition, lycopene plays no role as provitamin A precursor due to the absence of appropriate enzymes. In plants its function is considered important because it is an intermediate of carotenoid synthesis. All *trans*-isomer lycopene are usually present in plants. Isomerization of the all *trans*-isomer into the more bioavailable *cis*-isomer, occurs under acidic conditions (e.g. gastric acid), or due to exposure to light and thermal energy [35–37]. Lycopene content increases during different stages of ripening of fruits, for example, for tomatoes there is a steady increase in lycopene content from the breaker to the red stage [38]. Watermelon pulp can also be used for lycopene extraction [39] and is a rich source of *cis*-isomeric lycopene, abundant in higher concentrations than in tomatoes [40]. Another source that produces lycopene is the fungal plant pathogen *Blakeslee trispora* [41]. Several foods high in lycopene content are classified as functional foods [40]. Tomato juice, paste, puree, ketchup, sauce or soup represent lycopene sources with improved bioavailability due to thermal treatment, but also because processing releases lycopene from the fibrous cell structure matrix [42, 43].

One property of lycopene is an effective singlet oxygen quencher in the carotenoids group [35, 44]. Diverse studies have shown its importance as a potent antioxidant, ten times more potent than alpha tocopherol or twice than beta-carotene [35, 45]. Lycopene modulates also the production of antioxidant enzymes, such as superoxide dismutase and catalase [46, 47]. Oxidative stress causes endothelial dysfunction due to uncoupling of the nitric oxide synthase and oxidative injury of the endothelial cells [48]. Both are associated with inflammation. Lycopene is capable of reducing oxidative stress and reactive oxygen species. In consequence, it increases the bioavailability of nitric oxide (NO), improves endothelium-dependent vasodilation and reduces protein, lipids, DNA, and mitochondrial damage [40, 49–52].

Endothelial NO enables vasodilation, inhibits platelet functions and adhesion and transmigration of white blood cells, and reduces smooth muscle cell proliferation [53]. Lycopene supplementation improved endothelial-mediated vasodilation in cardiovascular disease patients, but not in healthy controls [36], suggesting the importance of lycopene in secondary cardiovascular prevention [54]. In summary, lycopene scavenges both reactive oxygen and nitrogen species, increases the production of antioxidant enzymes and protects the endothelial cells from oxidative damage.

Historically, the tomato traces its origins back to around 700 AD with the early Aztecs; therefore, it is believed that tomato is native to the Americas. It was not until around the sixteenth century that Europeans were introduced to this fruit, when the early explorers set sail to discover new lands. Tomatoes are an intensely nutritious plant food and are classified as a functional food. There are different types and sizes of tomatoes, and they can be consumed in different ways: raw, stewed in soups, as juice and puree, as ketchups, etc.. The health benefits can vary. For example, cherry tomatoes have higher beta-carotene content than regular tomatoes. Tomatoes also contain lycopene, lutein, beta-carotene and vitamin

C. Tomatoes can help protect the eyes from light damage. These are potent antioxidants that have been shown to protect the eyes against light-induced damage, the development of cataracts, and age-related macular degeneration.

Tomatoes are an excellent source of vitamin C and other antioxidants. With these components, tomatoes can help combat the formation of free radicals. Free radicals are known to cause cancer. A recent study [55] linked the high levels of beta-carotene to the prevention of tumor development in prostate cancer. Diets rich in beta-carotene may play a protective role against this kind of cancer. Tomatoes also have high fibre, potassium, vitamin C, and choline content and these compounds are associated with heart health. An increase in potassium intake, along with a decrease in sodium intake, is the most important dietary change the average person can make to reduce their risk of cardiovascular disease. Not only is high potassium intake also associated with a reduced risk of cardiovascular disease, but it is also known for protecting the muscles against deterioration, preserving bone mineral density, and reducing the production of kidney stones. High fibre diets in people with type 1 diabetes show lower blood glucose levels, while people with type 2 diabetes may have improved blood sugar, lipids, and insulin levels. The fibre may help hydration and support normal bowel movements. Tomatoes are often described as a laxative fruit. The age-related eye disease study recently found that people with high dietary intake of the carotenoids lutein and zeaxanthin, both present in tomatoes, had a 35% reduction in the risk of neovascular age-related eye disease [56]. Vitamin C is related with production of collagen in the body. Collagen is an essential component of the skin, hair, nails, and connective tissue. A deficiency of vitamin C can lead to scurvy. As vitamin C is a powerful antioxidant, a low intake is associated with increased damage from sunlight, pollution and smoke [57].

Carrots were probably first cultivated thousands of years ago, in the area now known as Afghanistan. It was a small, forked purple or yellow root with a bitter, woody flavour, quite different from the carrot we know nowadays. Purple, red, yellow, and white carrots were grown long before the appearance of the sweet, crunchy, and aromatic orange carrot that is now popular. This type was developed and stabilized by Dutch growers in the sixteenth and seventeenth centuries. This feature is part of a collection of articles on the health benefits of popular foods, and here are some key points about carrots. Carrots were first grown in Asia, and they were not orange. Carrots contain antioxidants, which may protect against cancer. While they may not help human to see in the dark, the vitamin A in carrots helps prevent vision loss. Carrots are available all year round and can be used in savoury dishes, cakes, and juices. Regular consumption in the diet of carotenoids has been shown to have anticancer effects, due to their antioxidant power in reducing free radicals in the body. Evidence suggests that eating more antioxidant-rich fruits and vegetables, such as carrots, can help reduce the risks of cancer and cardiovascular disease. Studies have found a possible link between diets rich in carotenoids and a lower risk of prostate cancer [58]. Carrots have important beta-carotene content. Former studies of beta-carotenes have concluded that the supplementation with them may reduce the risk of lung cancer. There is evidence of an inverse association between lung cancer incidence and β-carotene intake (and with serum concentrations of β-carotene). This data led to the initiation of several large-scale randomized chemoprevention trials to test the hypothesis that β-carotene supplements protected against lung cancer, but those trials had disappointing results. Indeed, β-carotene supplementation actually was found to increase the risk of lung cancer in high-risk populations [59]. The same pattern was not true for any individual carotenoid, such as beta-carotenoid. Among smokers, beta-carotene supplementation may increase the risk of lung cancer. A 2011 study found that carrot juice extract could kill leukemia cells and inhibit their progression [60]. Besides, carrots contain vitamin A. Vitamin A deficiency can lead to xerophthalmia, a progressive eye disease that can damage normal vision and result in night blindness, or the inability to see in low light or darkness. The main preventable cause of blindness in

children is a lack of vitamin A (according to the National Institutes of Health).

Carrots are also rich in vitamins, minerals, and fibre.

Papayas grow in tropical climates and are also known as papaws or pawpaws. Their sweet taste, vibrant colour, and the wide variety of health benefits they provide make them a popular fruit. The papaya, a previously exotic and rare fruit, is now available at most times of the year. The possible health benefits of consuming papaya include a reduced risk of heart disease, diabetes, cancer, aiding in digestion, improving blood glucose control in people with diabetes, lowering blood pressure, and improving wound healing.

Papaya is native to Mexico, but it grows naturally in the Caribbean and Florida too. According to the Food and Agriculture Organization of the United Nations (FAO), India produces the most papayas. Papayas are a soft, fleshy fruit that can be used in a wide variety of culinary ways. Here we will focus more on the health benefits, uses, how to incorporate them more into the human diet, and the nutritional value papayas have. It can be added to salads, smoothies, and other dishes.

Papayas have nutrients that are thought to have a range of health benefits. They may help protect against a number of health conditions such as age-related macular degeneration, asthma prevention and even anticancer properties. Zeaxanthin, an antioxidant found in papaya, filters out harmful blue light rays. It is thought to play a protective role in eye health, and it may ward off macular degeneration, reducing the risk of and progression of this pathology. Relationship between papaya and asthma prevention are associated with people who consume a high amount of certain nutrients. One of these nutrients is beta-carotene, contained in foods like papaya but also in apricots, broccoli, cantaloupe, pumpkin and carrots [61, 62].

Papaya also possesses beta-carotene and consequently its intake may reduce the risk of cancer. Among younger men, diets rich in beta-carotene may play a protective role against prostate cancer. Papaya has the highest content of vitamin K. Low intakes of vitamin K have been associated with a higher risk of bone fracture. Adequate vitamin K consumption is important for good health, as it improves calcium absorption and may reduce urinary excretion of calcium, meaning there is more calcium in the body to strengthen and rebuild bones. Studies have shown that people with type 1diabetes who consume high-fibre diets have lower blood glucose levels, and people with type 2 diabetes may have improved blood sugar, lipid and insulin levels. One small papaya provides about 3 g of fibre, which is equivalent to just 17 g of carbohydrates. Papayas contain an enzyme called papain that aids digestion; in fact, it can be used as a meat tenderizer. Papaya is also high in fibre and water content, both of which help to prevent constipation and promote regularity and a healthy digestive tract. The fibre, potassium, and vitamin content in papaya all help to ward off heart disease. An increase in potassium intake along with a decrease in sodium intake is the most important dietary change that a person can make to reduce their risk of cardiovascular disease. Choline is a very important and versatile nutrient found in papayas that aids our bodies in sleep, muscle movement, learning, and memory. Choline also helps to maintain the structure of cellular membranes, aids in the transmission of nerve impulses, assists in the absorption of fat, and reduces chronic inflammation [61, 62].

Papaya is also great for hair because it contains vitamin A, a nutrient required for sebum production, which keeps hair moisturized. Vitamin A is also necessary for the growth of all bodily tissues, including skin. Adequate intake of vitamin C, which papaya can provide, is needed for the building and maintenance of collagen, which provides structure to skin. Papayas are an excellent source of vitamin C, and one single medium fruit provides 22.4% of recommended daily intake.

Nowadays, the mangoes are cultivated in diverse countries including the United States, Mexico and the Caribbean. Originally, Mangoes are from Southern Asia. Mangoes are a tropical fruit and are refreshing, juicy and delicious. They have an inedible skin that ranges in colour depending on the variety from yellow to green through to red-green, whilst inside is a soft, edible yellow flesh and a hard inedible stone. It has some great nutritional benefits. The principal

benefits are: they are high in fibre content, vitamins and minerals composition [63].

Lutein, Fruits and Vegetables

Lutein is a xanthophyll, which belongs to the group commonly known as carotenoids. Lutein is synthesized only by plants and like other xanthophylls is found in high quantities in green leafy vegetables such as spinach, kale and yellow carrots. In green plants, xanthophylls act to modulate light energy and serve as protection agents. Animals obtain the lutein from ingest.

Different studies suggest that lutein and its isomers play important roles in ocular development in utero and during the life span, in vision performance in young and later adulthood, and in lowering risk for the development of common age-related eye diseases in older age. These xanthophyll (oxygen-containing) carotenoids are found in a wide variety of vegetables and fruits, and they are present in especially high concentrations in leafy green vegetables, egg yolks and human milk. The prevalence of lutein, zeaxanthin, and meso-zeaxanthin in dietary supplements is increasing. Particularly in pregnant and lactating women, setting optimal and safe ranges of intake requires additional research. Accumulated evidence about variable interindividual response to dietary intake of these carotenoids, based on genetic or metabolic influences, suggests that there may be subgroups that benefit from higher levels of intake and/or alternate strategies to improve lutein and zeaxanthin status.

Spinach contains several important plant compounds, including lutein, kaempferol, nitrates, quercetin, and zeaxanthin. Lutein and zeaxanthin are linked to improved eye health. Kaempferol is an antioxidant and is thought to be associated with a decreased risk of cancer and chronic diseases. Also, spinach contains high amounts of nitrates, which may promote heart health. Another antioxidant present in spinach is quercetin; this antioxidant may ward off infection and inflammation. Spinach is one of the richest dietary sources of quercetin. It is an extremely healthy vegetable and has been linked to numerous health benefits as it decreases oxidative stress, improves eye health, aids in cancer prevention and helps regulate blood pressure levels [64].

Remember that free radicals are by-products of normal metabolism. When their productions are increased they can cause oxidative stress, which triggers accelerated aging. Correspondingly, this increases the risk of cancer and diabetes [64]. However, spinach contains antioxidants, which fight oxidative stress and help reduce the damage it causes. One controlled trial on healthy people found that spinach helped prevent oxidative damage [65]. Although the study mentioned above was quite small, the findings are backed up by other animal and human studies [66, 67].

Zeaxanthin and lutein are the carotenoids responsible for colour in some vegetables. Human eyes also contain high quantities of these pigments. They help protect our eyes from the damage caused by sunlight [68]. In addition, numerous studies have indicated that zeaxanthin and lutein work to prevent macular degeneration and cataracts, which are the leading diseases that cause blindness [69]. These compounds may even be able to reverse existing damage [70]. There is evidence that these compounds helped to slow tumor growth in a human's cervix. They also decreased the size of the tumor [71]. Several human studies link spinach consumption to a reduced risk of prostate cancer. Eating this leafy green may also help prevent breast cancer [72]. A number of reactive oxygen species are produced during normal aerobic metabolism and these oxidants totally participate in aging and degenerative diseases such as cancer and atherosclerosis by oxidation of DNA, proteins and lipids [73]. Oxidation is not the only reason for cancer; rather inflammation is the other factor for carcinogenesis. Inflammation causes cancer by several mechanisms including the production of free radicals by inflammatory cells [74]. Spinach contains high amounts of antioxidants, which may also aid in cancer prevention [75]. It contains approximately 250 mg of calcium per cup. However, it is less easily absorbed than calcium obtained from dairy sources and makes it more difficult for our bodies to use it. Moreover, it has a high oxalate content, which binds to cal-

cium. Because of the calcium oxalate often present in this vegetable, people who tend to develop kidney stones should not eat large amounts of it [76]. On the other hand, spinach contains very high amounts of vitamin K1. Vitamin K1 serves several functions in the body, but is best known for its role in blood clotting. People who are taking blood-thinners, such as warfarin, may want to closely monitor their vitamin K intake or avoid leafy greens altogether [77]. Additionally, an ion present in this vegetable is iron and this can affect how efficiently the body uses energy. Make sure to combine vitamin-C-rich foods such as citrus fruits with plant iron like spinach to improve its absorption. Magnesium is also present in spinach which is considered the best dietary source of it. This ion is necessary for energy metabolism in maintaining muscle and nerve function, regular heart rhythm, a healthy immune system, and preserving a regular blood pressure. Magnesium also plays a part in hundreds more biochemical reactions that occur in the body.

Spinach has the following possible health benefits: diabetes management because contains an alpha-lipoic acid, which has been shown to lower glucose levels, increase insulin sensitivity, and prevent oxidative, stress-induced changes in patients with diabetes. Studies on alpha-lipoic acid have also showed decreases in peripheral neuropathy and autonomic neuropathy in diabetics [78].

Flavonoids, Fruits and Vegetables

Flavonoids are compounds with the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and a heterocyclic ring (C). This carbon structure can be abbreviated C6-C3-C6. They are widely distributed in plants, and are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. Flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. These compounds may also act as chemical messengers, physiological regulators and cell cycle inhibitors.

Flavonoids are naturally occurring compounds. They are considered secondary metabolites of plants and have numerous therapeutic activities: anti-allergic, anti-inflammatory, antioxidant, antimicrobial (antibacterial, antifungal and antiviral), anticancer and antidiarrhoeal activities [79–84]. Flavonoids have also been shown to inhibit topoisomerase enzymes [84]. However, in most of the above cases no follow-up in vivo or clinical research has been performed, leaving it impossible to say if these activities have any beneficial or detrimental effect on human health.

Flavonoids are difficult to absorb orally because there are highly lipophilic compounds. They experience excessive first pass metabolism on oral administration that hampers their oral bioavailability. In consequence, an alternative topical route of administration has been explored for distribution of several flavonoids [85].

Flavonoids include different subclasses of compounds such as flavones, flavonols, flavanol, isoflavones and anthocyanins.

Flavonols

Flavonols are *O*-glyosidic, kenotic compounds with sugar moiety at the 3-position. Flavonols act as antioxidants and protect reactive oxygen species (ROS) formation. Skin is the most common aim for oxidative stress due to UV, ozone radiations, and other harmful chemicals. The antioxidant property of flavonols is due to the combination of conjugated double bond presenting the C-ring along with neighbouring hydroxyl group in their B-ring.

Quercetin, kaempferol and myricetin are the main compounds in this class. Quercetin is a flavonol abundantly found in leafy vegetables, citrus fruits, berries, etc. Quercetin reduces inflammation and inhibits various inflammatory mediators such as interleukins (IL), prostaglandins, produced by COX, LOX and LPS [86]. It triggers tissue regeneration by promoting the growth of new collagen fibres and producing ground substance to restore the skin structure [87]. Also, quercetin helps in inhibiting all the mediators which cause oxidative stress producing, thus, the

antioxidant effect. It prevents cell death by inhibiting caspase-3 pathways and decreases the level of histidine decarboxylase [88].

Kaempferol is mostly found in berries and plants belonging to species of allium and brassica. This compound possesses anticancer, antioxidant, anti-inflammatory, and anti-allergic activity [89, 90]. Kaempferol inhibits nitric oxide synthase which forms nitric oxide, a pro-inflammatory mediator, and therefore acts as anti-inflammatory agent. However, this favonol undergoes excessive first pass metabolism and therefore, bioavailability is only 2% and hence, topical route is preferred for its delivery. Kaempferol acts as novel agent in treating the UVB-induced tumorigenesis and photo-inflammation.

Flavanols

Flavanols include catechin, epicatechin, epigallocatechin, gallocatechin, and their gallate derivatives (monomeric flavanols) and their polymerization products, proanthocyanidines. In general, they are present in important concentrations in cocoa powder and chocolate, teas, grapes, citrus fruits and in celery [91].

Grape and grape juices are rich in catechin and epicatechin, which are capable of reducing glutamate excitotoxicity and utilize potent antioxidant activity and thus ameliorate endothelial function and reduce platelet aggregation and low-density lipoprotein (LDL) oxidation [92]. In consequence, they reduce the risk of onset or progression of cerebrovascular damage. Results from preclinical and human studies on flavanol-rich cocoa administration (in which epicatechin is the most represented flavanol) have shown that it could result in the reduction of age-related cognitive decline and depression. A high-flavanol dietary supplement administered to elder adults was found to enhance activity in brain regions involved in age-related cognitive decline and to improve performance at cognitive testing [93]. Epigallocatechin gallate, the most abundant flavanol in green and black tea, has shown promising preclinical results in cognitive decline induced by vascular damage [92].

Catechins are the most readily absorbable flavonoids because they are the only form not bound to sugars (flavonoids glicosides are more easily absorbed after transformation in aglycan form) [94]. FDA has approved the use of catechins and its derivatives in various pharmaceutical formulations. Catechins act as antioxidants and are photoprotective, antiaging, anti-inflammatory, anticancer, neuroprotective, cardioprotective, antiviral and antibacterial [95]. Grape seed extract and tea polyphenols are rich in epigallocatechin and epicatechin, showing antioxidant effect by scavenging free radicals [91]. Catechins promote wound healing by scavenging free radicals at the injury site.

Anthocyanins

The beneficial pharmacological activities and possible health benefits of the anthocyanins confer a distinct advantage for their use as food colorants. These phenols have some positive therapeutic effects as anti-inflammatory agents, the prevention of cholesterol-induced atherosclerosis, including micro-circulation diseases [96, 97]. Anthocyanins are predominant pigments of grape skins, which are responsible for the colour of red wine, and also of blueberry, raspberry, black rice, and black soybean, among many others that are red, blue, purple or black. Some of the colours of autumn leaves are derived from anthocyanins and their colour may appear red, purple, or blue, depending on their pH. Usually, anthocyanins are associated with fruit but they are also present in vegetables, roots, legumes and cereals as well [98, 99]. They are water-soluble pigments and this characteristic is important for their large-scale extraction [100]. Red grape and wine have phenolic compounds present in the berry skin and wine and they are responsible for their blue and red colours.

These compounds are excellent antioxidants because they are easily oxidized under circumstances of stress, with reactive oxygen species present. This capacity is very important for the humans because contribute to the fruits and vegetables protective effect regarding degenerative

and chronic diseases [101–103]. The extracts of some plants and fruits with high phenolic compounds content have effects after human intake as mutagenesis and carcinogenesis inhibitors, anti-inflammatory agents, to prevent cholesterol-induced atherosclerosis, including micro-circulation diseases [96, 104–106]. Indeed, pills or powders rich in polyphenols made out of grape berry skins are already available on the market, and are preferred by naturists over conventional medicine.

In 1998, Renaud and Guegen reported a comparatively lower incidence of coronary heart disease in France in the face of high levels of saturated fat in the traditional French diet, commonly called 'The French paradox'. The wine contributes to this effect, but a moderate daily consumption of red wine [107]. Supplemental epidemiological studies from diverse populations have revealed that individuals who habitually consume moderate amounts of wine experience a 20–30% reduction in all-cause mortality, particularly cardiovascular mortality, when compared with individuals who abstain or who drink alcohol to excess [108]. There are studies performed in animals and humans that support a connection between regular moderate wine drinking and improved health. Although, there is evidence that supports the health benefits derived from grapes, grape juice, and grape seed extract. These products have been used to treat a variety of conditions, including cancer, cardiovascular disease, ischemic stroke, neurodegenerative disorders, aging, hypertension, hyperlipedemia and dental caries [109–111]. While wine has similar chemical constituents as grapes, the therapeutic effects have been attributed to wine more than the grapes. Some investigators believe these benefits may actually be enhanced in wine, possibly due to additive effects with the alcohol component of wine and/or to an increased bioavailability of wine polyphenols as a result of the fermentation process.

The cherry fruit is a nutrient-dense food with relatively low caloric content and significant amounts of important nutrients and bioactive food components including fibre, polyphenols, carotenoids, vitamin C, potassium, tryptophan, serotonin and melatonin [112, 113]. There are many cultivars of cherries, but they are grouped in two major types: sweet (*Prunus avium* L.) and tart (*Prunus cerasus* L.) cherries. The majority of sweet cherries are consumed fresh, and approximately 20–25% is processed: brined, canned, frozen, dried or juiced. The cherries are rich in polyphenols [112, 114]. Polyphenols concentration and composition of cherries varies in relation with many factors including the cultivar, stage of ripening, portion of fruit, storage, among others [97]. The major anthocyanins are cyanidin-3-glucoside and cyanidine-3-rutinoside. In addition to anthocyanins, cherries are also rich in hydroxycinnamates and flavin-3-ols. Hydroxycinnamates and flavin-3-ols, respectively, make up about 25–50% and 5–40% of the total phenolic in cherry depending on the cultivar.

Consequently, there is varied range in the concentration of anthocyanins in the different cultivars of cherries which may be due to the factors listed above and the precision of the analytical methods used. Further analyses under identical conditions are needed to compare the phenolic composition of specific cultivars of cherries.

In addition, cherries have another antioxidant which is associated to sleep regulation: melatonin. The antioxidant capacity of cherries varies but it have the oxygen radical absorbing capacity (ORAC) and ferric reducing ability of plasma (FRAP) assays, however, in a liposome-based system; the sweet cherries exhibited the highest antioxidant activity [115, 116].

Both anthocyanins and hydoxycinnamates are believed to be rapidly absorbed in humans reaching maximum plasma concentrations in less than 2 h and are quickly eliminated [117, 118]. Diverse findings suggest that anthocyanins have a minimum of 12.3% bioavailability and their metabolites continue in circulation longer than previously believed. Further studies are needed to confirm the bioavailability of anthocyanins. Given the high concentrations of bioactive compounds (e.g. anthocyanins, hydoxycinnamates, flavin-3-ols) in cherries, it is not surprising that cherry consumption promotes health. Numerous authors showed in animal and human studies that consumption of cherries may reduce the risk of

several chronic inflammatory diseases including, arthritis, cardiovascular disease (CVD), diabetes, and cancer. Additionally, there is evidence that cherry consumption may improve sleep, cognitive function, and recovery from pain after strenuous exercise [112, 119–121].

Antioxidants help neutralize the free-radical cellular damage that accumulates in the body through factors such as stress, pollution and a poor diet. Free-radical damage can result in degenerative diseases such as cancer. Two of the most potent anticancer agents – the flavonoids isoquercitrin and queritrin – are found in tart red cherries.

Cherry juice is also thought to be effective in preventing heart disease. In addition to its antioxidants, cherries are high in potassium, an important mineral for those with high blood pressure. The anthocyanins found in this fruit may also protect artery walls from damage that leads to plaque buildup and heart disease similar to what was described above.

Blueberries are not actually blue, but deep purple, which is the colour of anthocyanin, a pigment that is especially rich in blueberries.

Blueberries are known to be high in antioxidants, anthocyanins, phenols and polyphenols which are good for the human body. Anthocyanin, phenols and polyphenols and your health benefits were described earlier.

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Prospects of Herbal Remedies in Neuropsychiatric Diseases from the Gut–Brain Communication Perspective

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The Gut Microbiota in Health and Disease

The human gastrointestinal tract is colonized by a vast dynamic ecosystem of over 100 trillion (10^{14}) microorganisms comprising mostly bacteria, but also fungi, protozoa and viruses [1]. The number of microbial cells we carry vastly outnumber our own cells (3.72×10^{13}, [2]). In terms of genomics, the gut microbiota possesses a gene set 150-fold larger than the human genome [3, 4]. The biological significance of the gut microbiota is now well acknowledged – it has been described as an essential "virtual organ" of the body. Hence, a healthy ecosystem of gut microbiota is considered necessary for the normal development and health of its host. Conversely, its imbalance, known as dysbiosis, is associated with dysfunction and disease. Local effects of dysbiosis are readily implicated in intestine-related diseases such as irritable bowel syndrome, inflammatory bowel diseases [5], and metabolic diseases such as diabetes and obesity [6], but there is increasing evidence that dysbiosis may also contribute to a wide spectrum of chronic conditions. These range from hypertension [7], atherosclerosis [8] and cancer [9] to mental disorders such as depression, anxiety and schizophrenia [10].

First, the gut microbiota is necessary for optimum health and basic function of the gastrointestinal tract. It contributes directly to the metabolism of undigested and undigestible carbohydrates and proteins, and synthesis of vitamins and other essential bioactive substances, such as the short-chain fatty acids (SCFAs) [11, 12]. Butyrate, one of the SCFAs, is a key regulator of the assembly of tight junctions crucial to the integrity of the intestinal mucosal barrier [13, 14]. The gut microbiota also assume a critical protective role against invasion of pathogenic bacteria through *colonization resistance* [15]. Our resident gut bacteria not only act as a physical barrier to exogenous microbes, but also release antimicrobial substances to curtail incursion by foreign microorganisms as well as inhibit the growth of harmful indigenous species. It is now known that *colonization resistance* also

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depends on interaction with the host's systemic immune responses [16]. Indeed, the development and maintenance of the intestinal immune system are tightly regulated by the gut microbiota. Some of the most convincing demonstrations are derived from germ-free (GF) mice raised through generations in laboratory conditions devoid of all microorganisms.

Notably, GF mice are known to develop Peyer's patches and mesenteric lymph nodes in the gut indicative of deficient development of gut-associated lymphoid tissues. The levels of secretory IgA, the numbers of CD4+ T-cells, including T_H17 cells and $CD4^+$ $CD25^+$ T_{reg}-cells are all reduced in the guts of GF mice. Beyond the impact on the intestinal barrier and intestinal immune system, GF animals also show defects in metabolism, vasculature and behaviour [17, 18]. Although the extent to which their behavioural defects (e.g. altered stress response, anxiety-like behaviour) reflect nervous system dysfunction (rather than being secondary to the presence of other systemic dysfunctions) arguably still remains uncertain, structural changes in the brain are clearly evident in these animals. Brain morphological changes reported in GF mice include increased volume of the amygdala and hippocampus, even though the total volume of their brain is largely comparable with normal *microbe-colonized* mice [19].

Additional evidence for a possible causal relationship between gut microbiota and neural development comes from microbiota transplant experiments. Transplanting microbiota harvested from infant mice with phenotypes of retarded growth to the guts of GF mice could reproduce the retarded growth phenotypes in the recipient animals, including suppression of neuronal development and myelination [20]. This study isolated the importance of gut microbiota in modulating brain development. Secondly, given the defects in vasculature in GF mice, and that normal tight junction assembly in the intestine requires the presence of gut microbiota, it is not surprising that the blood-brain barrier (BBB) of GF mice is also reportedly "leaky" with increased permeability [21]. The specific link of leaky BBB with the absence of gut bacteria is supported by rescue experiments, in which the expression of tight junction proteins in the BBB of GM mice could be successfully upregulated following transplantation of gut microbiota harvested from normal mice [22].

The Gut–Brain Axis

The gut microbiota is an integral component of the "gut–brain axis". The term "gut–brain axis" has been around for decades, which encapsulates the interaction and communication between the enteric nervous system and the central nervous system [23]. Considering the pivotal roles played by gut microbes mentioned above, the "gut–brain axis" may be more accurately understood as a "microbiota–gut–brain axis" [24, 25]. Signalling within this triadic network is bi-directional (Fig. 26.1). While the gut microbiota may influence brain activity and modify behaviour, the gut microbiota are also modifiable by signals originating from the brain. Thus, the brain can alter the composition of the gut microbiota and their influence within the axis [26].

The direct as well as indirect connections between the gut microbiota and the brain have been implicated in psychiatric and behavioural disorders. The seminal paper by Sudo et al. [27] has often been cited as the first demonstration of a potential causal link. They showed that GF mice exhibited a stronger response to stress stimuli; and that the elevated stress response via the hypothalamic–pituitary–adrenal (HPA) stress axis as well as the associated anxiety traits could be partially normalized by re-colonization of the gut [27]. Although one may not exclude the contribution of microbiota in other organs to the aetiology of the abnormal psychophysiology in the GF mice, the normalization of anxiety-related behaviour pinpoints the importance of gut bacteria – a critical finding that has been consistently replicated [28, 29].

Attempts to manipulate the gut microbiota in normal animals have also yielded evidence for specific behavioural and neural effects. The chronic administration of the bacterial strain *Lactobacillus rhamnosus* (JB-1) in normal mice

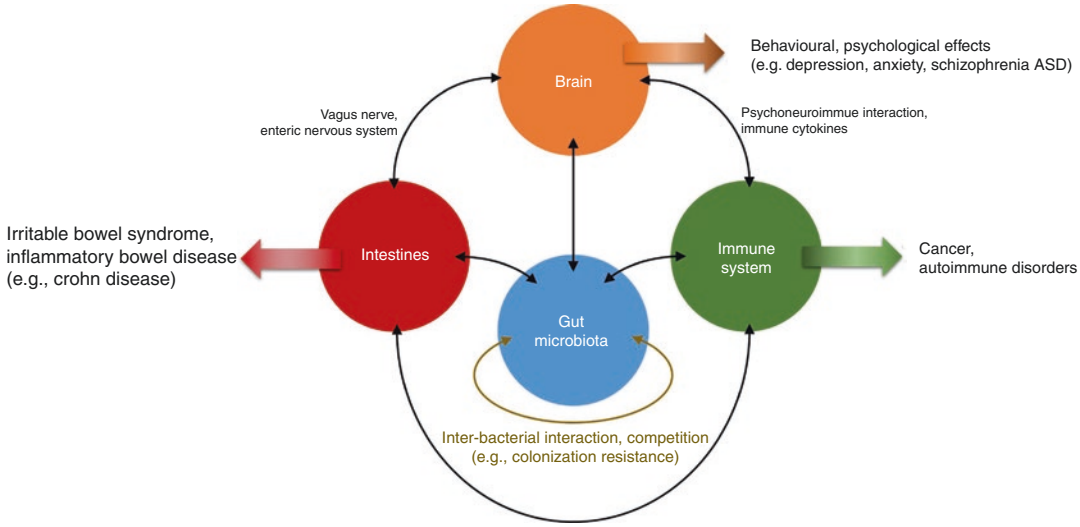

Fig. 26.1 The conception of the "microbiota–gut–brain" triadic network

was found to produce an antidepressant-like effect. The behavioural change has been attributed to the modification of inhibitory neurotransmission mediated by gamma-aminobutyric acid (GABA) in the multiple brain regions, leading to increased excitation in cortical regions and decreased signalling in the hippocampus, amygdala and locus coeruleus [30]. The antidepressant effect can be abolished by vagotomy, suggesting that the vagus nerve could be a major conduit for a direct communication between the gut bacteria and the brain [30].

Increased intestinal permeability and higher expression levels of intestinal inflammatory mediators have been shown in a mouse model of autism spectrum disorder (ASD) based on the neurodevelopmental impact of gestational immune activation. While one may not conclude from this correlative finding alone that the intestinal phenotypes may be causally related to the behavioural ASD-related phenotypes of this mouse model, the demonstration of a successful rescue by gut microbiota intervention has lent some stronger support – which highlights incidentally a novel risk factor of ASD. In the mouse gestational immune activation model of ASD, treatment with the human commensal bacterium *Bacteroides fragilis* not only restored the defective intestinal barrier and altered the serum metabolomic/cytokine profile but also ameliorated multiple ASD-related anxiety and attentional deficits of the resulting offspring. *B. fragilis* was effective in suppressing the massive increase of one specific metabolite, 4-ethylphenylsuphate, produced by *Clostridium* spp. in the ASD mouse model. These findings support a connection between the microbiome, gut and brain, whereby the composition of gut bacteria can affect the host's metabolome to alter behaviour from a neurodevelopmental perspective [31]. This study has been recognized as an important proof-of-concept that microbial metabolites provide a molecular connection between the gut and the brain [32].

Mechanisms of Gut Microbiota in Modulation of Neuropsychiatric Disease

The composition and diversity of gut microbiota has been linked to many major neuropsychiatric disorders, including autism spectrum disorder [33, 34], depression [35, 36], anxiety, attention deficit hyperactivity disorder [37], bipolar disorders, and schizophrenia [38].

However, it remains unclear the extent to which specific changes in the microbiota causally contribute to disease aetiology and progression,

or if such changes are secondary consequences of the disease in question. Support for substantive mechanistic links has been strengthened by recent studies highlighting the multiple pathways involved. The crosstalk between the gut microbiota and brain could be mediated through neural (neurotransmitters), immune (cytokines), neuroendocrine (hormones) pathways, and bacterial metabolites [39–41]. However, a given change in gut microbiota may often influence all pathways in parallel – directly or indirectly. It is therefore challenging to delineate the precise mechanism of any single effect, especially when the complexity of the intestinal microflora is further scrutinized. Furthermore, most of the current mechanistic knowledge is derived from experiments in laboratory rodents. Inter-species divergence in gut microbiota is a potential barrier to translation. To overcome this, epidemiological studies as well as clinical trials in humans, across regions and ethnicity, would be necessary.

Neurotransmission Gut bacteria are capable of modifying communications mediated by neurotransmitter and neuromodulator molecules at synaptic junctions through multiple mechanisms. Neurotransmission is essential for computational processes and neural plasticity central to all nervous system functions. Dysregulation of brain neurotransmission is implicated in the pathophysiology of neuropsychiatric conditions – from depression, anxiety, schizophrenia, mania, to Parkinson's disease – and the receptors, synthesis, and re-uptake of specific neurotransmitters are common targets for nearly all major neuropsychiatric drugs. Within this neurocentric view of psychiatry, *probiotics* for mental health has become a distinct possibility, with the ability of gut bacteria to modulate multiple neurotransmission pathways in the host's nervous system. Dysbiosis is associated with changes in receptor expression and neurotransmitter turnover in the brain across multiple neurotransmitter systems,: including catecholamines, GABA, serotonin, melatonin and acetylcholine. In addition, the expression of brain-derived neurotrophic factor (BDNF) is also sensitive to changes in the gut microbiota [42], which is closely involved in some forms of memory and learning as well as the pathogenesis of depression and anxiety.

Some bacteria have evolved the ability to respond and release neurotransmitter-like molecules to "communicate" with the host nervous system. Relatively high levels of catecholamine neurotransmitters have been observed in biomasses formed by *Escherichia coli, Proteus vulgaris, Serratia marcescens, Bacillus subtilis or Bacillus mycoides* [43]. A specific serotype of *E. coli* (O157:H7) can locate their presence in the intestines by sensing adrenaline and activating essential genes for colonization, which can be blocked by α- and β-adrenergic antagonists [44]. It has been suggested that such bacteria have adapted their inter-bacterial communication (quorum sensing) system to communicate with the host. Indeed, the levels of dopamine and noradrenaline in gut lumens are determined by gut microbiota composition. Compared with typical mice raised in specific-pathogen-free (SPF) conditions, the levels of free dopamine and noradrenaline are lower in GF mice [45]. Introduction of *E. coli* into the guts of GF mice could restore gut catecholamine content to SPF mice levels, and this restoration was dependent on the activity of the enzyme, β-glucuronidase.

Another monoamine neurotransmitter in the host that may be regulated by gut microbiota composition is serotonin. This is mediated by specific metabolites produced by spore-forming bacteria indigenous to humans and mice. Such bacterial metabolites could stimulate the release of serotonin by enterochromaffin cells and thus interfere with the regulation of GI motility and platelet function in the colon [46].

Finally, chronic treatment of common probiotic lactic acid bacteria – *Lactobacillus reuteri* and *Lactobacillus rhamnosus* – could lead to an anxiolytic and antidepression profile, together with a reduction in the release of corticosterone in response to stress [30, 47, 48]. These effects are attributed to multiple region-specific changes in GABA receptor expression in the brain. GABA-B 1b subunit expression was elevated in the cingulate and prelimbic cortices but reduced in the hippocampus, amygdala and locus coeruleus. On the

other hand, the expression of α2 GABA-A receptors was elevated in the hippocampus but reduced in the amygdala and prefrontal cortex [30]. The pivotal role of the vagal connection in mediating these diverse behavioural and molecular pharmacological effects is demonstrated by the ability of vagotomy prior to probiotic treatment to prevent such effects.

Immune Cytokines The gut microbiota's influence on the immune system is readily detectable through changes in the blood levels of immune cytokines. The resulting changes in the circulating levels of pro-inflammatory and anti-inflammatory cytokines are capable of bringing about structural as well as functional changes in the nervous system, including the brain. Chronic inflammation is associated with dysfunction in serotonergic, noradrenergic, and dopaminergic transmission as well as neurogenesis in the brain – all have been linked to various psychiatric conditions [49–52]. In addition, increases in the gene expression of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, and TNF-α) in the brain have been associated with hippocampal and cerebral damage [53].

Mechanistically, one immediate impact of dysbiosis is to undermine the intestinal epithelium and mucous layers as a physical barrier to bacterial incursion. The resulting increase in epithelial permeability or "leaky gut" accelerates the diffusion of bacterial metabolites into the systemic blood circulation, which may cause immune reactions [54]. It has been suggested that chronic low-grade inflammation present in some patients with depression is associated with leaky gut [55]. This is in keeping with the inflammatory hypothesis of depression, which suggests that persistent increased inflammation plays a causative role in depression [49], including relapse [56], and underlies drug resistance in some cases [57, 58]. The contribution of leaky gut in other psychiatric conditions may include bipolar disorder, schizophrenia and autism. Increased genes expression of inflammatory cytokines has been reported in post-mortem brain materials in people who had depression, bipolar disorder and schizophrenia [59]. Similarly, elevation of pro-inflammatory cytokines (e.g. TNF, IL-6, and monocyte chemotactic protein 1) has been reported in autism [60, 61].

Parkinson's disease is a neuropsychiatric condition with evidence for microbiota imbalance. The relative abundance of *Parabacteroides*, *Verrucomicrobia*, *Akkermansia*, *Butyricimonas*, *Veillonella*, *Odoribacter*, *Mucispirillum*, *Bilophila*, *Enterococcus*, and *Lactobacillus* was elevated in patients relative to controls. Amongst these changes, plasma levels of TNF-α and IFNγ correlated with the abundance of *Bacteroides* (a genus of Gram-negative, obligate anaerobic bacteria) and bacteria of the phylum *Verrucomicrobia* [62]. A mechanistic link between gut microbiota and α-synuclein pathology in the brain of Parkinson's disease patients has been proposed [63]. It may open new interventions utilizing prebiotics, probiotics and synbiotics [64].

Neuroendocrine Signalling Molecules The composition of the gut microbiota is sensitive to multiple endocrine factors including brain–gut peptides, leptin, corticotrophin-releasing factor (CRF), adrenocorticotropic hormone (ACTH), corticosterone and other hormones [65]. Activation of the HPA axis by stress and the release of corticosterone into the blood circulation could modulate how the gut microbiota may affect our bodily functions. For instance, stress may increase the permeability of the gut (thus compromising its barrier function and result in a "leaky gut") and the production of inflammatory cytokines by immune cells [52] as summarized above. In addition, the composition and activity of the microbiota, including bacterial gene expression and inter-bacteria signalling, can be modulated by stress hormones (e.g. noradrenaline) released by the host [66]. The bacteria-host signalling system is therefore clearly reciprocal when we take into account that the sensitivity of the stress axis can be modified by the composition of the gut microbiota. For instance, combined treatment with *Lactobacillus helveticus* and *Bifidobacterium longum* could effectively suppress the release of corticosterone in response to stress in mice [67].

Gut Bacteria Metabolites The microbiota can exert substantial impact on the host's metabolomic profile through the production of molecules, including bacterial metabolites and neuroactive signalling molecules [68]. A host of microbial-derived molecules are responsible for communication from the bacteria to the host's brain. Some metabolites are capable of crossing the intestinal barrier into systemic circulation, and can also pass through the BBB [26, 46, 69]. These include tryptophan metabolites, secondary bile acids, and microbial-derived SCFAs (e.g. acetate, butyrate, isobutyrate, hexonate, and propionate) [70, 71]. They may bind to receptors on enteroendocrine cells and/or be taken up by the intestinal epithelial cells with consequence in the host's mucosal immune and nervous functions. SCFAs are primary bacterial metabolites with potent anti-inflammatory properties, and are commonly associated with *Eubacterium, Rosebery, Faecalibacterium, Bifidobacterium, Lactobacillus and Enterobacter* species [72]. They typically decrease intestinal permeability, enhance immune system functioning, alter lipoprotein profiles, and decrease colonic pH [73]. They also bind to G-protein-coupled receptors (e.g. GPR41) and stimulate the release of neuropeptides, thus modulating enteroendocrine signalling [74]. Beyond that, more extensive influence on energy homeostasis and even behaviour may be realized through the actions of enteroendocrine neuropeptides on both enteric and afferent vagal pathways [75], as mentioned before.

Gut Microbiota in Depression and Anxiety

Although notable changes in gut microbiota associated with specific psychiatric diseases are often reported between specific patient groups, it remains difficult to identify consistent differences. Table 26.1 summarizes the major differences reported in three studies examining faecal samples obtained from patients with major depressive disorder (MDD) or general anxiety disorder (GAD) in comparison with healthy controls [76–78]. Whilst each study could identify significant changes in the relative abundance of specific groups of bacteria in both directions, these are hardly consistent across the selected studies. At the genus level, there is little overlap, although a reduction in *Prevotella spp*. appeared consistent [76, 77]. At the phylum level, only the increase of Actinobacteria in MDD patients was commonly reported in these two studies, but changes in three other phyla (Bacteroidetes, Proteobacteria, Firmicutes) identified were opposite between these two studies. The changes reported in MDD patients in these two studies are as different from each other as they are in comparison with the pattern reported in GAD patients. Similarly, neither the correlation between the abundance of *Faecalibacterium* and severity of depressive symptoms [77] nor the correlation between *Holdemania* abundance and anxiety symptoms in MDD patients [76] was replicated between studies. It seems premature to rely on such findings to direct therapeutic microbiota treatment. The diversity in diets amongst humans as well as systematic differences between geographical populations could add to the difficulty in comparison between studies. Nonetheless, correlative links between microbiota changes and symptom severity [76, 77] or remission [78] are encouraging when considered against the proof-of-principle studies in animals. However, it remains contentious whether the microbiota differences reported in human epidemiological studies may be consequential to changes in mental state instead of a causal factor. A new approach seems necessary to overcome this roadblock.

Animal models help overcome some of these interpretative difficulties of human data despite limitations of their own. First, laboratory animals of comparable age and genetic background may be used under highly regulated and stable diets between conditions. Second, baseline differences can be effectively minimized by thorough randomization of group allocation. Third, the experimental manipulations used to induce behavioural changes relevant to mental dysfunction are well

Table 26.1 Summary of three human studies examining gut microbiota differences in patients with major depressive disorder or general anxiety disorders

Psychiatric conditions	Microbiota (by phylum) Change in relative abundance		Microbiota (by genus) Change in relative abundance		
Major depressive disorder [77]	Bacteroidetes	↑	*Alistipes*	↑	
	Proteobacteria	↑	*Parabacteroides*	↑	
	Fusobacteria	↑	*Phascolarctobacterium*	↑	
	Actinobacteria	↑	*Rosebery*	↑	
	Firmicutes	↓	*Dialister*	↓	
			Faecalibacterium	↓	
			Prevotella	↓	[a]
			Ruminococcus	↓	
Major depressive disorder [76]	Actinobacteria	↑	*Bifidobacterium*	↑	
	Firmicutes	↑	*Blautia*	↑	[b]
	Proteobacteria	↓	*Prevotella*	↓	
	Bacteroidetes	↓			
General anxiety disorder [78]	Fusobacteria	↑	*Bacteroides*	↑	[c]
	Bacteroidetes	↑	*Escherichia-Shigella*	↑	
			Ruminococcus gnavus	↑	
			Lactobacillus	↑	
			Fusobacterium	↑	
	Firmicutes	↓	*Faecalibacterium*	↓	
			Eubacterium rectale	↓	
			Roseburia	↓	
			Subdoligranulum	↓	
			Lachnospira	↓	

Upper (↑) and lower (↓) arrows indicate the direction of change in the relative abundance of the respective genus and/or phyla of bacterium in the gut

[a] *Faecalibacterium* negatively correlated with depressive symptoms severity

[b] *Firmicutes Holdemania* positively correlated with anxiety symptoms in patients

[c] Lower relative abundance of *Bacteroides spp*. was related to remission

characterized and repeatable across laboratories. Table 26.2 summarizes a selection of seven rodent studies using diverse approaches to induce anxiety and depression-like behaviour and standard preclinical tests. Across a range of animal models of depression involving chronic stress as well as high-fat diet, the relative abundance of bacteria belonging to the phylum Proteobacteria (including genus: *Helicobacter*, *Flexispira*, *Helicobacter*, *Parasutterella* and *Klebsiella*) was consistently elevated [79–85]. In this list, the chronic alcohol exposure model in rats appears to be the exception – in which a decrease of Proteobacteria (*Helicobacter*) is reported instead [85]. In contrast, phyla Bacteroidetes, Firmicutes and Verrucomicrobia yielded less consistent changes across this set of studies.

A more exhaustive meta-analysis is certainly warranted, but this brief selection serves to illustrate the daunting task of identifying the signatures of dysbiosis in relation to specific psychiatric conditions in order to guide therapeutics with prebiotics, probiotics or symbiotics. The gut microbiota is a dynamic ecosystem, such that fluctuation of its composition in response to both internal and external factors (including the host) is the norm rather than the exception. Hence, the impact of any prebiotic/probiotic manipulation on the gut microbiota, no matter how specific it may seem, must be considered holistically. We may not exclude the possibility that apparent divergent patterns of microbiota changes could exert similar impacts on the host's physiology and behaviour. Such empirical considerations

Table 26.2 The impact of seven rodent models of depression and/or anxiety on gut microbiota

Animal models of anxiety/depression	Model species	Microbiota (by phylum) Change in relative abundance		Microbiota (by genus) Change in relative abundance	
Chronic unpredictable mild stress [80]	Mouse	Verrucomicrobia	↑	*Akkermansia*	↑
		Proteobacteria	↑	*Helicobacter*	↑
				Parasutterella	↑
				Alistipes	↑
				Odoribacter	↑
				Turicibacter	↑
				Barnesiella	↓
				Bifidobacterium	↓
				Lactobacillus	↓
				Olsenella	↓
Chronic mild stress [79]	Mouse			*Lactobacillus*	↑
Chronic mild social defeat stress [82]	Mouse	Deferribacteres	↑	*Oscillospira*	↑
		Proteobacteria	↑	*Bacteroides*	↑
				Flexispira	↑
		Actinobacteria	↓	*Akkermansia*	↓
		Verrucomicrobia	↓	*Ruminococcus*	↓
				Paraprevotella	↓
				Dorea	↓
Chronic restraint stress [81]	Mouse	Bacteroidetes	↑	*Bacteroides*	↑
		Firmicutes	↓	*Lactobacillus*	↓
Chronic restraint stress [83]	Mouse	Proteobacteria	↑	*Klebsiella*	↑
				Helicobacter	↑
		Bacteroidetes	↓		
High-fat diet [84]	Mouse	Proteobacteria	↑		
		Deferribacteres	↑		
		Bacteroidetes	↓		
		Tenericutes	↓		
Chronic alcohol exposure [85]	Rat	Bacteroidetes	↑	*Adlercreutzia*	↑
		Actinobacteria	↑	*Allobaculum*	↑
		Cyanobacteria	↑	*Turicibacter*	↑
		Firmicutes	↓		
		Proteobacteria	↓	*Heliobacter*	↓

Upper (↑) and lower (↓) arrows indicate the direction of change in the relative abundance of the respective genus and/or phyla of bacterium in the gut

[a]*Akkermansia* negatively correlated with depressive-like behaviour

[b]The relative abundance of *Adlercreutzia* negatively correlated with alcohol preference and locomotor activity

are in keeping with traditional herbal medicines, which, by nature, comprise multiple active ingredients and target multiple targets. Next, we examine how herbal therapies with potential psychiatric application could resolve dysbiosis as part of their mechanisms.

Herbal Modifications of Gut Microbiota

Herbal supplements have gained considerable popularity as complementary and alternative medicines. Many are derived from traditional

Chinese medicines, with documented applications in psychiatric conditions or the maintenance of mental well-being. Herbal supplements or medicines are consumed in a form comprising a multitude of active ingredients, whether they take the form of single herbs or formulae comprising specific mixtures of herbs (Table 26.3). They are thus intended to act on multiple targets including, not surprisingly, the gut microbiota [86].

Many candidate gut bacteria strains involved in psychiatric disorders and behavioural changes in animals (see Tables 26.1 and 26.2) are also sensitive to common herbal medicines or herbal formulae that take the form of single herbs as well as formulae consisting of mixtures of herbs (Table 26.4). Notably, Gegen Qinlian Decoction is highly effective in reducing the abundance of Bacteroidetes strains while elevating Proteobacteria strains and the Firmicutes strain, *Faecalibacterium*. These include changes that parallel those that accompany remission in general anxiety disorders, and less severe depressive states (see Table 26.1) [77, 78, 87]. Daesiho-Tang was shown to be effective in increasing the abundance of several Firmicutes strains, which may potentially counter its downregulated status found in MDD and GAD (see Table 26.1) [77, 78, 88]. The elevation of the relative abundance of *Akkermansia spp.* by *Flos Lonicera*, *Antrodia cinnamomea*, Mulberry leaves, and Daesiho-Tang [82, 89–92] may counter the increased

Table 26.3 The names and composition of herbal ingredients of common herbal decoctions and formulae and with claimed therapeutic efficacy for treating behavioural and mental symptoms

Formula	Major ingredients by plants	Ref
Rhubarb Peony decoction	*Rhei Radix et Rhizoma* *Moutan cortex* *Persicae semen* *Natrii sulfas* *Benincasae semen*	[116]
Gegen Qinlian decoction	*Radix Puerariae* *Radix Scutellariae* *Rhizoma Coptidis* *Honey-fried Radix Glycyrrhizae*	[87]
Bawei Xileisan	*Exocarpium Citrulli Galx* *Calculus Bovis* *Margarita* *Sal Sedatirum* *Borneolum Syntheticum* *Sal ammoniac* *Indigo Naturalis*	[117]
Daesiho Tang	*Bupleuri radix* *Pinelliae rhizome* *Zingiberis rhizome* *Scutellariae radix* *Paeoniae radix* *Zizyphus fructus* *Ponciri fructus* *Rhei undulati rhizome*	[89]
Hugan Qingzhi tablet	*Rhizoma Alismatis* *Fructus Crataegi* *Pollen Typhae* *Folium Nelumbinis* *Radix Notoginseng*	[118]
Shenzhu Tiaopi	*Codonopsis pilosula* *Rhizoma Atractylodis* *Pinellia* *Poria cocos* *Pericarpium Citri Reticulatae* *Coptis chinensis Franch* *Pueraria*	[119]
Tiansi liquid	*Morinda officinalis* *Cuscuta chinensis*	[98]
Xiaoyaosan	*Radix Angelicae Sinensis* *Radix Paeoniae Alba* *Poria* *Radix Bupleuri* *Radix Glycyrrhizae* *Rhizoma Atractylodis Macrocephalae* *Herba Menthae* *Rhizoma Zingiberis Recens*	[99, 120]
ZiBuPiYin recipe	*Radix Ginseng* *Radix Paeoniae Alba* *Rhizoma Dioscoreae* *Semen Lablab Album* *Poria* cum *Radix Pini* *Lignum Santali Albi*	[95, 121]

Table 26.4 The effects of herbal treatment either as single herbs or formulae on selected gut microbiota by phyla and genus

		Single herb						Herbal formula						
Phylum	Genus	Flos Lonicera	Ganoderma lucidum	Berberine	Panax ginseng	Antrodia cinnamomea	Mulberry leaves	Rhubarb Peony	Gegen Qinlian	Bawei Xileisan	Daesiho-Tang	Xiaoyao-san	Hugan Qingzhi	Shenzhu Tiaopi
Actinobacteria								↑						
Bacteroidetes								↓			↑		↑	↓
Firmicutes							↓	↑			↓	↓	↓	
Proteobacteria			↓		↑		↓	↓				↓		
Bacteroidetes	*Bacteroides*	↑	↑	↑			↑		↓	↑	↑			
	Alistipes								↓				↑	
	Barnesiella								↓					
	Odoribacter								↓					
	Parabacteroides								↓					
	Prevotella										↑		↓	
Firmicutes	*Blautia*												↓	
	Roseburia										↑			
	Ruminococcus										↑		↑	
	Allobaculum													↓
	Faecalibacterium								↑					
	Lachnospira					↓							↑	
	Lactobacillus			↑	↑					↑	↑			
Proteobacteria	*Escherichia-Shigella*								↑					
	Parasutterella				↑				↑					
Verrucomicrobia	*Akkermansia*	↑				↑	↑				↑			
	Ref	[91]	[122]	[123]	[124]	[92]	[90]	[116]	[87]	[117]	[89]	[120]	[118]	[119]

Upper (↑) and lower (↓) arrows indicate the direction of change in the relative abundance of the respective genus and/or phyla of bacterium in the gut

abundance of this strain in association with the presence of depressive symptoms. In addition, reduced *Lactobacillus spp*. counts that have been associated with MDD [93] were demonstrated to be increased by Berberine, *Panax ginseng*, Bawei Xileisan, and Daesiho-Tang.

The impact on the gut microbiota by commercially available herbal mixtures (viz., ZiBuPiYin, Tiansi Liquid and Xiaoyaosan) that are currently being consumed for their potential neuropsychiatric effects have been investigated in animal models of depression and anxiety. Table 26.5 summarizes the associated gut microbiota that might be mechanistically linked to their claimed ability to modify behaviour in the relevant animal models of mood disorders.

ZiBuPiYin – a recipe derived from the Zicheng Decoction first recorded by Cheng Wu [94] – has been prescribed for the treatment of memory loss in people with diabetes. Its potential efficacy against psychological disturbances common amongst diabetes has also been demonstrated in Zucker diabetic fatty rats. The stress-induced expression of anxiety and depression-like behaviour as well as the suppression of two bacteria strains (*Roseburia* and *Coprococcus*) in this rat model could be reversed by ZiBuPiYin [95]. This raises the possibility that the normalization of gut microbiota may contribute to the reported pro-cognitive effects of ZiBuPiYin in addition to its antioxidant, anti-amyloid and neuroprotective effects at the cellular and structural levels [94].

Another herbal formula, known as Tiansi Liquid, contains extracts from plants *Morinda officinalis* and *Cuscuta chinensis*, and is also prescribed for depression in China [96, 97]. Similarly, Tiansi Liquid was reported to reverse gut microbiota changes in a rat model of hydrocortisone-induced depression, which paralleled its antidepressant efficacy [98]. It is suspected that the antidepressant effect of Tiansi Liquid is linked to elevated serotoninergic activ-

Table 26.5 Gut modulation in animal models of mood disorders by commercially marketed herbal medicines

Commercial Formulae	Preclinical animal models	Reversal of gut microbiota changes associated with the depression models	
		At phylum level	*At genus / group level*
ZiBuPiYin [95]	Psychological stress in Zucker diabetic fatty rats	Increased abundance of phylum **Firmicutes**	*Roseburia*
			Coprococcus
		Reduced abundance in phylum **Bacteroidetes**	
Tiansi Liquid [98]	Hydrocortisone model in Sprague Dawley rats	Reduced abundance in phylum **Bacteroidetes**	*Bacteroides*
		Reduced abundance in phylum **Firmucutes**	*Ruminococcaceae UCG-013*
			Ruminococcaceae UCG-014
			Lactobacillis
			Lactococcus
Xiaoyaosan [99]	Chronic restraint stress in Sprague Dawley rats	Increased abundance in phylum **Bacteroidetes**	*Prevotellaceae Ga6A1*
		Increased abundance in phylum **Proteobacteria**	*Desulfovibrio*
		Reduced abundance in phylum **Firmucutes**	*Ruminiclostridium 6*
			Ruminiclostridium
			Anaerotruncus
		Increased abundance in phylum **Chloroflexi**	
		Increased abundance in phylum **Planctomycetes**	

ity resulting from the suppression of rate-limiting catabolic enzymes in the breakdown of serotonin: tryptophan-2,3-dioxygenase and indoleamine 2,3-dioxygenase [98].

The Chinese herbal formula, Xiaoyaosan, is also marketed for its potential use in mental diseases, especially depression. This decoction can be traced back to the Song Dynasty in China (960–1127 AD) and it is primarily composed of eight herbs. Modifications of the original recipes are common. Again, the antidepression effect of Xiaoyaosan seen in a chronic restraint stress model of depression in rats is accompanied by the normalization of gut microbiota [99].

In addition to decoctions and recipes derived or revived from historical Chinese medicine texts, development of a novel composition has also been attempted – specifically with the intention to modify gut microbiota in order to alleviate depression symptoms [100]. Although the derivation of the new formula was not fully explained, except that it is loosely based on the Gegen Qinlian decoction and another recipe, its application in a chronic mild stress model of depression in rats had demonstrated a behavioural efficacy. The new formula was able to reverse two out of six significant gut microbiota changes (increased abundance of *Ruminococcus 2* and reduced abundance of *Roseburia*) induced by the chronic mild stress model [100]. This study illustrates how novel herbal supplements may be developed with a focus on microbiota modification. The enhancement of multiple cellular and molecular processes (including lysine biosynthesis, protein export, expression of diverse cancer-related microRNAs, and the bacterial secretion system) in the microbiota analysis have been highlighted but the precise mechanism remains elusive, and the interpretation of these changes not entirely straightforward. An emphasis of a more stringently driven hypothesis approach rather than ad hoc speculation would boost the impact and value of similar attempts in developing new microbiota-modifying herbal interventions for psychiatric conditions or general mental well-being.

Future Perspectives

We contend that there is sufficient evidence to place gut microbiota as a critical determinant of mental health such that its imbalance or disturbance, that is, dysbiosis, could contribute to behavioural and cognitive dysfunctions. At the same time, increasing evidence suggests that interventions that may rebalance the gut microbiota could be of therapeutic potential. Although there are significant gaps in our knowledge of the intricate and complex interactions underlying how the microbiota–gut–brain axis may shape the initiation, development and progression of psychiatric diseases, they should not deter the development of new therapeutics targeting gut microbiota with an aim to improve the treatment of neuropsychiatric disorders. The efforts will no doubt also clarify the aetiological significance of gut microbiota in mental illnesses. While current animal and human studies have mostly targeted anxiety and depression, the list of mental illnesses is anticipated to grow.

Although we are still unable to provide the precise composition and functional characteristics of healthy gut microbiota, technical advances and improvements in study designs may bring us closer to answering this million dollar question.

It is known that the human gut microbiota naturally undergo changes as a function of age, body mass index, food intake, medications, and differences do exist as a function of ethnicity, culture and lifestyle, such as levels of regular exercise [101, 102]. Similar individual differences can also be identified in laboratory animals related to differences in genetic background (e.g. between mouse strains), housing conditions, diets, age [103–105]. In designing intervention studies or comparison between psychiatric conditions, consideration of such factors is imperative. Basal gut microbiota composition, diversity and specific bacteria levels should be performed. The scale of the study may be increased if necessary. A longitudinal design with multiple sampling of stool samples is always preferred, whether it is to monitor gut microbiota changes that accompany dis-

ease progression or randomized controlled trials for prebiotics, probiotics and synbiotics. Finally, the experimenter must also exercise caution over faecal collection and extraction methods which represent extra sources of variability complicating interpretation [106, 107].

Until now, metagenomics analysis using next-generation sequencing (NGS) remains the mainstream for the analysis of gut microbiota. Commonly used NGS platforms such as Illumina genome sequencer, Roche 454 genome sequencer and ABI SOLiD system can produce reasonable data. However, the cost, resolution, length for analysis and potential errors still hinder gut microbiota analysis. Advances have led to the introduction of third-generation sequencing technologies, for example, Single Molecule, Real-Time (SMRT) sequencing from Pacific Bioscience, and nanopore sequencing from Oxford Nanopore Technologies. Their wider adoption could facilitate the analysis of gut microbiota with increased accuracy in amplification and reduced detection times [108]. With such technical innovations and the standardization of analytic protocols to support systematic, large multi-centre, longitudinal studies, it will become possible to more precisely determine the constituents of disease-altering gut microbiota.

In this review, we propose that the impact of existing herbal medicines and supplements on gut microbiota must be considered as one of their therapeutic mechanisms [86]. There exist herbal medications, including those based on the tradition of Chinese medicines, being prescribed for the treatment of psychiatric diseases as well as the maintenance of mental well-being for millennia [109]. Their increasing popularity in the past decades [110] should provide opportunities to investigate their impact on gut microbiota composition and the physiological and biological pathways known to be relevant to the regulation of mental functions. This review has highlighted current evidence that at least some herbal formulae do modify the gut microbiota and potential mechanisms that may underlie their claimed efficacy. However, we must not overlook that the impact on the abundance of different gut bacterial species does vary between herbal treatments despite similarity in their claimed psychiatric efficacy (see Tables 26.4 and 26.5). While similar variations could also be identified in epidemiological studies (Table 26.1) or animal models of psychiatric conditions (Table 26.2), any further development of herbal drugs in mental illnesses is associated with some unique challenges.

One must overcome the difficulties inherent to the use of herbs, or mixtures of multiple herbs, which necessarily includes a myriad of potentially active ingredients. Indeed, a single herb may contain chemical components numbering into the thousands. The identification and systematic screening of bioactive compounds, including their interactions can be a daunting task. Yet, it is necessary for dissecting the relevant biochemical and molecular cascades whereby the desired changes in gut microbiota are achieved by diverse herbal treatment. Only then could we expect to develop more efficient and reliable therapeutics. First we must address the lack of standards in the cultivation, sourcing and preparation of the herbs [110, 111], including the differentiation of subspecies, place of origin, harvesting season, and methods of processing and storage, which could determine the presence of contamination and artificial ingredients. To ensure robust and reproducible experimental outcomes, quality control of herbal drugs is of paramount importance [112]. It ultimately must be based on advanced separation and chromatography techniques [113] but not quantitative assays of only a handful of chemical markers in the herbal samples [114]. A combination of multiple chromatographic fingerprinting and bio- and meta-fingerprinting is essential for independent authentication of herbal medicines and the accurate detection and documentation of any lot-to-lot variation [115]. International standards at the production levels, such as Good Agricultural Practice and Good Manufacturing Practice, may not be sufficient when compared with the current standards of pharmaceutical manufacturing.

The past several decades have seen the wider acceptance of complementary and alternative medicine by patients and medical practitioners, including both probiotics and herbal treatments. Their potential application to psychiatric conditions must be guided by sound evidence and mechanistic understanding. Evaluation of their impact on gut microbiota may offer a hypothesis-driven approach.

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27 Biological Strategies, Adjunct to the Current Antidepressant Treatment

Eric Wainwright

Introduction

The Depression burden in our times is growing as it is an important and frequent cause of morbidity and mortality. Even with a wide a variety of modern pharmacological alternatives, most patients do not reach adequate response even in the presence of correct treatment efforts, hence the search for alternative treatment strategies. Usually, cognitive disorders and elderly patient groups pose further difficulties in the treatment of Depression.

Compliance with antidepressant treatment is generally poor: studies report that between 20% and 59% patients adequately comply with pharmacological treatment. This is mostly due to treatment side effects, and an incomplete disease understanding by patients and family [1]. We now know that mental well-being is intimately related to physical well-being. The concept above, links mood with immune status, particularly in uncompliant patients, this being specifically associated with deteriorating general state. These concepts further stimulate research for new strategies, involving general physical well-being, as a link to immune function.

When treating a new patient, we usually explain the treatment strategy, both pharmacological, and psychotherapeutical, but rarely include psychoeducation. This must be solved, as patients may benefit with simple changes, regarding the following topics, as undoubtedly our brain is intimately associated with the rest of our body. These activities, which are beneficial both to our brain and bodily health, must be discussed from the beginning of the psychiatric treatment. We must highlight the importance of exercise, relaxing meditation (mindfulness, or other), and improving nutritional style and sleep pattern optimization, among other topics.

It is now clear that physical health problems are related to mental health disorders by inflammation mechanisms. In chronic inflammatory conditions, the patient's mood tends to deteriorate.

Recent research further confirms the relationship of inflammation and mental health, and there is a gradual increase in publications illustrating anti-inflammatory strategies to be included in the daily practice of mental health practitioners.

Many studies indicate that the efficacy of antidepressive treatment is under scrutiny, particularly following the STAR-D study, that alerted us regarding the use and real effectiveness of antidepressant strategies in use today [2].

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The currently used antidepressants have proved unable to achieve sustained remission in a significant proportion of depressed patients. Furthermore, placebo studies have planted further doubts on their efficacy. Since the STAR-D study was published, new treatment alternatives have been researched. Some of them have proven to be useful; the results have been evaluated elsewhere, in an effort to explain previous treatment failures.

Apparently, antidepressant drugs are less efficacious in patients with high levels of inflammatory markers. This theory has recently been scrutinized. Among these studies, SIRT1, an anti-inflammatory polyphenol, has been extensively studied, in relation with inflammation in CNS. When SIRT1's levels fall, a parallel reduction has been seen in the Brain-Derived Neurotrophic Factor *(BNDF)*. These changes are related to ageing, particularly levels of the 75 kDa fragment of SIRT1 (110 kDa), in areas associated with cognitive processes [3, 4].

We must not forget that an early improvement of modifiable lifestyle factors may prevent functional decline during normal ageing. This may be especially useful when Depression or other neurodegenerative processes are present. Modifiable lifestyle factors include diet and physical and cognitive activities. Besides, depressive symptoms have been associated with higher intake of fast food and high-calorie sweets together with lower consumption of fruits and vegetables [3, 4].

The combination of these lifestyle findings, together with the reports on limited antidepressant effects and the safety of the biological strategies, have prompted the diversification of treatment alternatives in the search for more effective treatment strategies.

Some of the Biological Strategies – aiming at anti-inflammatory effects – that may be found useful in daily practice are enumerated in the following:

Dietary Improvements

Emerging evidence suggests an association between dietary habits and emotional and cognitive performance. It has long been suspected that the relative abundance of specific nutrients can affect cognitive processes and emotions. Newly described influences of dietary factors on neuronal function and synaptic plasticity have revealed some of the vital mechanisms that are responsible for the action of diet on brain health and mental function. Several gut hormones that can enter the brain, or that are produced in the brain itself, influence cognitive ability. In addition, well-established regulators of synaptic plasticity, such as brain-derived neurotrophic factor, can function as metabolic modulators, responding to peripheral signals such as food intake [5].

Consumption of food, rich in trans fatty acids, for example junk or fast food, meat, refined grains and sweets or commercially produced baked goods, have been recently associated with greater risk of depression and neurodegeneration [4, 6].

It is a usual practice to recommend limited intake of excessively elaborated food, bread derivates, cold cuts, etc. but this practice is not adequately stressed during psychiatric interviews. We should start highlighting the importance of fruit, vegetables, varied nuts, and fish in the diet, including limited amounts of meat, improving in this way the patient's nutrition following the recommendations of traditional medicine. A 2016 study reported the association between Mediterranean diet and improvements in inflammatory processes; the authors recommended "micro" nutritional changes, including vitamin D, L-metilfolate, S-adenosyl methionine (SAMe), and omega-3 in everyday diet. As a sideline, in patients suffering neurodegenerative disorders, the rhythm of hippocampal atrophy slowed down after these nutritional changes, the atrophy progressing 4.25 times slower for patients under treatment with medically designed nutrients as compared with the control group. Another very appealing publication reports that SAMe supplements were similar in efficacy to a first-line SSRI, as monotherapy or in combination with an antidepressant [7, 8].

Both studies remind us that nutritional changes may safely improve mood symptoms and associated neurodegenerative and inflammatory changes in depressed patients.

Further in this line, the results of a comparative study between healthy snacks and chocolate-derived snacks showed that consumption of fruit as a healthy snack was associated with lower anxiety, depression, and emotional distress than consumption of crisps/chocolate [9].

On behalf of chocolate use, we must mention that experimental studies on animals and cultured human cell lines have demonstrated the role of polyphenols, especially flavonoids (contained in Cocoa) in the prevention of cardiovascular and neurodegenerative diseases such as cancer, diabetes, and osteoporosis. It has also demonstrated effectiveness in Hypertension and Chronic Fatigue Syndrome [10].

Particularly in the elderly, flavonoid-rich diets have been associated with better cognitive performance. These findings are in line with previous research, indicating these changes are dose dependent for chocolate and wine (approximately, 10 g daily and 85 ml daily respectively) but linear for tea [11].

The ingestion of dark chocolate (flavonol-rich cocoa) is associated with increased gray matter blood flow and it has been suggested that it may be beneficial in dementia and stroke. But highly processed chocolate-derived snacks lose their flavonoid content, particularly when alkalinized for color or in milk chocolate [11].

Chocolate ingestion patterns require a special viewpoint, particularly when consumed as an emotional eating strategy. It is more likely to be associated with increased dysphoric mood. Chocolate and chocolate-derived snacks can provide their own hedonistic reward by satisfying cravings but this is short-lived, and usually guilt ridden [12].

Coffee consumption requires a special mention. Caffeine is the world's most widely used central nervous system stimulant, most of it (80%) consumed as regular coffee. Coffee consumption has demonstrated a strong inverse association with suicide in different populations. A recent review found that depression risk decreases with increasing coffee consumption. But more studies are required to know whether caffeinated coffee consumption really contributes to depression prevention, proposing further research [13].

Chronic treatment with polyphenols (resveratrol, silimarin, quercetin, and naringenin) may improve the altered function in Central Monoaminergic systems, generating changes in protein levels, related to inflammation. These compounds are anti-inflammatory and antioxidant. Besides normalizing Central Monoaminergic systems, they may regulate levels of SIRT1 y NF-$_{K}$B, both core proteins in neuro-inflammation and ageing processes [14].

Flavanol-rich foods have been shown to cause significantly increased cerebral Blood Flow (CBF) in areas irrigated by the middle cerebral artery in human subjects. Foods rich in three specific flavonoid subgroups, the flavanols, anthocyanins, and/or flavanones, possess the greatest potential to act on human cognitive processes. These components are found most commonly in fruits such as apples, berries, and citrus. Unfortunately, there is still limited evidence for the actions of such flavonoids on cognitive behavior and related cellular function and architecture, but current findings are stimulating [15].

Weight Control

Weight alterations have also been directly associated to mood disorders and changes in brain structure. Particularly, obesity was found to increase the risk of depression; this finding is most pronounced among females, Americans, and particularly for clinically diagnosed depression. Additionally, depression is predictive of developing obesity. Interestingly, this association was found in women but not in men [16].

These changes occur previously to the appearance of metabolic syndrome or diabetes, implying these CNS regions may be specifically vulnerable to obesity in early stages of the disease.

A meta-analysis in humans confirms the reciprocal connection between depression and obesity:

Obesity increases the risk of depression; 55% of overweight patients had an increase in this risk.

Also, Major Depression predicted obesity; female depressed patients had a 58% increase in

risk of obesity. These findings alert on the importance of weight control in depressed patients [2, 17, 18].

Dietary improvements may directly influence patient's weight. Both strategies have been associated with reduction in depression symptoms, and as described previously, also improving general well-being. These findings can be related to the following topic.

Probiotics

Depression has been associated to an increase in gut Actinobacteria, with a concomitant reduction in Bacteroidetes and Firmicutes types. Antioxidant-rich diets including vitamins and antioxidant supplementation have been proposed as an add-on to treatment of mood disorders and work-related stress, and may be useful as a side-effect-free adjunct treatment [18].

Probiotics, combined with Prebiotics, may be useful additions to the first topic in this review, as current research investigates different strategies within the Dietary Improvements. The inclusion of probiotics in the diet has been associated with improvements in the Leaky Gut Syndrome, and other studies reported probiotics supplementation reduced negative emotions in healthy volunteers, with additional improvements in Anxiety, Major Depression and Chronic Fatigue Syndrome. These findings are particularly interesting and warrant further studies regarding the gut/brain relationship, and its implications in Mood Disorder treatment [5, 6].

Restriction of Calorie Intake

Brief periods of calorie restriction may improve mood and may also reduce anxiety. The recommendations are to reduce intake initially by 10%, continuing to 25% if it is successful. Complete fasting should be restricted. Patients should be instructed to start with short fasting periods, with gradual calorie restrictions between 300 and 500 calories during fasting days. Greater percent weight loss at month 24 was associated with increased vigor and less mood disturbance, together with improved general health, and better sleep quality. In non-obese adults, calorie restriction, marked by approximately 10% weight loss, can be undertaken with little concern about negative effects on quality of life, mood, sexual function, and sleep [16, 18, 19].

These initial findings may also be associated with improved auto perception by the patients, as a beneficial side effect. Future and well-designed studies are warranted, since the causal factors have not been adequately differentiated and extreme calorie restrictions have been associated with nutritional imbalances.

Improvement in Sleep Behavior

Sleep as many other autonomic processes, occurs rhythmically, and is intimately related to mood disorders. This environmental 24 h synchronization, the Circadian Rhythm, is usually disordered in depressive patients. Together with the previously depicted topics it should be detected and addressed in everyday practice.

The Circadian Rhythm can be influenced by exogenous or internal cues, (zeitgebers) including light, sounds, temperature, digestive and urinary tract content, and recent physical activity and other symptoms.

Most sleep cues are regulated through behavior, and may be improved by an adequate psychoeducation, in similar fashion to most of the behavioral issues addressed in this review.

The sleep process is mediated by Melatonin levels (peaks at night), involving ACTH and cortisol (peaks in morning). Alterations in Circadian Rhythm have been linked to psychiatric illness, particularly related to inflammation, as Major Depressive Disorder (MDD) and Bipolar Disorders, also with disruptions of immune, metabolic and cardiovascular systems [3].

Depression and anxiety disorders, as previously stated, have been related to sleep symptoms. Moreover, these disorders are one of the main causes of sleep disorders. Many community studies reported sleep disorders as important risk factors for the development of new depres-

sive episodes in the year following evaluation. As many as 70% of MDD patients reported sleep problems. Reporting insomnia or ineffective sleep presents a higher risk of depression during the patient's lifetime. Related studies associate sleep disorders, in particular, sleep deprivation with an increase in inflammatory signaling. Preliminary research has suggested that Cognitive Behavioral Therapy for insomnia may contribute to durable anti-inflammatory effects [20].

Emerging results indicate that subjects presenting sleep disorders present a higher risk of mood disorders. This is one of the classical symptoms of depression and monitoring is mandatory in these individuals, when present [20, 21].

Irregular sleep habits, noisy surroundings, being thirsty and being worried when going to sleep, may cause deterioration in sleep effectivity and lead to various consequences including drowsiness and cognitive symptoms. Some studies suggest exercise may help reduce sleep abnormalities in MDD. It is understood that neuroplasticity requires adequate sleep, particularly in MDD.

Health providers, when highlighting efforts in improving sleep disorders, may be preventing mood disorders. Improving patient's sleep pattern has proven to be a safe and "non-toxic" health intervention, and should be prioritized in mood disorders daily treatment [18, 21].

Exercise

Current evidence supports anti-inflammatory and neuroplastic effects of exercise. Most researchers accept that a brief reminder may be quite effective for an improvement in the frequency and duration of exercise in the short term. There is no confirmation yet on the persistence of these effects in the long term. [22]

No dose/response effect was found in relation to increasing exercise intensity. At least 30 min of moderate daily exercise sessions, 5 days a week, has been recommended. On the other hand, some studies recommend 2 h daily over a 6-month period, mixing aerobics, strength, and other types. It appears that too much or excessive exercise may not be as beneficial as previously believed (DAPA trial) [22–24].

Some findings associated to exercise include improvements in immune function and feedback, in circadian rhythmicity, hypothalamic/pituitary/adrenal axis feedback, hippocampal neurogenesis, increased BDNF, increased telomere length, adaptive epigenetic signaling, and synaptic number and function, associated with improvements in neurotransmitter functionality.

Physical Activity restores the synthesis, release, and metabolism of serotonin, noradrenaline, glutamate, and dopamine, mainly in cerebral cortex, hippocampus, hypothalamus, and also in the brainstem.

Exercise is also involved in the noradrenergic system, limiting the output of NE from the Locus Ceruleus, also modulating the balance between the Serotonin Pathway and the Kynurenine Pathway, due to inactivation of indolamine 2,3 dioxygenase and tryptophan 2,3 dioxygenase. Physical Activity also dampens reactivity to stress, stimulating the conversion of Cortisol to Cortisone [23].

It also modulates the turnover of Dopamine and calcium levels. Lately Dopaminergic function has been associated with Depression and Bipolar Disorder [3].

Adequate exercise as a psychoeducational topic should always be included in daily practice as its effects may improve patients' well-being, neuroplasticity, and involvement in the treatment process.

Socialization

Several recent studies have reported interesting associations between social activity and mood disorders, one of them being between isolation in childhood and inflammatory disorders in adults, as lower social functional scores have been related to increased inflammation. Other studies have found associations between voluntary social activities and reduction in inflammation. Increasing social skills may be a simple way to reduce inflammation, and depressive symptoms [3, 25–28].

It is generally understood that isolation is not an effective rearing strategy. Pediatricians usually encourage socialization efforts in families not usually active in this sense. In our consultation social activity should be reviewed, so as to be able to include sensible advice in this regard.

Meditation Techniques

Meditation practice has demonstrated anti-inflammatory effects, particularly Mindfulness-based Stress Reduction (MBSR) and Tai Chi practice. Training in these techniques has shown benefits both for depression and substance use disorders. Mindfulness-based Cognitive Therapy (MBCT) achieved a reduction of 44–50% in the relapse rate in recurrent depression.

Rumination, usually included in cognitive problems in mood disorders, has been associated to longer periods of depression, and poor response to treatment. Mindfulness meditation may break the ruminative cycle, decreasing dysphoria, and, therefore, the duration of the depression episode [29–32].

A recent study in Asian nursing students showed that mindfulness interventions and stress management programs were effective, in the short-term, on depressive patients, as well as other no pharmacological interventions that also showed improvements in depressive symptoms [33].

Tai Chi Chuan techniques have been associated with increased psychological well-being, including decreased stress, anxiety, depression symptoms, and increased self-esteem. Emerging results suggest that frequent practice of Tai Chi (40 min to 2 h, 1–4 days a week) resulted in significant reduction of depressive symptoms as compared to various controls *(ES, 0.56; IC del 95%, 0.31 a 0.80) con I2 = 62%* [34].

These results may also be associated with improvement in somatic symptoms and physical function in patients with rheumatoid arthritis and multiple sclerosis, as well as improvement in the immune response of healthy elderly participants [34].

Hypothetic mechanisms explaining the effects of Tai Chi in the treatment of depression include direct modulation and indirect effect on activity and connectivity in the CNS regions involved in depression and mood regulation, reducing neuroinflammatory sensitization associated with these disorders and modulating the autonomous nervous system activity. This type of mind–body exercise, in combination with pharmacologic treatment may provide additional improvements of clinical outcomes in the treatment of depression, particularly in geriatric populations. Meditation interventions, particularly combined with exercise require further study as each individual intervention has proved useful in these patients [35, 36].

All of these strategies are interesting in that they have no obvious pharmacological interactions or untoward effects in themselves, as long as used adequately. They have also proven to have an excellent cost–benefit ratio: sleep interventions, nutritional improvement, meditation, and exercise would be effective strategies for the reduction of depressive symptoms in the short term. Further research on the longer term in clinical populations and prevention of recurrences is warranted. These strategies should be included in our usual treatment as an adjunct to pharmacological and psychological treatment strategies as a simple and extremely safe way to improve treatment efficacy.

Conclusions of the Present Study

The usual treatment options have not proven satisfactory, even when aggressive treatment regimens are used. With the introduction of adjunct biological strategies, we may help patients become active participants in their own treatment, besides the expected effects that the studies included in this review show, on affective, somatic, and cognitive symptoms of these diseases.

However, the majority of studies had inadequate controls, warranting further study.

These strategies are a simple, inexpensive, and safe way to include the patient as an active participant in his or her own treatment. In view of the current success rate with modern treatment options, it is probably time to look elsewhere for

new (or maybe ancient) strategies to help alleviate patients' sufferings.

Even in the light of limited results, these strategies can be effective add-ons to traditional treatment strategies, improving patient's compliance with regular treatment options.

The lack of effective treatment for cognitive decline and dementia symptoms, often related to mood disorders, points to the need of preventive strategies that may delay the onset and/or minimize the effects of both these devastating conditions. The present results with the Mediterranean diet are encouraging, as are other strategies presented in this review. Mental Health practitioners tend to run behind in the use of these health-related strategies, as other areas of medicine have been using them for some time now.

Definitive conclusions are limited as studies present variation in designs, heterogeneous outcomes, and controls. Further high-quality trials are needed to better inform clinical decisions.

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28

Potential Interactions Between Environmental and Psychoneurobiological Factors in the Interface Between Stress and Depression: A Road Map to Resilience

Gustavo Eduardo Tafet and Diego Javier Feder

Introduction

The role of stress in the origin and development of depression and anxiety disorders has long been demonstrated, including the long-lasting effect of early life stress and the impact of chronic stress, later in life [1–5]. In this regard, sustained and prolonged exposure to environmental stressors may stimulate a repertoire of adaptive responses, mediated by different neural structures involved in emotional and cognitive processing in the central nervous system (CNS), and the subsequent activation of the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal axis (HPA) [6, 7]. Environmental stressors may be bio-ecological, when associated to the natural environment, and psychosocial, when associated to the interaction between individuals in a social environment. Chronic stress has been mostly studied in social conditions, where it has been possible to describe different disorders produced by the prolonged and sustained impact of psychosocial stressors. It has been demonstrated that exposure to chronically stressful conditions may lead to the origin and development of different symptoms of anxiety and depression, with different characteristics according to specific features associated with stressors, diverse aspects associated with each individual, and the resulting interactions between them. Stressors may be acute or chronic, mild or severe, positive or negative, predictable or unexpected, controllable or not. Each individual may perceive environmental stressors in a different manner, according to cognitive and emotional features involved in their subjective appraisal, their personal resources, and the resulting coping strategies. In this regard, according to their personal characteristics, each individual may be affected in a different manner, which in turn may differ according to their potential vulnerability or resilience. Therefore, different interactions between stressors and individuals may be translated into different conditions, which in turn may depend on individual factors of vulnerability or resilience.

Stress: From the Environment to the Brain

Stressors are perceived as environmental stimuli, which are transmitted through sensory pathways to different structures in the CNS, including the

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thalamus, the amygdala, the hippocampus, and cortical areas, including sensory and associative cortices, which in turn convey information to the prefrontal cortex (PFC) [5, 7]. Direct projections from the thalamus may reach the amygdala, to stimulate arousal and early alarm reactions, which in turn may lead to the activation of the ANS, more specifically its sympathetic branch, and the HPA system. The amygdala may also receive indirect projections from sensory, associative and transitional cortices, which in turn are known to send projections to the hippocampus, where sensory input is integrated with contextual information to be subsequently conveyed from the hippocampus to the lateral nucleus of the amygdala (LNA) [8]. Projections from the hippocampus may also reach the para-ventricular nucleus (PVN) of the hypothalamus, where it plays an inhibitory role [9, 10]. The LNA is also the source of multiple projections to other ports of the amygdala, such as the basal, accessory basal, and central nucleus of the amygdala (CNA) [8, 11]. The CNA sends projections to the lateral nucleus of the hypothalamus (LNH), which activates the sympathetic branch of the ANS [12], the dorsal motor nucleus of the vagus, which activates the para-sympathetic branch, and the PVN, with the consequent activation of the HPA axis [12, 13], as part of the adaptive response to stress. The amygdala also shares connections with different areas of the PFC, including the orbito-frontal cortex (OFC) and the medial PFC (MPFC) [14]. The OFC participates in the integration and evaluation of sensory stimuli, including the primary appraisal of their positive or negative value and, subsequently, in the integration of their emotional and cognitive appraisal [14]. The MPFC, together with the anterior cingulated cortex (ACC), participates in the regulation of emotional responses, particularly those related to the amygdala [15]. All these cortices are also connected with the dorso-lateral PFC (DLPFC), which is involved in cognitive control and voluntary regulation of emotional responses. The DLPFC is known to be critically involved in executive aspects of cognitive processing [16], such as conscious processing and working memory. It receives projections from the amygdala through the OFC and ACC, and projects back to limbic structures through indirect connections to specific areas of the MPFC, namely, the ventromedial PFC (VMPFC), which projects to specific areas of the ACC, such as the subgenual ACC (sgACC) [16]. Projections from the VMPFC and the sgACC may reach the amygdala to exert a modulatory effect [16, 17], which in turn is also connected with the hypothalamic PVN. Therefore, the HPA axis may be regulated by stimulatory projections from the amygdala and inhibitory projections from the hippocampus, which in turn may be reflected in the adaptive response to stress.

The Role of the HPA Axis

It has been shown that the HPA axis is modulated by limbic components, such as the amygdala and the hippocampus, and cortical areas also involved in the regulation of these neural structures. Therefore, the amygdala sends stimulatory projections to the PVN [6], where the corticotropin releasing hormone (CRH) is synthesized and released to the hypophyseal portal blood to reach the anterior pituitary. There, CRH stimulates the transcription of the pro-opio-melanocortin (POMC) gene, a precursor for the adrenocorticotropic hormone (ACTH), which in turn is released into the bloodstream to reach the adrenal cortex, where it stimulates the biosynthesis and release of cortisol (see Fig. 28.1). At the molecular level, cortisol binds to mineralocorticoid receptors (MRs or type I) and glucocorticoid receptors (GRs or type II), constituting a hormone–receptor complex, which in turn may be ready to interact with a glucocorticoid response element (GRE), in the promoter region of target genes [18], to participate in transcription regulation. This molecular mechanism may explain the down-regulation of the POMC [19] and the CRH genes [20], where cortisol regulates its own synthesis and release through negative feedback circuits involved in the regulation of the HPA axis. In addition, cortisol may also bind to hippocampal GRs, which in turn may inhibit the PVN [21, 22]. It has been shown that chronic stress may abolish these negative-

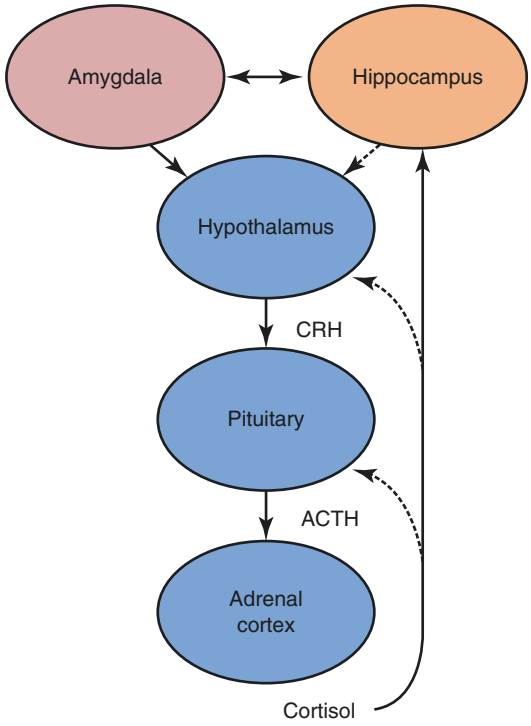

Fig. 28.1 A schematic representation of the hypothalamic–pituitary–adrenal axis, with excitatory projections from the amygdala, inhibitory projections from the hippocampus, and ther negative feedback circuits mediated by cortisol. (Modified from Tafet and Nemeroff [5])

feedback circuits, therefore resulting in sustained activation of the HPA axis [18]. Chronic stress may also reduce the expression of brain derived neurotrophic factor (BDNF) in the hippocampus, therefore decreasing its capability to inhibit the HPA axis [23, 24]. These alterations in the regulation of the HPA axis, associated with chronic stress, have been involved in the origin and development of depression and anxiety disorders, where hyperactivity of the HPA axis, with the consequent increase in CRH and cortisol levels, are commonly observed [25, 26]. In addition to the PVN, CRH neurons have been also described in the amygdala [27, 28], particularly in the CNA, which activates the HPA axis through projections to the PVN. Reciprocal connections have been also described between these and aminergic nuclei, such as the locus coeruleus (LC) and the raphe nuclei (RN) [3], which may be involved in reciprocal interaction between the noradrenergic and the serotonergic systems with the HPA axis [3, 29], probably involved in the pathophysiology of mood and anxiety disorders [30].

It has been shown that a history of traumatic events during childhood represents a critical factor of vulnerability in the origin and development of depression later in life [3, 31]. These stressful conditions have been associated with enduring alterations in different neural structures, reflected in hyperreactivity of neural and neuroendocrine responses to stress, with the consequent increase in CRH concentrations, glucocorticoid resistance, and reduced volume of the hippocampus [31, 32].

The Role of the Serotonergic System

Among the aminergic systems involved in the stress response, it has been long demonstrated that alteration in the serotonergic system plays a critical role in the origin and development of anxiety and depressive symptoms [29]. This system has its main sources in the RN, which project to different neural structures, including projections to the forebrain from the dorsal (DRN) and medial raphe nuclei (MRN) [33] (Azmitia 1987). Projections from the DRN may reach different neural structures involved in adaptive responses to stress, and the origin of anxiety-related symptoms [34–36], including the amygdala, particularly the CAN [37], the bed nucleus of the stria terminalis (BNST) [38], the PVN and different areas of the PFC [39]. Projections from the DRN may also reach other neural structures related to regulation of fight-or-flight responses, such as the periaqueductal grey (PAG) [40, 41] and the striatum, which have been shown to be involved in passive coping behavior [42], and the state of anticipatory anxiety that plays a critical adaptive role in threatening situations, informing the amygdala about current negative stimuli and emotional reactions associated with them [10]. On the other hand, projections from the MRN may reach other neural structures, including the hippocampus and the hypothalamus [43, 44], which have been associated with tolerance to per-

sistent aversive stimuli [45], such as those perceived during chronic stress, and adaptive control on negative emotional experiences [10]. Hence, alteration of the MRN-hippocampal projections has been associated with decreased tolerance to aversive stimuli, learned helplessness, and subsequent depression [43].

At the molecular level, serotonin (5-hydroxitryptamine, 5HT) is normally released to the synaptic cleft, where it binds to specific 5HT receptors. Serotonergic neurotransmission is regulated by the serotonin transporter (5HTT), which is responsible for reuptaking 5HT from the synaptic cleft, therefore regulating the concentrations of the neurotransmitter. This represents the target of different antidepressants, including the tricyclics (TCs) and the selective serotonin reuptake inhibitors (SSRIs), which have been shown to block the 5HTT, therefore leading to increased concentrations of 5HT in the synaptic cleft, hence allowing the activation of 5HT receptors [46]. These molecules may also induce adaptive changes, including desensitization or down-regulation of somato-dendritic $5HT_{1A}$ auto-receptors in the RN and up-regulation of postsynaptic $5HT_{1A}$ and desensitization of $5HT_{2A}$ receptors [47], particularly in the MRN-hippocampal tract. Postsynaptic $5HT_{1A}$ receptors may be down-regulated or desensitized in different limbic structures by cortisol or exposure to chronic stress [48–50]. Alteration of 5HT neurotransmission by cortisol may be exerted tonically through binding to MRs, while sustained and prolonged increased levels of cortisol, such as the observed during chronic stress, bind predominantly to GRs, which in turn may interact with GREs in the promoter region of target genes, therefore inhibiting the expression of the 5HT1A gene [48]. Interestingly, it has been shown that cortisol may stimulate 5HT uptake in vitro, due to increased expression of the 5HTT gene [51], providing additional evidence about the reciprocal regulation between the HPA axis and the serotonergic system, and their potential interactions in the interface between stress and depression.

The Role of Cognitive Vulnerability

The influence of stress in the origin and development of depression and anxiety disorders has long been observed at the clinical level, where cognitive processing has been shown to play a critical role. In this regard, the harmful effect of environmental stressors may depend on various aspects associated to stressful events, including their length and strength, and the availability of cognitive resources to cope with them. The availability of these resources may also depend on subjective cognitive processing, aimed at assessing the potential efficacy of these resources, and the resulting balance between them and environmental stressors, which is part of the cognitive appraisal, and the resulting coping strategies [52]. This adaptive cognitive processing is necessary to assess diverse characteristics of environmental stimuli, which may lead to the assumption that many of them may be noxious or negative, while many others may be perceived as positive stressors. In this regard, certain environmental stimuli, assessed and perceived as desirable, predictable, and controllable situations, may constitute positive conditions, hence distinguished as pleasant or exciting challenges, known as "eustress," or positive stress. On the other hand, "distress," which is commonly known and identified as negative stress, is characterized by undesirable, unpredictable, and uncontrollable conditions, generally provoked by more intense and persistent stimuli, which may be perceived as dangerous or threatening, and consequently may lead to maladaptive responses, which in turn may be associated with the origin and development of various disorders [53]. In this regard, distress may engender an array of adaptive responses, including a defense reaction, which represents the active mode of adaptive responses, or a defeat reaction, which represents the passive mode. Active responses may result as a consequence of perceived threat, when subjective feelings of control are threatened by environmental stimuli, and are usually associated with effortful coping strategies. On the other hand, passivity is associated with per-

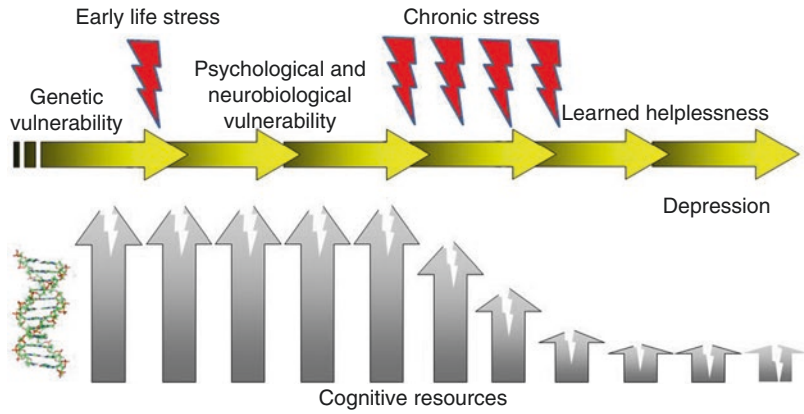

Fig. 28.2 A schematic representation of the effect of stress in the origin and development of depression, including factors of vulnerability

ceived loss, when subjective feelings of control are considered null and void, which in turn may lead to inability to cope. Accordingly, sustained and prolonged impact of undesirable, unpredictable, and uncontrollable stimuli may lead to the subjective feeling of loss, associated with the belief that the available resources are not effective or not enough, with the consequent impossibility to cope with the situation, which in turn may lead to subjective feelings of helplessness [54]. In this regard, chronic distressful conditions may lead to learned helplessness, which in turn has been associated with increased vulnerability to develop depression or anxiety disorders [54], and this is more evident when chronic stressful conditions occur during childhood [3, 31] (illustrated in Fig. 28.2).

The Role of Early Adverse Experiences

It has been shown that, in addition to chronic stress, the harmful impact of adverse conditions, a hostile environment, and traumatic events suffered during early periods of life represent a significant factor of vulnerability in the origin and development of depression [3, 31]. In this regard, the impact of adverse experiences, such as abuse, neglect, or loss, has long been associated with increased vulnerability to stressful conditions later in life, and the consequent development of depression [30, 31]. An increasing body of research has demonstrated the association between early adverse conditions and diverse alterations in different neural structures, including cortical and limbic areas, and the HPA axis. In this regard, CRH neurotransmission may be particularly affected [25], which may lead to increased reactivity to stress [3, 31]. Increased levels of CRH have been associated with hyperactivity of the HPA axis and the consequent increase in cortisol levels, which in turn may lead to functional and structural changes in the hippocampus [55]. It has been shown that increased levels of cortisol, in a sustained and prolonged manner, may affect GRs availability in the hippocampus [56]. It has been shown that early adverse experiences may lead to decreased availability and reduced efficacy of hippocampal GRs [31, 57], which in turn has been associated with glucocorticoid resistance and increased reactivity of the HPA axis in response to further stressful situations. In addition, it has been shown that decreased GRs induced by early adverse experiences, along with increased levels of cortisol, may lead to decreased hippocampal function and volume in adulthood [58]. In this regard, decreased function and volume of the hippocampus, along with hyperreactivity of the amygdala, have been associated with a history of early life stress [31, 59]. Changes in cortical thickness have been also described in patients exposed to specific stressors during childhood [60]. Therefore, early life stress

may lead to functional and structural changes, including hyperreactivity of neural and neuroendocrine responses to stress, which may affect potential responses to upcoming stressful situations later in life (illustrated in Fig. 28.2).

The Role of Genetic Polymorphisms

The role of stressors has been extensively elaborated; however, some individuals may be more vulnerable to stress, while some others may be more resistant or even resilient [61]. The influence of stressors may depend not only on their attributes, but also on the interactions between them and the characteristics of each individual, including psychological conditions, including their cognitive resources, and biological conditions, including their genetic background [31]. The relationship between different genetic polymorphisms and possible alterations, either functional or structural, in the CNS is an important aspect to better understand the molecular mechanisms underlying potential gene–environment interactions. Various polymorphisms have been studied in different candidate genes, critically involved in diverse molecular pathways closely involved in the origin and development of depression. These genetic variations may participate in the development of depression, either in response to adverse experiences during childhood or environmental stressors during adulthood, therefore representing important factors of vulnerability [31, 61–63]. Among these genetic variations, a polymorphism was identified in the promoter region of the 5-HTT gene [64]. Activity in the promoter region may be regulated by sequence elements located in the upstream regulatory region, termed the 5-HTT gene-linked-polymorphic-region (5-HTTLPR), where a short (5-HTTLPR-S) and a long (5-HTTLPR-L) variant have been described [63]. The short allele was associated with decreased transcriptional efficiency, compared to that observed in the long one, resulting in down-regulation of the 5-HTT gene [64] with a resulting decrease in the 5-HTT availability. It has been shown that the potential effect of various antidepressants, including the serotonin reuptake inhibitors, may depend mostly on 5-HTT blockade, therefore increasing 5-HT concentrations in the synaptic cleft, and also on down-regulation of presynaptic 5-HT_{1A} auto-receptors [47, 65]. Hence, it has been proposed that down-regulation of the 5-HTT gene, with the resulting effect on 5-HT concentrations in the synaptic cleft, may be different between the expressed by congenital conditions, and the triggered by environmental stressors. Congenital alterations, such as that observed in the short allele carriers, may result in increased concentration of 5-HT, which in turn may lead to down-regulation of postsynaptic 5-HT receptors, with the resulting desensitization of the serotonergic system [65], which may provide a mechanism to explain the vulnerability expressed by carriers of the 5-HTTLPR-S allele. It has been demonstrated that alterations in the regulation of the 5-HTT gene may be involved in the modulation of serotonergic activity in response to stress, and this was further supported by clinical and preclinical studies [66]. In addition, it has been observed in functional brain imaging studies that 5-HTTLPR-S carriers expressed an increased reactivity in the amygdala, in the presence of fearful and threatening stimuli, comparing to that observed in 5-HTTLPR-L carriers [67], strongly suggesting that these genetic variations may be involved in psychological responses to stress [61]. It has been shown that the amygdala participates in the regulation of emotional reactions, including anxiety and mood regulation, and also participates in the activation of the HPA axis, with the resulting hyper-cortisolism, therefore explaining the role of this polymorphism as a potential factor of vulnerability. Another important polymorphism has been investigated in the brain-derived neurotrophic factor (BDNF) gene, particularly in its coding region at nucleotide position 196, where a guanine base is replaced by an adenine, which in turn is translated into the substitution of valine (Val) by methionine (Met) at codon 66, therefore termed "Val66Met." This substitution has been associated with altered intracellular trafficking and decreased availability of BDNF [68–70] (Duman and Monteggia 2006; Egan et al. 2003; Gatt et al. 2009). It has

been shown that BDNF plays a critical role in the stimulation of neuroplasticity and neurogenesis in the hippocampus, which may be critical in the treatment of mood disorders [70], while decreased concentrations of BDNF have been associated with depressive symptoms [71]. In this regard, it has been observed that carriers of the Met-BDNF allele exhibited reduced hippocampal volumes, compared with carriers of the Val-BDNF allele [70], and decreased hippocampal activation [69], with the consequent deficient cognitive performance [70], which in turn have been associated with emotional instability and vulnerabilitsy to develop depressive symptoms. Many other polymorphisms have been also studied, and are currently investigated, therefore representing additional factors of vulnerability (illustrated in Fig. 28.2).

Neurobiological Circuits of Vulnerability and Resilience

It has been shown that serotonergic projections from the RN may reach different targets in the CNS. Projections from the MRN may reach the hippocampus to stimulate 5HT1A receptors involved in adaptation to chronic aversive stimuli, with the consequent tolerance to adverse events, such as that experienced during chronic stress. Therefore, impairment of this serotonergic pathway has been associated with learned helplessness and subsequent depressive symptoms [35]. Projections from the DRN may reach the extended amygdala, including the BNST and certain nuclei of the amygdala itself [36, 43] to stimulate 5HT2A receptors. Activation of these serotonergic pathways has been shown to be sufficient and necessary to produce increased fear and anxiety [72, 73]. In acute stress conditions, fear represents an adaptive emotion, necessary to implement and sustain active behavioral responses, such as the activation of fight or flight. During chronic stress, excessive fear and anxiety become more intense and less adaptive, as it is usually described in anxiety disorders and depression. In addition, serotonergic projections from the DRN may also reach additional neural structures, such as the striatum and the periaqueductal gray (PAG), which are known to be involved in active coping behavior [74]. Serotonergic input from the DRN to these areas has been shown to exert inhibitory effect, which may lead to inhibition of active responses, such as fight or flight, which in turn may be replaced by passive responses [72, 73]. The lack of active responses, also known as passivity, combined with increased fear and anxiety, represent core symptoms of learned helplessness [75], and therefore are usually observed in anxiety disorders and depression. The DRN may be modulated by multiple inputs, including excitatory projections from the noradrenergic LC, which participate in adaptive responses to stress, and inhibitory projections from cortical sources, particularly from the prelimbic region of the ventromedial prefrontal cortex (VMPFC) [76]. Input from the VMPFC is mediated by glutamatergic neurons, which synapse to GABAergic interneurons in the DRN, therefore inhibiting serotonergic neurons. It has been shown that neurons in the VMPFC participate, with the dorsal medial striatum, in a circuit involved in detection of control, which in turn may lead to inhibit the DRN [42, 77]. Therefore, passivity, associated with chronic stress and learned helplessness, may be overcome by learned control, which may be understood as a result of VMPFC inhibition of the DRN. In addition, prelimbic and infra-limbic neurons in the VMPFC have been shown to extend glutamatergic projections to synapse GABAergic interneurons in the amygdala, therefore exerting inhibitory effect on this neural structure. Therefore, increased fear and anxiety, associated with hyperactivation of the amygdala, may be also overcome by learned control, which has been associated with activity in the VMPFC. Learned control also requires the concerted activity of other PFC areas, such as the DLPFC, which is involved in working memory and cognitive processing, and therefore participates in cognitive aspects of inhibitory control, the OFC, which participates in emotional aspects of inhibitory control, and the ACC, which is involved in emotion regulation [78]. Hence, cognitive processing may exert control on limbic structures, such as the amygdala, by means of inhibitory projections

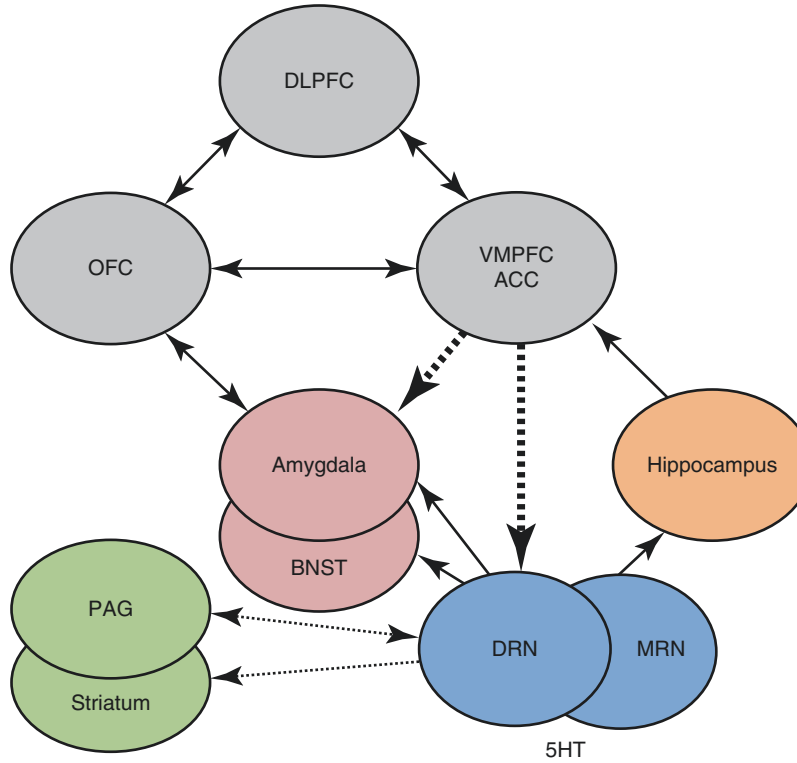

Fig. 28.3 A schematic representation of stimulatory serotonergic pathways from the DRN to the amygdala and BNST, involved in increased anxiety, inhibitory pathways to the striatum and PAG, involved in passivity, and projections from the MRN to the hippocampus, involved in increased tolerance to stressors. Reciprocal connections between the DLPFC, the OFC, and the VMPFC, together with the ACC, which in turn send inhibitory projections to the DRN and the amygdala. (Modified from Tafet and Nemeroff [5])

from the VMPFC to the amygdala. In addition, increased controllability, exerted by inhibitory projections from the VMPFC to the DRN, may be followed by synthesis of new proteins, therefore reinforcing these adaptive connections involved in controllability and predictability, both aspects of resilience (illustrated in Fig. 28.3).

From Distress to Eustress: The Road Map to Resilience

The role of stress in the origin and development of anxiety and depressive symptoms has long been investigated, demonstrating to be a multifactorial relationship. Among these, it is possible to identify an array of features corresponding to the impact of different environmental stressors, where it is also possible to differentiate between the characteristics of stressors themselves and their environment, either bio-ecological or psychosocial. To successfully cope with these stressors, it is important to know the characteristics of each individual, which in turn may explain their potential vulnerability or resilience. In this regard, it is possible to identify biological factors, including genetic and epigenetic mechanisms, and psychological aspects, including those related to the biography, where a history of traumatic events in early periods of life has been shown to represent an important factor of vulnerability. Traumatic events during adulthood may also predispose to increased vulnerability, although it has been shown that many subjects with such a traumatic history exhibit certain ability to develop an extraordinary strength and additional skills to cope with stressful events, therefore becoming less vulnerable and more resilient. This has been shown to depend on different factors, mostly related to their ability to perceive stressful events in a different manner and appraise them according to their potential resources. Cognitive appraisal may provide cognitive resources to increase predictability and controllability, which in turn may lead to more effective coping strategies. The feeling of controllability may be suf-

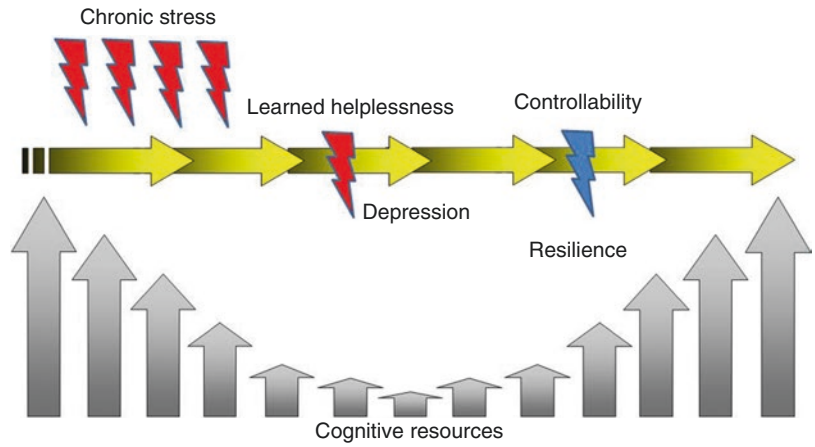

Fig. 28.4 A schematic representation of the effect of stress, learned helplessness, and controllability

ficient and necessary to transform distress into eustress. Therefore, cognitive strategies aimed at improving appraisal and related information processing may be highly effective to improve subjective feelings of controllability (illustrated in Fig. 28.4). More effective approaches to improve controllability, therefore avoiding learned helplessness, are currently investigated, which in turn may provide effective strategies for the treatment of depression and anxiety disorders, but also to promote resilience, providing novel approaches for the prevention of anxiety and depression in vulnerable individuals, including those exposed to chronic stress and those with a history of traumatic events in early periods of life.

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29

The Impact of Apolipoprotein E Allelic Variants on Alzheimer's Disease

Ezequiel Serrano, Pablo G. Sanz, and Francisco J. Barrantes

Introduction

Alzheimer disease (AD) was described for the first time in 1907 by Alois Alzheimer, a German neurologist, psychiatrist and neuropathologist [1], and today is the most frequent form of dementia. It has a huge global occurrence with 47 million affected individuals in 2016, expected to reach 130 million by the year 2050 [2]. The disease has a predominant late-onset, "sporadic" form (~95%) in the population older than 65 years [3, 4]. Besides its characteristic neurological and psychological dysfunctions, AD has additional negative impacts on the quality of life of the patient [4, 5], affects the familial environment, exhibits high morbidity and mortality – reaching 85,000 annual deaths in the United States alone [6, 7] – and involves enormous costs for the healthcare system [8].

Clinically, AD is characterized by an amnesic syndrome, cognitive decline, and no initial focal signs [9]. Cerebral atrophy is a macroscopic sign characteristically found in necropsies, and in more recent times fully corroborated in patients using imaging techniques such as positron emission tomography (PET), single-photon emission computed tomography (SPECT) or computer tomography (CT), nuclear magnetic resonance imaging (MRI), or its variant volumetric brain MRI [10, 11]. At the light microscope level changes involve regional loss of neuronal cells, insoluble amyloid plaques, which we now know are made up of extracellular amyloid β (Aβ) deposits (see below), and neurofibrillary tangles made up of microtubule hyperphosphorylated tau protein, predominantly found in memory-processing brain regions. PET with appropriate labeled ligands can detect specific metabolites such as Aβ or tau. These neuropathological changes are presumably initiated by loss of synapses and subsequent death of neuronal cells [9], initially in the entorhinal cortex and hippocampus. At the cellular level, the cholinergic neurons at the basal forebrain region are attacked at early stages [12–14]. At more advanced stages of the disease, essentially all cerebral cortex and other brain areas are affected. Mass spectrometry-based proteomic studies of postmortem brains from patients with AD, asymptomatic AD, and controls show correlations between neuropathological changes, cognition, and AD risk loci identified by genome-wide association studies (GWAS) and transcriptome analyses [15, 16]. Transcriptomics and proteomics can be successfully combined as diagnostic tools to provide a more comprehensive description of the physio-

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pathological mechanisms involved in AD and the underlying risk factors.

Two forms of AD can be distinguished based on the age of onset of the clinical manifestations: early-onset AD (EOAD <65 years) and late-onset AD (LOAD >65 years) [17]. In both presentations of the disease, the "sporadic" form accounts for 95% of cases [17], comprising an amalgamation of environmental factors and genetically complex risk factor combinations with up to 80% hereditability [17]. Although EOAD is the most common neurodegenerative dementia of early onset, it only represent 4–6% of all AD cases [18]. EOAD often shows autosomal dominant history (14.2%), the majority of the cases are nonfamilial, and the identification of determinant AD genes in this population is rare [19]. The EOAD form of the disease is associated with mutations in the genes coding for the presenilin (PS) variants PS1, PS2, and the amyloid precursor protein (APP) [20], with concomitant alterations in the processing of APP and increased deposition of Aβ. We will not deal with EOAD in this chapter.

Lipoproteins are a class of proteins whose major role is the transport of lipids in the circulatory system, involving lipid delivery to cell-surface receptors and lipid transfer from one cell type to another. They comprise various families: very-low density (VLDL), low-density (LDL), intermediate-density (IDL) and high-density (HDL) lipoproteins, chylomychrons, chylomicron remnants, and lipoprotein-A. The link between abnormal levels of plasma LDL complexed with cholesterol, the pathogenesis of atherosclerosis, and the increased risk for ischemic cardiopathy hypothesized in the mid-1970s evolved to become a fundamental element in current views regarding the leading cause of death in Western civilization and concurrent approaches to preventive clinical cardiology. Dyslipidemias are by now undisputable risk factors for atherosclerotic cardiovascular disease, carotid artery plaque, and peripheral artery disease (see recent review in Ref. [21]). Lipoproteins interact with a variety of actors: lipids, enzymes, cell-surface specific lipoprotein receptors and surrogate molecules, and lipid transfer molecules.

Among plasma lipoproteins, the apolipoproteins E (ApoE) have gained relevance in relation to their negative or positive effect on a key group of neurodegenerative diseases, particularly AD. The burden conferred by carrying certain alleles of the ApoE-coding gene (*APOE*) has been documented as the major genetic risk factor for LOAD and for shifting the age of onset of AD, homozygous *APOEε4* carriers developing AD earlier than heterozygous and non-*APOEε4* carriers, although the contribution of this genetic factor to AD pathogenesis is still far from clear [22]. *APOE* has been postulated to be related to both Aβ-dependent and Aβ-independent pathways [23, 24]. *APOE* is also considered as a potential diagnostic tool and therapeutic target, though these two aspects are still understudied [25]. In this review, we address the current status of ApoE isoform biochemistry, expression in brain, and the various impact modalities of *APOE* status on AD.

APOE Polymorphism and ApoE Isoform Expression

The *APOE* gene is localized in chromosome 19, more precisely in the 19q13,32 sector. The gene comprises four exons and three introns [26]. Genetic analysis has disclosed a characteristic of *APOE* uniquely observed in humans: three gene alleles, termed *ε2*, *ε3*, *and ε4*, which code the expression of three isoforms of the protein: ApoE2, ApoE3, and ApoE4, respectively. The frequency of the three alleles is 2–10% (*ε2)*, 70–80% (*ε3*), and 10–15% (*ε4*), respectively [27]. Genotyping has also uncovered that single-nucleotide polymorphisms (SNPs) confer high risk or protection from AD, as we will discuss below.

APOE is not homogeneously expressed in the body, its expression varying from tissue to tissue. The organ with the highest expression is the liver, followed by the brain, kidney, adrenal gland, adipose tissue, and spleen. The brain has a high production of ApoE, though it only amounts to about a fourth of that produced by the liver [28]. ApoE supply in the central nervous system (CNS) relies entirely on local biosynthesis, as the blood–brain

barrier (BBB) precludes the entrance of peripheral ApoE [29]. ApoE is synthesized by a variety of cell types, its expression varying among cells of the same organ; this is particularly apparent in the CNS, where ApoE is mostly produced by astrocytes [30]. Neurons do not normally express the *APOE* gene (see the next section), but may do so upon injury [31].

ApoE Isoforms: Structure and Functional Implications

ApoE is a relatively small glycoprotein made up of 299 amino acids, with an average molecular weight of 34.000 Daltons [32]. Peripheral blood ApoE subjected to isoelectric focusing can be separated into its three isoforms based on minor differences in their charges: ApoE2 differs from ApoE3 in a single amino acid at position 158 (Arg^{158} is replaced by a Cys residue in ApoE2) and ApoE4 differs from ApoE3 also by a single amino acid (Cys^{112} replaced by Arg^{112}) [33]. These single amino acid substitutions suffice to produce significant changes in the lipid binding ability of the three isoforms.

The ApoE3/E3 phenotype is the most abundant one, constituting between 50% and 70% of the total, whereas ApoE2/E2 makes up only 1–5% of the circulating ApoE [34, 35]. In their lipid-free (apo) state, the three ApoE proteins share two distinct, independently folded structural domains: the N-terminal domain, comprising residues 1–191, the C-terminal domain, extending from residues 210-(225) to 299 [36], and a protease-sensitive hinge region – a loop– connecting the two domains [37]. The N-term possesses a four-strand antiparallel structure containing the LDL-R (and other surrogate receptors) binding region (residues 134–150 and Arg 172) in its fourth helix, enriched in Lys and Arg residues [38, 39]. The C-term domain is the one carrying the major lipid binding region (between residues 244 and 272) [37]. These structural studies aimed at identifying functionally relevant domains were carried out using various combinations of biophysical techniques, including X-ray diffraction and small-angle X-ray diffraction studies [40, 41], complemented by electron-spin resonance (ESR) [42], circular dichroism (CD) and differential scanning calorimetry (DSC) [43], and recently, nuclear magnetic resonance (NMR) and "fluorescence spectroscopies" [44].

Even more recently, two alternative conformational states of (lipidated) ApoE4 at the surface of nanodiscs and their possible transition upon binding to the ApoER were identified using a combination of cryoelectron microscopy, chemical cross-linking, mass spectrometry, and in silico molecular modeling [37]. By varying the position of the hinge, the opened hairpin and the compact hairpin models were generated. According to the authors, they may represent distinct states of lipidation of ApoE4 coexisting in solution. The models also account for the lipid-dependent ApoE conformation relevant for recognition by the LDL receptor: extended helix 4/opened hairpin (see Fig. 29.1).

ApoE Receptors

ApoE proteins bind to specific cell-surface macromolecules, the ApoE receptors, which belong to the evolutionarily ancient LDL receptor (LDL-R) family [45–47]; these latter also include the LDLR-related protein 1 (LRP1), megalin (LRP2), ApoE receptor 2 (ApoER2 or LRP8), LRP4, and LRP1b [47]. All these receptor proteins bind ApoE, but ApoE isoforms do not bind equally well to all receptors: ApoE2, for instance, binds much less strongly to its receptors than the two other isoforms, its binding capacity being 50–100 times lower than that of ApoE3 or ApoE4 binding to the LDL-R [48]. This is because Cys^{158} abolishes the salt bridge between residues 158 and 154 in ApoE2, generating a new bridge between residues 154 and 150 (LDLR-binding region 136–150) [49]. These differences have been associated with a higher probability of developing type III hyperlipoproteinemia, abnormally high levels of plasma cholesterol and triglycerides and clinical manifestations such as cutaneous disorders, premature coronary disease, and peripheral atherosclerosis in ApoE2 carriers [50].

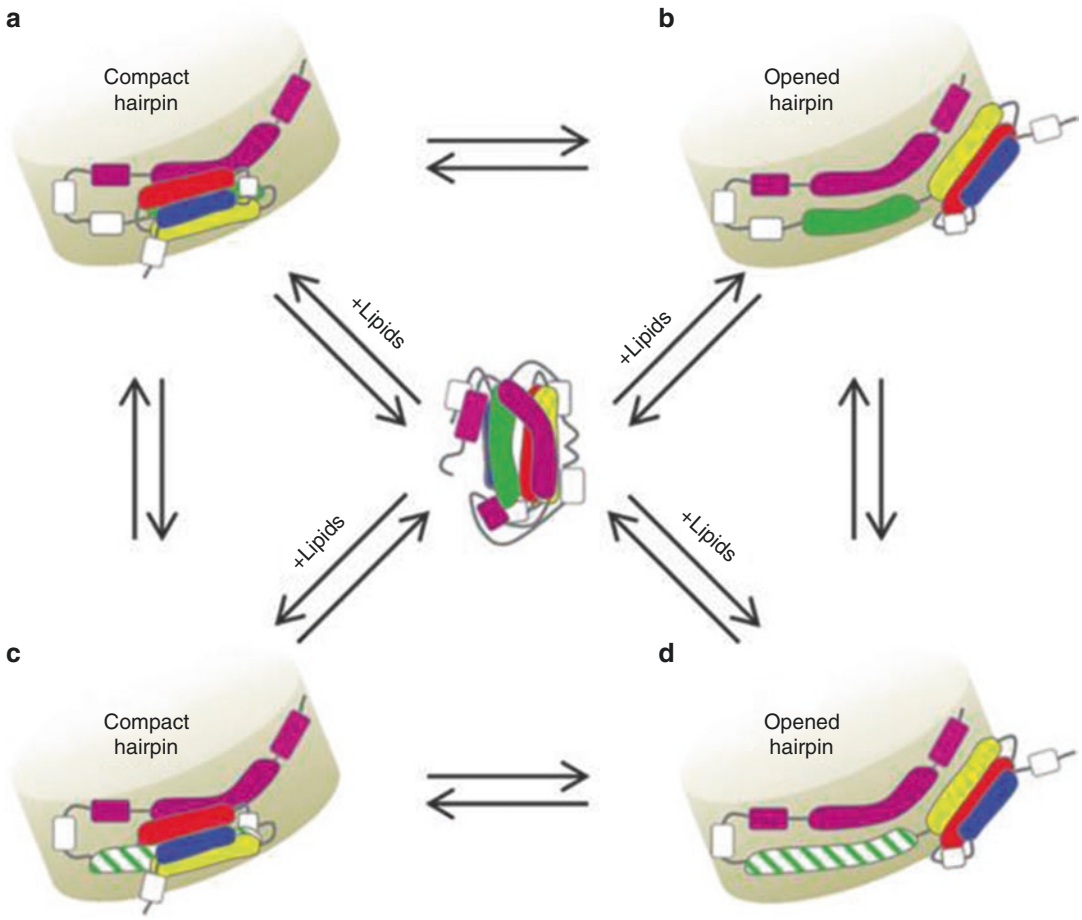

Fig. 29.1 The two models of ApoE4 (compact and opened hairpins) at the surface of lipid nanodiscs. According to these models, upon binding lipids two ApoE4 molecules adopt either the compact (**a**) or the opened (**b**) hairpin conformation as a "belt" partially surrounding the lipid nanodisc. Subsequently (or concomitantly), the N-terminal helix 4 elongates (**c**, **d**). The elongated N-term helix 4 is accessible and recognized by the LDL receptors (see below) in the opened state (**d**). C-term helices are colored purple; the N-term helices 1,2,3, and 4 are shown in blue, red, yellow, and green, respectively. (Reproduced from Ref. [37], following the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/))

The binding of a lipid is essential for ApoE high-affinity binding to the LDL-R [36, 41, 51]. When ApoE binds lipids, it undergoes substantial conformational changes, adopting a hairpin-like structure that wraps around the polar section of a phospholipid discoidal bilayer [52–55]. This places the LDL-R binding region into the apex of the hairpin, facilitating interaction between the binding region and the LDL-R [40]. ApoE isoforms also bind to other lipoproteins, albeit with different affinities. ApoE 2 and 3 bind to small HDLs whereas ApoE4 exhibits preference for binding to large VLDL [56].

ApoE Properties and Functions

ApoE is one of the major components of the circulating lipoproteins involved in the transport of the neutral lipid cholesterol. It is also involved in the control of the normal chylomicron and VLDL metabolism, particularly the removal from circulation of remnants generated by the metabolism of dietary lipids, transporting them to the liver where they are internalized by the hepatocytes, in a process mediated by ApoE-ApoE/chylomicron remnant receptors

[57–59]. Correspondingly, ApoE phenotypes constitute ligands for the three types of cell-surface receptors mentioned earlier: the LDL-R, the low-density lipoprotein-related protein receptor, and the VLDL receptor, albeit with different abilities. The ApoE2 isoform, for instance, is not effective in the receptor-mediated remnant lipoprotein removal process: its binding capacity to the LDL-R is only 2% that of ApoE3. *APOEε2/ε2* homozygous individuals therefore have much higher levels of remnant lipoproteins.

ApoE, together with the major HDL protein Apo A-I, is involved in the efflux of cholesterol from cells and ApoE actively participates in the transport of cholesterol produced by hepatocytes to peripheral tissues. Triglycerides, ApoE and ApoB-100-rich molecules are produced in the liver and are metabolized by plasma lipoprotein lipase from VLDL to intermediate-density lipoproteins (IDL) and LDL, losing ApoE along these processes. The uptake of the remnant VLDL and IDL is mediated by ApoE, whereas the uptake of LDL is facilitated by ApoB-100 [57].

The transport of cholesterol from peripheral tissues to the liver (reverse cholesterol transport) also requires the participation of ApoE [57, 58, 60]. Cells with high levels of cholesterol can transfer cholesterol to acceptors like high-density lipoprotein (HDL), which upon forming a complex with ApoE, facilitated by the high cholesterol in the HDL, incorporate cholesterol at a faster rate. The complex displays additional cholesterol binding sites on its surface and stronger binding to LDL-receptor [61, 62]. HDL lacking ApoE does not bind to LDL receptors. Humans possess another reverse cholesterol transport mechanism, in which high-cholesterol HDL transfers cholesterol to lipoproteins with lower density (VLDL, LDL, IDL), which deliver cholesterol to the liver [57, 63, 64].

ApoE also participates in the transport of lipids between cells within the same tissue. Cells with lower than normal levels of cholesterol express high levels of LDL-R in their membranes; ApoE-cholesterol complexes deliver the neutral lipid to these cell-surface receptors, and this is followed by cholesterol internalization [57]. Damaged neurons, for instance, require lipids for the restoration of their dendrites and axons. Peripheral nerve injury activates macrophages inducing the production of ApoE, while the tips of the nerve endings express high levels of LDL-R to acquire ApoE-cholesterol [65].

ApoE was found to display heparin-binding properties [66], helping cells to interact with the extracellular matrix, stimulating their adhesion, growth, and division [67]. ApoE can prompt or inhibit other interactions between cells and the matrix [57]. These interactions have important consequences on neuronal growth and smooth muscle cell migration and differentiation (e.g., in atherosclerosis) [57, 66, 68, 69]. ApoE also has an important role in immunoregulation, having the capacity to induce or inhibit T-lymphocyte activation and proliferation depending on the T-lymphocyte receptor and concentration and type of lipoprotein [57]. ApoE can also bind to exogenous lipid antigens, assisting their presentation in the immune system [70]. ApoE is involved in inflammatory response pathways in an isoform-dependent and dual manner, with anti-inflammatory effects in the presence of Aβ and pro-inflammatory responses in the absence of Aβ [71].

ApoE4 has the least resistance to thermal and chemical denaturation and proteolytic degradation as compared to the other isoforms, which follow the order E4 > E3 > E2. This difference correlates with the propensity to unfold in a less cooperative manner following the sequence E4 > E3 > E2 [43, 72, 73]. ApoE3 and ApoE4 also generate intermediate states, unlike ApoE2 [73]. The low unfolding capacity of ApoE4 is responsible for a higher rate of occurrence of stable intermediate conformations that are still compact (compared with the properly folded state) but with a larger radius of gyration (expanded), characterized as molten globules [73, 74]. The molten globule states have been related to various disease conditions and have been proposed as a possible explanation for the relationship between ApoE4 and AD [73].

The rate of transport of β-amyloid$_{1–42}$ has been shown to be slower when complexed with ApoE4 compared with the other ApoE isoforms [75].

APOEε4 carriers have been reported to present reduced cerebrospinal fluid $A\beta_{1–42}$ levels, which are considered biomarkers of amyloid plaque deposition, and a cause–effect relationship has been attributed to this finding [76].

APOE Alleles and Risk of LOAD and Other Dementias

There have been considerable advances in our understanding of the relationship between *APOE* alleles and AD during the last few decades. One important emerging concept is that *APOEε2* is essentially a protective allele and *APOEε4* a high-risk allele [77, 78], particularly for the late-onset form of Alzheimer's disease (LOAD) [79]. In LOAD, both environmental and genetic influences are invoked [80]. The presence of the *APOEε2* isoform, for instance, lowers the probability of developing AD to 0.6 [77]. The incidence of LOAD varies not only with the *APOE* allele [81], but also with homozygocity/heterocygocity: *APOEε4*/ε2–3 heterocygotes have a fourfold higher probability to develop LOAD, and homozygous *APOEε4*/ε4 carriers are 12–20 times more likely to develop LOAD compared with *APOEε3/Eε3* homozygous carriers [77, 82]. Furthermore, homozygous *APOEε4* (*APOEε4/ε4*) carriers have ca. 30% higher chances of developing AD with an earlier age of onset of clinical symptoms than heterozygous *APOEε4* carriers [83, 84], who still exhibit a higher risk than carriers of other *APOE* variants. The risk is therefore dose-dependent [84]. The memory decline in Aβ-positive *APOEε4* carriers begins much earlier than in Aβ-positive *APOEε4 non*carriers; the difference is quite substantial: memory decline had an age of onset of 64.5 versus 76.5 years of age, respectively [85].

The genetic probability factor also becomes apparent when one compares the frequencies of occurrence of polymorphic alleles in affected individuals with those in the general population: *APOEε2* is present in 3.9% of AD patients (8.4% in the general population), whereas *APOEε4* is found in 36.7% of the AD population (13.7% in the general population) [86, 87]. It is noteworthy, however, that a proportion of elderly homozygous *APOEε4* carriers are not affected [88], making it clear that the correlation between *APOEε4* and AD remains poorly understood. Patients lacking ApoE expression due to mutations in both of their *APOE* alleles exhibit severe dysbetalipiproteinemia, but lack cognitive or neurological impairments [89, 90], an observation that has raised the hypothesis that ApoE does not normally play a major role in brain physiology but acquires relevance under physiopathological conditions.

It is worth noting that the occurrence of the *APOEε4* allele is not pathognomonic of AD, although its frequency in this disease is quite high (38.1%) in comparison to other forms of dementia: Alzheimer–Lewy Body dementia mix (40.6%), pure Lewy Body dementia (31.9%) and PD dementia (19.1%) groups and to the control group (7.2%) [91]. An attempt to find clinical correlates with the occurrence of *APOEε4* in a cohort of 500 PD patients did not show any statistically significant relationship with psychotic symptomatology [92]. Noteworthy is the fact that for still unknown reasons, many *APOEε4* carriers (53%) never develop AD and only 47% do so [87]. *APOEε4* affects cognitive function in other brain pathologies, undermining white matter integrity and neurovascular regulation, possibly leading to deep cortical white matter hypoxic-ischemic damage, and increased risk of small vessel (microvascular) disease in carriers [93]. *APOEε4* is also a risk factor for intracerebral hemorrhage (see, e.g., [94]). According to Mahley [95], two structural features are responsible for ApoE4 pathogenicity: an abnormal domain interaction, in which the amino acid residue Arg^{61} interacts ionically with Glu^{255}, and the less stable conformation of ApoE4 as compared to ApoE3 or ApoE2.

ApoE 4 has also been found to be correlated with an increase in the incidence of pugilistic dementia by chronic traumatic brain injury (CTBI). In 1997, a study on boxers found that those individuals carrying at least one *APOE4* allele and with high exposure to traumatic injuries (boxing) had greater Chronic Brain Traumatic scores. Moreover, all studied patients who ended up with severe impairments were carriers of this allele [96]. Several other studies support this

association: ApoE4 has been linked with an increase in the deposition of Aβ after TBI [97]; severe complications after TBI [98]; greater contusial damage and ischemic brain damage in fatal TBI [99]; tenfold increases in the incidence of AD with ApoE4 and TBI, twofold with ApoE4 alone, and no correlation with TBI alone [100]. However, there are several articles that show inconclusive results in terms of the relationship between ApoE and dementia induced by CTBI [101–104].

ApoE is also strongly associated with vascular dementia: a recent large meta-analysis including 69 studies with a total of 16,045 participants (446 cases; 11,583 controls) supported this association [105]. The meta-analysis found a 3.1 -fold increase in the risk of developing vascular dementia in *APOE4* homozygous patients compared with *APOE3* individuals.

In terms of sex differences, according to a recent meta-analysis study, male and female individuals carrying the *APOEε4* or the *APOEε3* isoform have the same chances of developing AD from age 55 to 85 years, but women have increased risk at younger ages [106]. Female patients with mild cognitive impairment (MCI) as well as fully developed AD patients appear to have negatively regulated behavioral traits like anxiety when the two alleles are expressed [107]. However, this is still a contentious subject, and in addition to the need for further studies on gender differences and other population-associated issues related to *APOE* variants, there are still meta-analytical approaches to be settled.

A cross-sectional study to assess the effect of the *APOE* allelic genotype was conducted on a cohort of 989 cognitively normal elderly adults. A subset of these individuals underwent PET for Aβ and MRI of hippocampus to evaluate its volume. *APOE4* homozygotes (*ε4/ε4*) exhibited significantly worse episodic memory and higher Aβ levels than *ε4* heterozygotes. No differences were found in hippocampal volume between different *APOE* allele carriers. *APOE* acts in a codominant form on Aβ levels, episodic memory and hippocampal volume in normal old individuals and *APOE4* homozygotes [108].

Psychological stress-reducing perceptions or cultural conditions like "positive age beliefs" have been invoked as possible factors reducing the incidence of AD in *APOEε4* carriers and healthy individuals [109]. A recent study analyzed a cohort of >900 healthy subjects considered as "exceptional elderly individuals," that is, without any signs of AD physiopathology, in the search for protective factors (*APOE* genotype, demographics, normolipidemia, etc.) but no clear conclusions were reached, warranting further investigation [110]. A more recent study of a cohort of 300 cognitively healthy Dutch centenarians showed a 2.1-fold enrichment in *APOEε2* and 2.3-fold lower values of *APOEε4*, without apparent changes in *APOEε3* [111].

The TREM2/APOE4 Risk Factor Combination

In addition to age and the *APOE* pathogenic isoform *ε4*, rare genetic variants of other proteins apparently linked to AD have been discovered. The so-called triggering receptor expressed on myeloid cells 2 (TREM2) [112], a single-pass transmembrane receptor protein present in microglia, has recently emerged as a potential risk factor (reviewed in [113]). TREM2 binds ApoE in brain, where this protein is selectively expressed by microglia [114–116], triggering a signal transduction mechanism in these cells [117]. Interestingly, the pathogenic ApoE4 isoform also causes amyloid-dependent microglial dysfunction [118]. *APOE4*-associated AD risk factor might therefore encompass, among other mechanisms, a combination of dysregulation of microglial function and hindrance of Aβ clearance (see the following section). *TREM2* knock-out microglia show reduced internalization of LDL and clusterin (CLU; also known as apolipoprotein J), two lipoproteins to which Aβ binds and are normally internalized by microglia [114]. Macrophages of humans carrying the TREM2 AD variant exhibit reduced endocytosis of Aβ-lipoprotein complexes [114]. ApoE activation of TREM2 in microglia would be a manifestation of CNS-contained innate immune response [119].

ApoE, Aβ, and AD Risk

The increased probability of pathogenic development associated with the *APOE4* allele and its antipolar *APOE2* protective character are by now relatively well documented [81, 84, 120–122]. We know that these effects are multifactorial; a first level of complexity is given by the association of these effects with Aβ, which is in turn associated with the lipidation status-dependent binding of Aβ to ApoE [121]. Early studies demonstrated that lipid-free ApoE forms complexes with Aβ; ApoE4 was found to be a faster acceptor than ApoE3 [123]. More recent work indicates that ApoE–lipid and ApoE–Aβ complex efficiency follows the order of ApoE2 > ApoE3 >> ApoE4 [124, 125]. The complex efficiency of ApoE–lipid and Aβ is inversely correlated with AD risk, lending support to the idea that ApoE aids Aβ clearance. Some studies indicate that lipid-poor and lipid-free ApoE4 can stimulate LRP1 and ApoeR2, increase amyloid precursor protein (APP) endocytosis and processing, and increase Aβ levels [126, 127] although other investigations do not find isoform-specific differences in these effects [128, 129].

Binding of the Aβ peptide to ApoE involves residues 12–28 of the peptide and residues 244–272 of ApoE [87]. ApoE4 has been characterized as the most pathogenic isoform, promoting Aβ deposition and hindering its clearance. Some studies also indicate that ApoE4 increases Aβ fibrilogenesis more than the other isoforms [130–132], whereas other studies suggest that ApoE4 is the least effective isoform in inhibiting Aβ deposition. When ApoE is associated with lipid, its capacity to inhibit Aβ fibril formation is reduced [133, 134]. Different Aβ fragments appear to exert different influences on this process: $A\beta_{40}$ was shown to inhibit Aβ deposition and $A\beta_{42}$ to augment it [135, 136]. ApoE4 appears to induce $A\beta_{40}$ aggregation in a dose-dependent manner [84, 137–139].

Affectation of the cholinergic system appears to be ApoE isoform-dependent. AD patients who are also *APOE-ε4* carriers have a more marked deficit in the ACh synthesizing machinery, as evidenced by their lower choline acetyltransferase (ChAT) levels in comparison with noncarriers [140–142]. In another study of postmortem AD brains, the *APOE-ε4* allele was found to be correlated in a dose-dependent manner with higher losses of ChAT activity in the neocortex than the other alleles. Furthermore, homozygous AD patients had more Aβ senile plaques in the temporal cortex compared to heterozygous patients with only one copy of the *ε4* allele. The former also displayed lower levels of M2 muscarinic receptor and nicotinic α4β2-type receptor densities [143].

The PDAAP transgenic mouse model of AD [144] has shown that expression of the different ApoE isoforms is associated with differences in the pattern of Aβ deposition and formation of neurite plaques. The tendency to undergo pathological Aβ deposition followed the order: mouse ApoE > knock-out mouse ApoE > human ApoE4 > human ApoE3 > human ApoE2. The protection given by the human ApoE in mice is dose-dependent, heterozygous human ApoE3 mice having a higher Aβ deposition than homozygous animals [145–150].

Aβ clearance follows three main pathways: proteolytic degradation, cellular clearance and clearance via the BBB. ApoE is involved in each one of these pathways. ApoE-dependent stimulation of Aβ degradation and phagocytosis follows the order: ApoE2 > ApoE3 > ApoE4 [151, 152]. ApoE has the capacity to activate microglia and stimulate its migration (ApoE3 > ApoE4), thus increasing Aβ clearance [153, 154]. Aβ can be transported through the BBB by LRP1-receptor binding; when bound to ApoE, Aβ clearance is slowed down, as ApoE inhibits the interaction with the receptor in the BBB (ApoE4 > ApoE3 > ApoE2) [75, 155]. ApoE4 also exacerbates Aβ plaque formation and cerebral amyloid angiopathology [156, 157]. For a comprehensive review on Aβ clearance the reader is referred to Ref. [121].

ApoE as a Biomarker of AD. Genome-Wide Association Studies and Other Approaches

As we have discussed in the preceding sections, *APOE* can safely be considered the key gene in relation to the risk of developing LOAD and numerous studies have addressed the issue to validate its use as an AD biomarker. Measurements of ApoE in cerebrospinal fluid (CSF) have produced inconsistent results, precluding the establishment of statistically significant values that differentiate cognitively normal elderly people from AD patients [158–163] or correlations between stages of AD progression ("clinical dementia rating") [164] and different ApoE isoforms [161, 163–165]. Attempts to measure ApoE from brain parenchyma have met with similarly discouraging results [166–172]. Although an isoform-dependent increase in ApoE was detected in brain and CSF of ApoE knock-in mice following the order E2 > E3 > E4 [126, 127, 173–175], these data need further validation.

A longitudinal study of CSF biomarkers in AD and MCI patients found that the ratio $A\beta_{42}/A\beta_{40}$ is modified by the presence of ApoE4, carriers presenting lower levels than noncarriers. All other biomarkers evaluated (T-tau, P-tau231, IP, T-tau/$A\beta_{42/40}$ ratio, P-tau/$A\beta_{42/40}$ ratios) did not show statistically significant differences [176].

Another study evaluating the influence of *APOE4* on AD biomarkers led to a different conclusion. A behavioral study [177] stated that *APOE4* carriers presented higher IP, P-Tau, and P-Tau/$A\beta_{42-40}$ ratios; the highest levels of these biomarkers were found in *APOE4* carriers with subjective memory decline (SMC, i.e., patients who perform correctly on standard memory tests but who complain of memory impairment). PET neuroimaging of cognitively normal, elderly *APOE4* homozygous individuals, that is, presymptomatic but at high risk of developing LOAD, has revealed decreased bilateral medial temporal fluorodeoxyglucose metabolism and increased lenticular amyloid (Florbetapir F^{18}) deposition, predominantly in the putamen, compared to noncarriers. The latter is also observed in preclinical carriers of autosomal dominant AD and in preclinical AD associated with Down syndrome [178]. It should be pointed out that the pattern of atrophy in EOAD and LOAD is similar [179].

In cognitively normal individuals, neuroimaging neurodegeneration biomarkers did not differ between β-amyloid+ and β-amyloid- carriers. In contrast, patients with MCI and SNAP with *ApoE2* exhibited markedly less temporal hypometabolism and temporal lobe atrophy [180]. The reader is referred to a recent review [181] relating *APOE* with metabolic cerebrovascular and metabolic dysfunction.

Epistasis is defined as the interaction phenomenon whereby the effect of one gene allele is dependent on the presence of other genes, termed "modifier genes." If a locus is studied without inclusion of other genes, its influence on disease might be missed [80]. The above loci can be used to generate a polygenic score prediction in AD [182]. The data obtained by this predictive exercise can help diagnose individuals at risk of developing AD or predict the possible development of AD in apparently healthy controls [183, 184] or MCI [185].

In addition to the well-known *APOE* gene, other genes are purported to influence the occurrence of LOAD. A GWAS study [186] revealed associations of the following risk genes with LOAD: *ABCA7, BIN1, CD2AP, CLU, CR1, EPHA1, MS4A6A, PICALM, APOE4, SORL1, CASS4, FERMT2, HLA-DRB5-DRB1, INPP5D, MEF2C, CELF1, NME8, PTK2B, SLC2A4, and ZCWPW1*. The vast majority of these genes can be grouped into one or more pathways involved in the physiopathology of AD (β-amyloid pathway, inflammatory immunity pathway, lipid transport and metabolism pathways, synaptic cell function and endocytic pathways, APP pathways, or tau pathway [80]). A recent GWAS in African North-American AD patients implicates novel AD risk genes, including F5, *ABCA7*, *KANSL1*,

CNN2, and *TRIM35* [187]. The study highlights the value of undertaking sequence analyses of multiple ethnic populations for the discovery of new impactful AD risk gene variants. Apparently, individual variants in genetic loci exert a modest influence on LOAD risk, well below that of *APOE4*.

A recent study of polygenic predictors of age-related decline in cognitive ability employed 14 polygenic scores for phenotypes that have been suggested as risk or protective factors for cognitive decline, respectively. The study found that only *APOE4* status provided a significant prediction of the rate of cognitive decline between ages 70 and 79, indicating that GWAS are not yet as developed as the *APOE4* predictor [188]. Attenuated cerebrovascular function during young adulthood appears to provide a mechanism whereby the *APOE4* risk allele confers susceptibility to AD [189].

The advent of techniques to produce experimentally induced pluripotent stem cells (iPSCs) has opened the possibility of exploring the early stages and pathogenesis of disease and attempting to establish correlations with the different ApoE isoforms. For instance, the iPSC lines reproduce, in vitro, some of the characteristic signs of AD observed in patients with the V717I mutation present in APP in the London familial form of AD, such as increased Aβ and tau [190]. Similarly, isogenic ApoE4 cell lines were shown to have elevated levels of Aβ and phosphorylated tau [191] accompanied by an increase in synaptogenesis and microglia and astrocyte dysfunction [191, 192]. More recently, iPSCs from a large cohort of sporadic AD patients were obtained [193] and the transcriptome of these cells suggests that upregulated genes are modulated by the transcriptional repressor REST (repressor element 1-silencing transcription factor or neuron-restrictive silencer factor [NRSF]), a key regulator of neuronal differentiation [194, 195]. Interestingly, this factor is also induced in human brain during normal aging but exhibits diminished levels in AD [196]. APOE4 expression in the iPSCs was found to be associated with lower nuclear translocation and chromatin binding of REST; the dysfunction of this factor may potentially contribute to AD pathogenesis and link altered transcription and altered differentiation with ApoE4 expression [193].

ApoE and Mimetic Peptides as Therapeutic Agents in AD

Given our still limited knowledge of AD etiopathogenesis, the putative targets for therapeutic intervention are still vague; it is highly likely that some of the multiple pathogenic mechanisms (see, e.g., [197] and possible therapeutic targets [198] will be discarded as we deepen our understanding of AD dysfunction. The long latency period during the presymptomatic stages of the LOAD form of the disease opens a considerable time window for attempting to reduce the risk burden associated with AD. A recent review analyzes AD risk factors and outlines six different mechanisms in AD pathogenesis and possible strategies to ameliorate such risks. The mechanisms fall into two categories: systemic factors (altered glucose metabolism, inflammation, and oxidative stress) and neurological mechanisms (trophic factors, amyloid burden, and calcium toxicity) [198].

Accumulating evidence of the roles played by the different ApoE isoforms in physiological and physiopathological mechanisms and of the risk and course of AD has given rise to several approaches aimed at reversing or inhibiting pathogenicity and/or improving beneficial properties. One such approach is the design and application of inhibitors of ApoE4 activity. A small molecule has been developed to inhibit the interactions between the N- and C-term domains of ApoE4, thus increasing its susceptibility to proteolytic degradation and converting ApoE4 into an ApoE3-like protein, devoid of the characteristic ApoE4 pathogenic properties [199]. The abnormal domain interaction that characterizes ApoE4, in which Arg 61 interacts ionically with Glu255, can be blocked using gene targeting approaches, for example, by replacing Arg61 with a threonine residue [95]. This leads to higher ApoE4 levels and lipid-binding capacity and decreases intracellular degradation of the protein.

ApoE4 lipidation has been reported to be deficient compared with the other isoforms [25]. Diminished lipidation affects the aggregation and clearance of Aβ. Exploiting this knowledge, a possible therapeutic approach is to increase ApoE4 lipidation. The retinoind X receptor agonist, Bexarotene, increases the activity of transporters involved in protein lipidation, such as the ATP-binding cassette (ABC) transporter 1 (ABCA1) and ABCG1 [200, 201]. ABC transporters are a large family of integral membrane protein transporters with high structural homology, consisting of six or 12 transmembrane helices and one or two ATP binding sites. These transporters are subdivided into seven subfamilies based on their structural kindredness [202]. ABCG1, which belongs to the ABCG subfamily, appears to play a major role in the export of cholesterol from cells to HDL through the "reverse cholesterol transport" mechanism, that is, the movement of cholesterol from peripheral tissues to the liver. It also appears to reverse poor ApoE4 lipidation and its associated physiopathology [201]. This therapeutic effect has also been reported to be accomplished with a docosahexaenoic acid-enriched diet [203].

ApoE proteins interact with Aβ, prompting its deposition; ApoE4 exhibits the strongest effect in Aβ deposition and aggregation and diminishes its clearance [22]. Amino acid residues 12–28 in Aβ are the ones that interact with ApoE [122]. Overload by exogenous $A\beta_{12–28}$ peptide reduces ApoE-Aβ interactions and their synergistic effects [204]. Increased ApoE lipidation also reduces this interaction [200].

Another therapeutic avenue is aimed at reducing the amount of ApoE4. ApoE4-specific monoclonal antibodies (mAb) have been studied in ApoE4-positive transgenic mice. When these mice were treated with anti-ApoE, antibody accumulation was observed in hippocampal regions, together with a reduction in hyperphosphorylation of brain tau and increased levels of ApoER2 compared with untreated control mice, together with a concomitant prevention of ApoE4-induced cognitive impairment [205].

Treatments with ApoE-mimetic surrogate proteins comprising the receptor-binding region of ApoE but not the lipid- or the Aβ-binding regions [206, 207] improve the outcomes of conditions such as traumatic brain injury and focal ischemia [208, 209] and reduce memory deficits and the occurrence of plaques and tangles [210]. These surrogate ApoE fragments have been proposed as therapeutic agents for sporadic AD [211].

The use of smaller peptide fragments has been suggested due to their likelihood of crossing the BBB. ApoE133–149 (COG133) has been assayed in a transgenic mouse model of AD, and shown to reduce inflammation and amyloid deposition while augmenting the enzymatic activity of protein phosphatase 2A [210]. Another ApoE-mimetic peptide, Ac-hE18A-NH2, was shown to restore ApoE levels previously reduced by in vitro application of ox-PAPC or $A\beta_{42}$ [212]. The major lipoprotein of plasma HDL (accounting for ca. 70% of the total HDL protein), ApoA-I, has also been tested as a putative therapeutic agent [213]. The rationale behind this approach is analogous. The high-density lipoprotein mimetic peptide, 4F, has been experimentally tested in the APP transgenic mouse model where it improved cognition and reduced Aβ burden upon oral application [214]. Intraperitoneal administration of the mitochondria-targeted tetra-peptide SS31 to the APP mouse model of AD over a period of 6 weeks reduced Aβ production and Aβ-induced mitochondrial and synaptic toxicities, from which the protective effect of SS31 was inferred [215]. More recently, another peptide, 4F, was assayed on human astroglial cells and was found to increase both the production, lipidation, and secretion of ApoE in an ABCA1-dependent manner, and also to restore the Aβ-induced inhibition of these processes [216]. ABCA1 is a lipid transporter operative in the endoplasmic reticulum–Golgi complex exocytic pathway. As recently discussed by Wang and Zhu [217], several issues remain to be elucidated in relation to these positive effects of 4F, particularly whether the effects are isoform-specific.

Another peptide therapeutic approach in an animal model showed that $A\beta_{12–28P}$ diminished

amyloid plaques in the APP/PS1 transgenic mouse model and vascular amyloid in TgSwDI mice with congophilic amyloid angiopathy [87]. Subsequent work from these authors showed that the peptide was also effective in tau-related pathology as tested on the triple transgenic AD mouse (3xTg, with PS1M146V, APPSwe, and tauP30IL transgenes) [218].

ApoE upregulation has also been suggested as a possible therapeutic approach in AD. Several candidates have been tested, such as bexarotene, a retinoid X receptor agonist, with apparently some promising outcomes reported [201, 219, 220], although results are still inconsistent [221–225] and some side effects have been reported [220, 226].

An additional therapeutic approach is based on gene transfer of *APOEε2*. The rationale behind this strategy is the notion that ApoE2 exerts a neuroprotective role in AD and the hypothesis that overexpressing this isoform might halt or mitigate ApoE4 pathogenicity. Preliminary studies in mouse have shown encouraging results [227].

Other therapeutic strategies currently being considered focus on the targets of ApoE. ApoE interacts with LRP1 and ApoeR2 proteins of the LDL receptor family in an isoform-dependent manner [120], ApoE4 being one of the most efficient effectors for modulating these receptor levels in the hippocampus [228]. The rationale here is that controlling the effect of ApoE4 on the expression of these receptors could counteract its negative effects.

ApoE4 has a distinctive protease pathway that generates neurotoxic fragments when expressed in neurons [199, 229]. Identifying and inhibiting the molecules involved in this pathway might constitute a potential therapeutic target.

The susceptibility of the BBB to damage is also affected by ApoE4 [230, 231]. The barrier proper and the neurovascular dysfunction generated by ApoE4 is an additional therapeutic target in AD.

Conclusions

Despite being the most common form of dementia worldwide, the etiology and pathogenesis of Alzheimer's disease is far from being understood. This review has attempted to provide an appraisal of our current knowledge on the influence of apolipoprotein E on AD. We have reviewed the biochemical and physiological aspects of the different Apo E isoforms, the protection, susceptibility, and high-risk factors associated with expression of the ApoE4 isoform, and the evolution and prognosis of AD. We have also discussed the current strategies involving the use of ApoE as a diagnostic tool, and the multiple approaches addressing this protein as a therapeutic target. The epidemiological data and projections of AD morbidity for the forthcoming decades make it of the utmost importance to find new paradigms to unravel other genetic susceptibility factors, possibly stemming from the use of genome-wide association studies and other multidimensional approaches, and find early diagnostic tools conservatively applicable at the prodromic stages of the disease.

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30

Fearing Parkinson's Disease: Relationships Between Cognition and Emotion

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Introduction

Parkinson's disease (PD) is a neurodegenerative disorder that results from a progressive dopaminergic neuronal loss in the nigrostriatal pathway. It is considered a multifactorial condition due to the multiplicity of symptoms experienced, that can be grouped according to which circuit malfunction is involved [1]. The types of symptoms present and their severity depend significantly on the length of time since onset, the speed of functional decline and treatment received [2]. While this condition is known for its characteristic motor deficits, patients also have a wide variety of non-motor symptoms (NMS), such as impaired learning and memory, mood changes and gastrointestinal problems, among others [3].

Cognitive dysfunctions and emotional disturbances are one of the most severe non-motor symptoms of PD, leading to an increasing caregiver burden [4]. The focus on cognitive–emotional impairment in neurodegenerative diseases, including PD, is shifting from end to earlier stages and could be stronger determinants of Health-Related Quality of Life (HRQoL) [5, 6]. The molecular mechanism responsible for degenerative processes in the nigrostriatal dopaminergic system remains unknown. A variety of research approaches linked genes associated with PD, participation of a reactive gliosis leading to a neuroinflammatory state, mitochondrial dysfunction, protein degradation, aggregation of neurotoxic oligomers and oxidative stress among others [7]. Emerging data suggest that dopamine release in mesolimbic areas could contribute to synaptic plasticity required for updating learning and memory [8]. Any failure in the connection of these circuits might be implicated in the genesis and progression of dementia, as well as other neuropsychiatric aspects of PD [9]. In this chapter, we will update the latest findings about neurocircuitries, regulation networks and possible therapeutic approaches in different experimental models of parkinsonism about non-motor symptoms of PD.

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Stages of PD-Related Pathology

PD is a multisystem and autonomic system disorder during the progression of which subcortical nuclei, cortical areas, spinal cord structures and portions of the peripheral and enteric nervous system become involved [10]. Almost 100 years have passed since Parkinson's first description when Fredrick Lewy described the presence of some unusual proteins in the brain that make some people act and think differently. His discovery became known as Lewy bodies (LBs), as published in the *Handbook of Neurology* in 1912. LBs became a defining pathological characteristic of Parkinson's disease and dementia with Lewy bodies (DLB) [11, 12]. A typical LB is an eosinophilic cytoplasmic inclusion of a dense core surrounded by a halo of 10-nm-wide radiating fibrils, and may vary among brain areas. It is composed of a primary structural component which is the protein alpha-synuclein associated with other proteins, such as ubiquitin, neurofilament protein and alpha B crystalline. Also observed is the presence of Tau proteins as well as neurofibrillary tangles, particularly in the amygdala [13]. Otherwise, Lewy neurites (LNs) are abnormal neurites containing granular material and abnormal α-synuclein filaments similar to those found in Lewy bodies and because of this they constitute a key feature of α-synucleinopathies and AD [14].

Thus, systematic studies of PD patients made possible setting up of a classification of six neuropathological stages (Table 30.1). Each of these stages is marked by a continuum development of distinctive bodies' inclusion and, in part, branching Lewy neurites (LNs) within cellular processes, granular aggregations and Lewy bodies (LBs) in the soma of involved nerve cells [15].

Besides several of these non-specific symptoms, the combination of specific vulnerable neuronal types and susceptible brain regions to inclusion bodies is specific to PD and makes its differential diagnosis and association from other possible neurodegenerative diseases [15, 16]. It is also true that the complete absence of detectables LNs/LBs in neuroimaging, could lead to misdiagnosis. There are several physiological changes that accompany both, the neurodegenerative process and the natural process of aging. such as protein aggregations. In PD, alpha synuclein aggregation is the hallmark of early presymptomatic disease-related PD stages [17, 18].

Table 30.1 Description of Braak's six stages of progression of Parkinson's disease, with specific characteristics of each stage. Green highlights the brain areas correlated with non-motor symptoms, red with motor symptoms and blue with cognitive impairments

	Characteristics
Stage 1	LBs and neurites in the dorsal IX/X motor nuclei and/or intermediate reticular zone. Involvement of myenteric plexus
Stage 2	Medulla oblongata and pontine tegmentum affected. Pathology of stage 1 plus lesions in the caudal raphe nuclei, gigantocellular reticular nucleus, coeruleus–subsceruleus complex and the olfactory bulb
Stage 3	Pathology of stage 2 plus midbrain lesions, specifically in SNpc
Stage 4	Basal prosencephalon and mesocortex pathology (cortical involvement confined to the transentorhinal region and allocortex, and CA2 plexus). Lesions in the midbrain, pons and medulla oblongata
Stage 5	Extension to sensory association areas of the neocortex and prefrontal neocortex
Stage 6	Pathology of the previous five stages plus lesions in first-order sensory association areas of the neocortex and pre-motor areas. Mild changes in primary sensory areas and the primary motor cortex

Non-motor Features in PD

Non-motor symptoms (NMS) are detrimental to health-related quality of life (HRQoL) of Parkinson's disease patients [19]. Therefore, it would appear that the first contemporary account on NMS appeared with the introduction of levodopa (LD) as a pharmacological strategy to compensate for or replace dopamine deficiency. Nevertheless, this disease is associated with other neurotransmitters deficits and, as a consequence LD could in fact improve several NMS. After several studies over time, NMS were taken into account and their impact was measured [20]; such is the case that, nowadays, one of the major therapeutic challenges for PD

is the development of effective symptomatic interventions for NMS both in the motor stages and the pre-motor stages [21].

NMS can be grouped according to the general traits of specific endophenotypes, brain areas and neural circuits involved. Among physiological disturbances, several studies reported dysautonomia – changes in activity of the autonomic nervous system – as a frequent occurrence in PD [22]. Probably the most common forms of dysautonomia are altered vascular/circulatory, digestive and urinary performances and are considered as secondary causes [23]. Not least, olfactory dysfunction is among the earliest NMS of PD, present in approximately 90% of early-stage PD cases and can precede the onset of motor symptoms by years [24]. PD patients also experience sleep disorders: sleep fragmentation, rapid eye movement behaviour disorder and complex paroxysmal nocturnal motor behaviour [23, 25, 26]. Other neuropsychiatric alterations experienced by subjects with PD with a significant statistic importance are visual and auditory hallucinations [27], agitated confusion [28], vivid [29] dreaming, delirium and delusions [30, 31]. All these alterations may precede parkinsonian symptoms and they usually increase in intensity with disease progression.

On the other hand, affective disturbances like anhedonia – lowered ability to experience pleasure – could be explained by dysfunction of dopamine reward pathway in the mesolimbic area, secondary to the neurodegenerative process observed in PD [32]. In correlation with anhedonia, 30–40% of PD subjects have significant depression [33] being one of the most frequently reported neuropsychiatric disturbances in PD. It is also true that depressive disturbances are underrecognized and frequently undertreated [34] and could lead to earlier initiation of dopaminergic therapy, greater functional disability, faster physical and cognitive deterioration, increased mortality, poorer quality of life and increased caregiver distress [34]. Furthermore, the observable phenomenon of apathy, increasingly recognized as a syndrome with distinct targets for pharmacotherapy, is related to four subdomains: (i) decrease in emotional resonance (reward deficiency syndrome), (ii) depression, (iii) decrease in cognitive interests (executive dysfunction) and (iv) absence of spontaneous activation of mental processes (auto-activation deficit), and their dysfunction could be due to the interrelationship with other subdomains and with different neural circuits [35].

Anxiety is present in up to 50% of PD patients [36] and it is frequently comorbid with depression [37]. In spite of its significant impact, the symptomatology, chronology and neurobiology of anxiety in PD are all poorly understood, which complicates accurate diagnosis and development of effective treatment strategies [38]. Moreover, anxiety and depression disorders are greatly related to other affective disturbances such as panic attacks and social phobias (for further review see [39]).

Of equal or greater importance, we found that moderate to severe cognitive deterioration is found in this neurodegenerative disease. The PD cognitive spectrum, it is observed, ranges from normal cognition, through mild cognitive impairment (MCI) to moderate and even severe PD dementia (PDD) [40]. Approximately, 25% of non-demented PD patients have mild cognitive impairment (MCI); besides, up to 80% of all PD patients will eventually develop dementia [41]. A great heterogeneity among cognitive deficits was reported and is characterized by subtle changes, difficult to detect and diagnose [21].

Given all that has been mentioned so far, it is very difficult to study a specific isolated symptom without the interaction with other NMS and the onset of motor deficits. These PD-associated changes are also very difficult to find in current PD animal models. In the following sections, we will delve into different experimental model systems and the main focus of this chapter, the interplay between cognition and emotion in PD.

Experimental Model Systems

Disease models have a fundamental role in the knowledge of neurodegenerative pathogenesis. In particular, the main focus of study in PD lies behind the causes of selective loss of dopaminer-

gic neurons at a cellular level, and its correlation at a behavioural level, since motor disturbances do not present clinically until 70–80% of striatal dopamine has been lost. However, addressing the pathological, pharmacological and clinical aspects of NMS will allow defining of the prediagnostic stages of PD.

It is expected that an experimental model must reproduce both clinical and pathological features of PD [42]. However, no animal or cell culture model is likely to be an exact phenotype of the human disease. For example, motor system organization differs in rodents and humans as well as their dopaminergic content and, consequently, a particular behavioural phenotype depends upon its correlation with striatal dopaminergic function, and not necessarily to a symptom of PD. Nevertheless, animal models are critical to achieve a better understanding of different brain circuits and neural networks that converge on a parkinsonian phenotype in order to establish potential therapeutic strategies. It is also true that experimental modelling of NMS of PD has serious limitations recapitulating the clinical and pathological characteristics and its progressive nature [21].

Approximately, 95% of PD cases are considered sporadic and cannot be correlated to a typically inherited condition. The remaining 5% of cases are linked to several monogenic mutations and gene-environment interactions [43]. To extrapolate these conditions, we can distinguish animal models based on toxins, such as 6-hydroxydopamine (6-OHDA), rotenone, paraquat, MPTP, reserpine, from those that are based on genetic forms, most frequently α-synuclein, parkin, ubiquitin C-terminal hydrolase L1 and DJ-1 transgenic mice. Schapira et al. state that none of this model type has shown consistency in the evaluation of any specific NMS and that the presence of motor symptoms may interfere with the ability to evaluate non-motor disturbances [21].

Behavioural Non-motor Impairments in Rodent Models of Parkinsonism

Rodent models, both rats and mice, constitute the most widely used and best-characterized experimental approach for this neurodegenerative disease [42, 44], especially those based on the injection of 6-OHDA (for review, see [42]). Moreover, Magnard et al. suggest the importance of therapeutical targets concerning pharmacological treatment to reverse specific NMS; for example, some pharmacological studies show that a certain medication for the treatment of motivational deficits had positive results not only for this NMS but also it is effective on affective disturbances, indicating a potential confluence of molecular mechanisms between these NMS in PD [32, 42, 45, 46]. Although motor symptoms can constitute a major bias in the study of neurobehavioural mechanisms, it has been considered in the last decades, the key role of the dopaminergic nigrostriatal pathway in the evaluation of motivational, affective and cognitive tasks [47]. For example, to evaluate neurobiological mechanisms underlying apathy, several studies assess motivational preparatory processes with the use of operant self-administrating procedure of food reward. behavioural-like, several studies assess evaluation of motivational preparatory processes preferably operant self-administrating procedure in order to get and eat palatable food [48]. In addition, depression-like state is commonly measured in the force-swim test [49] and in the tail-suspension test [50] giving a specific index when the animal stops swimming or stops turning to both sides. Besides, in rodent experimental systems, anhedonia is considered as a behavioural insensitivity to the rewarding or reinforcing properties of a specific substance (i.e. sucrose, drugs of abuse, alcohol) or event (i.e. copulation). For anxiety-like behaviours, animals are evaluated in their performance on the plus-maze task, on a light/dark avoidance box and the classical open field [51]. Regarding cognitive impairment, different learning and memory deficits were characterized using a variety of behavioural tasks such as object recognition [52], social recognition [53], Morris water maze [53, 54] and Y-maze [49, 55]. However, further studies will prompt complementary approaches for elucidating prominent non-motor, and pre-motor symptoms of PD throughout the progressive neurodegeneration of the dopaminergic nigrostriatal

pathway; especially those with overexpression of wild type α-synuclein, by viral vector induction or using transgenic strains [21]. These studies will be of significant importance in drugs screening, efficiency tests, revealing potential collateral effects and neurosurgical approaches [42].

Interaction Between Cognition and Emotion Disturbances

Parkinson's disease has a complex pathophysiology caused by the emergence of several risk factors with variable contributions to motor, cognitive and affective disturbances [56]. In the next sections of this chapter, we will focus on cognitive and emotional disturbances since a continuous interplay between these two NMS affects different aspects of the daily life of subjects with PD.

When the physician James Parkinson published his monograph *An essay on the shaking palsy*, it was the first statement of Parkinson's disease as a neurological disorder [1]. Nevertheless, this description was defined only on the basis of the presence of specific motor features [57]. Through the years, anatomy studies advanced towards the clinical delineation of PD and related disorders and, as consequence, identified and defined a prior phase to the time that motor diagnostic appear and pharmacological treatment was assessed. This prior phase suggests that advanced nigral dopaminergic degeneration is already present at the time of motor symptoms arise and several clinical biomarker and genetic biomarkers that, alone or in combination, can accurately identify a subset of individuals who eventually will fill motor criteria [58]. Prodromal PD is the name for this early state of neurodegeneration and specific diagnostic criteria were recently proposed. Indeed, recent evidence suggests that PD may have several endophenotypes and some of these endophenotypes are dominated by NMS (Table 30.1) [21]. Among the many clinical markers of the prodromal state, impaired cognition occurs in 20–30% of individuals with early PD [6, 33], and involves a failure in the corticoestriatal loop (ventrolateral prefrontal cortex, caudate nucleus, and thalamus), involving executive defects in planning, initiation and visuospatial memory [59]. This aspect is relevant because 25% of PD patients have working memory deterioration and short-term memory impairment [4, 60]. Furthermore, the hippocampus is involved in the allocentric and dorsolateral striatum in the egocentric spatial memory [61], suggesting that many aspects of cognitive alterations are between the striatum and the prefrontal cortex but also the dorsal hippocampal circuit [62–64].

On the other hand, and as mentioned previously, the cause of PD remains unknown. Although research has mostly focused on motor symptoms and cognitive impairments, the observation that emotional processing can be affected in PD has received growing attention [65]. In fact, Parkinson's disease provides a useful model for studying the neural substrates of emotional processing. Research experts in the field of emotions agree that these are episodes of synchronized changes in several of the organism's components in response to environmental events of major significance to the organism, like thoughts, feelings, memories – internal events – or reaction to a novel stimulus, other people's behavior – external events – including motor expression, action tendencies, cognitive processes and the different types of physiological activation of autonomic nervous system, hormone levels and certain neurotransmitters, known as physiological arousal [66]. Aside from the affective disturbances mentioned above, we also found other emotion processing affections like deficits in recognition of disgust and anger [33] and difficulties in the production of emotional responses and in the perception of both facial expressions and affective prosody [67]. Among the control mechanisms responsible for emotional processing, the limbic system leads the emotional orchestra with its two major structures such as the hippocampus and the amygdala. Emotion recognition is associated with the activation of a complex network comprising the occipito-temporal, the amygdala, and the orbito-frontal cortex [68]. The amygdala plays an important role in the regulation of emotional states that lead to behavioural expression of such states [69] and it is also a structure that registers emotional occurrences [70]. In correlation

with nigrostriatal dopaminergic neuronal loss observed in PD, also reported was a remarkable degeneration of nigromesolimbic dopaminergic system that originates in the ventral tegmental area (VTA) and medial SNpc, which innervates the amygdala complex [67].

For all this, Parkinson's disease has a complex pathophysiology caused by the emergence of several risk factors with variable contributions to motor, cognitive and affective disturbances [56]. A continuous interplay between cognition and sensorimotor processing is observed in the ability of PD patients to walk safely, especially in challenging conditions that require adaptation to environmental changes and obstacles [68]. In elderly people and those with neurological diseases, there is an increase difficulty in performing a cognitive task whilst walking, assuming the interference of shared networks within the brain [71]. A critical network affected in PD is the cholinergic system with a vital role in the top-down control of attention, orientation and stimulus discrimination [72]. Several studies reported associations between gait disturbances, cognitive impairment and emotional stage [68]. Gross et al. reported how emotional feelings influence walking performance and characterize two types in healthy adults: (i) "sad walking" – reduced walking speed, lower range of extremities motion and higher flexion of the neck and the trunk – and (ii) "joyful walking" – increased gait velocity, wider range of motion and greater trunk extension [73]. At the same time, Hamman and collaborators [74] described that amygdala activity is related to enhancing memory for pleasant and aversive stimuli where an appetitive stimulus (i.e. food and shopping) activates "approach" behaviour and an aversive stimulus (i.e. frightening animals or violence) activates "avoidance" behaviour. These behaviours suggest that level of arousal has a salient effect, as well as the valence of people's emotions [75].

It is important that clinical and epidemiologic studies have increasingly recognized and investigated the importance of NMS, and their interaction, in understanding the natural history, aetiology and clinical care of PD emphasizing on gender differences. PD disproportionately affects men and the male-to-female incidence ratio is 1.5 approximately [76]. In relation to motor symptoms, male patients tend to develop motor symptoms earlier and have faster progression while female patients are more prone to develop dyskinesia whereas in the NMS, women have a higher prevalence of fatigue, apathy, depression, anxiety and pain and men have severe sexual dysfunction and poorer performance in multiple cognitive domains around PD diagnosis [66, 77, 78]. Gender differences in cognition have not been extensively examined, some reports observed deficits in men in aspects of cognition that contribute to activities of daily living, that is, verbal fluency and recognition of facial emotion and in women in visuospatial cognition [79]. Knowledge about differences in the presentation of PD symptoms and about the pathophysiology underlying those differences may enhance the accuracy and effectiveness of clinical assessment and treatment of the disease [78].

Regulation Networks

Several neuropathological studies have shown that some of these NMS are the consequence of an imbalance between serotoninergic, cholinergic and dopaminergic systems of different regulation networks in different brain circuits. For example, a growing interest is emerging about the role of the amygdala and its neural connections in psychiatric symptoms in PD. A decreased responsiveness is found in the amygdala related to fearful facial expressions, prosodic, written verbal stimuli, decision-making, and facial recognition in parkinsonian patients [80, 81]. It is of remarkable importance that both emotional and social experience as retention and memory formation implies the fundamental anatomical and physiological basis of synapses that link neurons into networks. Through specific electrical signals, synaptic junctions allow the release of chemical neurotransmitters between a presynaptic and a postsynaptic neuron. A reduction in the number and quality of synapses in certain neuronal networks correlates with loss of memory in dementia disease states like Parkinson's disease.

Regarding this, Hopper et al. [82] suggests the essential role of the major transcription factor regulating expression of heat shock genes: heat shock factor 1 (HSF1). Besides establishing and sustaining synaptic fidelity and function in memory consolidation, HSF1 augments formation of essential synaptic elements – neuroligands, vesicle transport, synaptic scaffolding proteins, lipid rafts, synaptic spines and axodendritic synapses – and is potentially implicated in the regulation of huntingtin and α-synuclein aggregation [83–85]. Emotional events can alter neurotransmitters and hormones that can induce HSF1 activity, for example, serotonin, associated with danger recognition [86] is enclosed with activation of HSP1, suggesting that through this molecular pathway emotional experiences could become more memorable [82].

Moreover, a combination of dopaminergic and cholinergic deficiencies has been proposed as a crucial condition for the appearance of cognitive dysfunctions [87], since monoaminergic innervation from the locus ceruleus, serotonin neurotramistter release from neurons of the raphe neurons, and cholinergicic innervation provided by neurons of the nucleus basalis of Meynert are deficient in the neocortex in PD [88–91]. In correlation with this, Broussard et al. suggest that dopamine release in the hippocampus could contribute to synaptic plasticity required for updating learning and memory [8] and any failure in the connection of corticostriatal and hippocampal loops might be implicated in the genesis and progression of dementia, as well as, other neuropsychiatric aspect of PD [9].

Otherwise, the resultant spectrum of symptoms of a PD patient derives from the interplay between genetics and environment and involves multiple neuroanatomical structures. Among the multiple complex factors that contribute to the appearance of PD and that could be possible therapeutic targets, there are those of a mitochondrial nature. Experimental results, in different animal models, indicate that this neurodegenerative disease may be related to two interdependent conditions of the brain mitochondria: (a) mitochondrial dysfunction and (b) mitochondrial oxidative damage [92]. In both clinical PD and animal parkinsonism, mitochondrial dysfunction is frequently associated with a reduced activity of mitochondrial complex I in several brain areas such as substantia nigra [93] and prefrontal cortex [92]. This reduced activity may be reflected in a variety of symptoms expressed in PD and correlated with an increase in cell death processes as a result of a deficient production of adenosine triphosphate (ATP), an increase in the release of proapoptotic factors and reactive oxygen species (ROS) generation, which increase oxidative stress susceptibility [94]. An excessive oxidative stress leads to the production and accumulation of reactive and toxic aldehydes by lipid peroxidation and these metabolites may inhibit aldehyde dehydrogenase 2 (ALDH2) enzyme leading to a higher toxic damage in several brain areas. About this fact, several studies proposed the catecholaldehyde hypothesis of neurodegeneration [94–96], where impairments of cell metabolism and regulatory processes including mitochondrial dysfunction, vesicular transport alterations, lipid peroxidation, protein-cross-linking and oxidative stress generate higher levels of aldehyde accumulation and, as consequence, the deposition of neurotoxic metabolites such as 3,4-dihydroxyphenylacetaldehyde (DOPAL) and 3,4-dihydroxy-phenylglycolaldehyde (DOPEGAL) [97] that may contribute to the appearance of both motor and non-motor symptoms of PD.

Pharmacological treatments to relieve specific motor symptoms aim to increase dopaminergic concentrations or to stimulate dopaminergic receptors. However, the understanding of genetic modifications and mechanisms of neuroinflammation in cellular and animal models is crucial to identify potential targets or therapeutics-related approaches intended to relieve severe symptoms that affect the HRQoL.

Potential Therapeutical Approaches in Different Experimental Models

Several studies have been reported to recreate the characteristic motor symptoms of PD as consequence of nigrostriatal dopaminergic loss.

From, computational models, overexpression of α-synuclein (α-syn) to neurotocicity drugs inducing oxidative stress at michocondrial level such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6OHDA), Paraquat or rotenone have been used to investigate the dopamine deficiency on PD symptoms [98–100].

Important approaches were adopted to study progress linked to genes associated with Parkinson's disease, Parkinson-familial EP, alpha synuclein, parkin, LRRK2, etc. and possible overexpression therapies in substantia nigra and striatum (tyrosine hydroxylase, aromatic L-amino acid decarboxylase, GTP-cyclohydrolase I and growth factors of TGFbeta family-GDNF and neurturin), without yet reaching a significant clinical benefit [101]. There is a general consensus in the pathogenesis regarding the participation of a possible reactive gliosis, microglial activation, mitochondrial dysfunction, protein degradation, aggregation of alpha synuclein to neurotoxic oligomers, oxidative stress (triggering redox-dependent signals) and neuroinflammation as processes involved in the degeneration of dopaminergic neurons that are lost in the disease [7].

In all these experimental models, both innate and adaptive neuroinflammation immune responses may contribute to dopaminergic neurodegeneration and disease progression. Dopaminergic neurons and microglia are sensitive to even minor disturbances in the CNS, become microglial cells readily activated during neuropathological conditions [101]. So, in experimental animal models of PD, good approaches were conducted in order to downregulate immune and inflammatory processes using immunomodulatory agents. The modulation of inflammation could be important in slowing the progression of PD and might be exploited as treatments in patients with PD.

In this way, a therapeutic possibility of increasing clinical interest in the treatment of neurodegenerative processes is the use of neurotrophic factors that prevent degeneration and restore the function of the remaining neuronal populations. Interestingly, in the evolution of parkinsonian patients it has been reported that insulin growth factor I (IGF-I) is elevated in serum in moderate stages of the disease, and not in early stages [101], perhaps as a natural anti-inflammatory response. Rodriguez-Perez et al. demonstrated that IGF-1 participates, together with the local renin angiotensin system, to inhibit or activate neuroinflammation (transition from phenotype M1 to M2), oxidative stress and dopaminergic degeneration induced by the MPP + neurotoxin. It has also been shown that candesartan (Angiotensin II receptor antagonist, selective for AT1) managed to decrease inflammation and enhance/improve the functioning of nigrostriatal dopaminergic neurons in mutated forms of alpha-synuclein model [102].

Among the neurotrophic factors with a proven dopaminotrophic effect, glial cell line-derived neurotrophic factor (GDNF) and neurturin (NRTN) have been extensively tested in animal models of PD and indeed translated to early phases of clinical trials (for review, see [103]). Chronic infusion of NRTN ameliorated motor scores in MPTP-treated animals has been reported [75]. In addition, it has been demonstrated that overexpression of pleiotrophin partially rescued the immunoreactivity of dopaminergic neuron bodies and terminals in the degeneration model induced by 6-OHDA [104].

Moreover, several natural phytochemicals were evaluated in Parkinson animal models. Among them, naringin, which is found in grapefruits and citrus fruits in high concentrations [75], has pharmacological anti-inflammatory and antioxidant activities inducing also neuroprotective effects against neurodegenerative disorders [75]. The ability of naringin to prevent neurodegeneration through an anti-inflammatory effect increasing GDNF expression was reported [105, 106]. Naphthazarin, a naphthoquinone phytochemical, was also evaluated in a Parkinson's disease animal model. Seon Young Choi found that naphthazarin induced a neuroprotective effect, enhanced movement ability, prevented loss of dopaminergic neurons and attenuated neuroinflammation, mediated by the suppression of astroglial activation, in MPTP animal model [106]. Currently, the discovery of animal cloning and the subsequent development of cell reprogramming technology constitute a new paradigm shift in geron-

tology and neurodegeneration. Takahashi and Yamanaka demonstrated that the transfer of only four master genes, namely oct4, sox2, klf4 and c-myc (OSKM genes) to adult mouse fibroblasts was able to reprogramme them, taking the cells to a pluripotency stage in which they behave as embryonic stem cells [106].

In in vitro reprogramming studies, Kim et al. showed that mouse fibroblasts can be reprogrammed directly to dopaminergic precursors (iDP) through the transient expression of the four Yamanaka factors, culturing the cells in specific media enriched with sonic hedgehog (SHH) and fibroblast growth factor 8 (FGF8) [107]. To date, cell reprogramming for CNS repair had been born as a new approach, with the subsequent implementation on neurodegeneration process including PD [108].

Concluding Remarks

As we have described throughout this chapter, different Parkinson's disease experimental animal models have been evaluated to mimic pathognomonic cognitive–emotional disorders of the pathology. Numerous efforts have been made to prevent or restore the associated Parkinsonian symptomatology, among them approaches with trophic factors, phytochemicals, specific enzymes, anti-inflammatory, gene therapy and currently incorporated reprogramming cell studies. The combination and optimization of these therapeutic approaches could contribute, in the near future, not only to the knowledge of the progression of the pathology but also to improving the quality of life in patients with Parkinson's disease.

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Part III

Neurosciences, Learning, Teaching and The Role of Social Environment

31

Friendship Relationships in Children and Adolescents: Positive Development and Prevention of Mental Health Problems

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Introduction

In recent years the study of positive development in children and adolescents has taken great relevance. So much so that in the year 2017, the American Journal *Child Development* published a special issue on this subject dedicated to *Positive Youth Development*, being its main feature the explicit rejection of a focus centered in addressing what is wrong or deficient in the behavior of children and adolescents. It emphasizes the skills and tools of children and adolescents such as positive relationships, opportunities, values, and positive social relationships [1]. In this context, the most important peer relationship is friendship.

Developmental psychology defines friendship as a specific kind of relationship that has distinct properties. Some of its properties are, for example, a dyadic and horizontal relationship. This relationship is based on mutual affection, reciprocity of liking, and mutuality [2]. Reciprocity of liking is also called reciprocity of attraction or reciprocal liking. It is a particular type of reciprocity that refers to the tendency for people to like others who express liking for them [3]. According to Hartup and Stevens [2], the deep structure of friendship is reciprocity. This property is the same across the lifespan, but for those authors the surface structure changes with age.

Friendship can be defined as a voluntary, reciprocal, and egalitarian relationship in which both people recognize the relationship and treat each other as equals. It is a relationship characterized by companionship, a shared history, and mutual affection. In addition, throughout the life cycle, people usually choose as friends people who are similar to them in certain characteristics (e.g., sex, age, or behavior styles). Such similarities allow most friendships to be relatively equal in power and control, different from parent-child relationships, for example, which tend to be relatively asymmetric in power [4].

Developmental psychology theorists such as Harry Stack Sullivan [4] have postulated that friendships can provide a unique developmental context in which children and adolescents learn about conflict and negotiation and develop perspective taking and empathy. Also in that relationship, they satisfy social needs of companionship and intimacy. It is believed that friendships func-

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tion as important extra-family sources of social and emotional support that can help people overcome the challenges and stressors of life. Among the benefits of friendship, positive development results can be mentioned taking perspective, tools and socio-cognitive skills, positive feelings about oneself and others, as well as feelings of psychological well-being [4].

The purpose of this chapter is to develop the following issues: (a) the adaptation of the Friendship Quality Scale in Argentina and related studies; (b) the relationship between friendship and loneliness in childhood and adolescence; (c) values, empathy, and friendship quality in adolescents; and finally, (d) friendship and prevention of mental health problems.

Bukowski Friendship Quality Scale Argentine Adaptation

William Bukowski Friendship Quality Scale measures the quality of friendship in children and adolescents, taking into account the following dimensions: Companionship, Conflict, Balance, Help, Security, and Closeness. This instrument evaluates the dimensions mentioned in the relationship with their best friend. In the original scale, the subject must mention who his best friend is and then answer 46 items that describe friendship features, indicating the degree of agreement with them [5].

Friendships involve people who do things together; friends are people who share the stuff of life. Companionship is considered a basic feature or component of friendship relations. The Companionship dimension refers to the amount of voluntary time that friends share or spend together. It includes items such as: *My friend and I spend all our free time together*.

The Balance dimension refers to reciprocity. If in the friendship relationship, one of the subjects gives more than the other, there is no reciprocity. It includes items such as: *Being friends is more important to me than to my friend*.

Bukowski said that in spite of the idyllic views of friendship that can be found in endless number of sappy greeting cards and poems, many studies have shown that friends have more conflicts with each other than they do with other peers [6]. The Conflict dimension refers to fights, disagreements, or arguments within the friendship relationship. Conflict is an inevitable consequence of any relationship that requires decision-making. This dimension includes items such as: *My friend and I can argue a lot*.

The Help dimension refers to mutual help and assistance, as well as help in conflicting situations that can be experienced with others. It includes items such as: *If I forgot to bring my lunch or needed a little money my friend would lend it to me* (aid), or *My friend would defend me if another mate was causing me trouble* (protection).

Friendship has an enduring nature that assures its continuity across time and circumstance. The Security dimension refers to the belief that when you need it, your best friend is reliable and you can trust him (reliable alliance). This aspect includes items such as: *If I have a problem at school or at home I can talk to my friend about it*. This dimension also refers to the strength of friendship, regardless of the differences or disagreements that there may be within the friendship bond (transcending problems). This aspect includes items such as: *If I said I was sorry after I had an argument with my friend, he would still be mad at me*.

The Closeness dimension refers to the sense of affection or "specialness" that they experience with their friend and the strength of their attachment or bond to their friend. Fine [7] highlighted the work of Cooley and Mead (symbolic interactionists) to support an emphasis on closeness. These authors argue that the main provision of relationships is information about the goodness or value of the self. Inherent in their ideas is the notion that the closeness of the affective bond in a friendship relationship gives children and early adolescents the opportunities for reflected appraisal and affection that indicate that they are important to and valued by their friend. It includes items such as: *I feel happy when I am with my friend*, which is an *Affective Bond item* that refers to feelings about their friend; and *Even if other people stopped liking me, my friend would still be my friend* or *My friend puts our friendship ahead of other things*, these are

Reflected Appraisal items that refer to the feelings derived from friendship and their impression of how important they are to their friend.

Resett et al. [8] adapted the scale to Spanish in Argentinian children. They used a sample of 195 children from 9 to 12 years old (M = 10.44 SD = 0.59). This version consists of 33 items. An exploratory factorial analysis was carried out using the principal component method and the oblique rotation. Prior to it, the following results were obtained: KMO = .802; Bartlett's test of sphericity coefficient X^2 = 1944.53; p = .000. The Cattell sedimentation test revealed that the optimal number of factors was six, equal to the original scale number. These six factors explained 50.74% of the variance. Regarding the internal consistency, in this study Cronbach's alpha coefficients for the subscales were the following: Companionship, .61; Help, .80; Security, .70; Closeness, .81; Conflict, .80; and Balance, .65.

Rodriguez et al. [9] carried out new studies to evaluate the psychometric properties in a population of 509 children from 10 to 12 years old (M = 10.65 SD = 0.69), of which 251 were males and 258 were females. They carried out a confirmatory factorial analysis with the method of estimation of parameters by maximum likelihood. In this study, the 6-factor model with 33 items was found to have an adequate fit (X^2/df = 1.98 GFI = .90 CFI = .90 $RMSEA$ = .044). The standardized regression coefficients of the items ranged between .321 and .775. Regarding the internal consistency, in this study Cronbach's alpha coefficients for the subscales were Closeness, .83; Conflict, .73; Balance, .63; Help, .82; Companionship, .66; and Security, .63, which show a satisfactory reliability.

In this study, Pearson correlations between the dimensions of friendship quality and being victimized or being an aggressor in peer relationships (bullying) were analyzed. It was found that at a higher level of Help and Companionship, there are lower levels of being victimized or attacked. In addition, at a higher level of Closeness, a lower level of peer aggression was observed. At the same time, higher levels of Conflict in friendship indicate higher levels of being victimized and attacked [9]. These results highlight the importance of friendship quality for the prevention of peer victimization and aggression.

While studying Bukowski Friendship Quality Scale translation and adaptation into Spanish, Moreno and Resett [10] used the scale together with sociograms. In a sample of 161 both sexes children from 10 to 11 years old, they found that more than 40% of the sample participants were chosen as a friend by more than half of the class. In addition, more than 97% of the sample (almost the whole) chose an individual of the same sex as their best friend. At the same time, more than 80% of the sample chose as their best friend an individual of their class at school. These studies highlight the importance of schooling for the generation of friendship bonds.

Rodriguez and Moreno [11] evaluated the relationship between parenting styles and friendship relationships among peers in childhood and preadolescence. In a sample of 841 students aged 8–13 years old, they found that parents who act firmly, but with love and acceptance (affection) instead of a strict or pathological control, facilitate friendship bonds of children with others. In this study it was observed that the higher the pathological control, the lower the level of companionship and friendship. This empirically confirms the need to improve parent-child relationships for a better social interaction among peers.

Loneliness and Friendship in Childhood and Adolescence

There are many studies focused on children and adolescents who lack friends in school. Hartup and Laursen consider that poor peer relationships are predictive of serious adjustment problems in later life [12].

Loneliness and health have been investigated in adolescents, and, in line with adult literature, lonely adolescents report lower perceived health status and increased symptoms of psychosomatic manifestations of psychological distress, such as headaches and loss of appetite [13].

Harris, Qualter, and Robinson [14] examine in preadolescents the association between chronic loneliness and perceived health, school absence

due to illness, sleep duration, and disturbance. Loneliness was measured in three collection waves that were 18 months apart and covered the ages 8–11 years. Using growth mixture modeling, two groups were identified with discrete growth patterns of loneliness: (a) relatively high, reducing loneliness (48%) and (b) low, stable loneliness (52%). At age 11 years, those in the relatively high, reducing lonely group reported higher levels of depressive symptoms, poorer general health, took longer to get to sleep, and had greater sleep disturbance than children in the low, stable loneliness group. The authors consider that these findings suggest that there may be long-term health effects of experiencing high loneliness in middle childhood, even when loneliness levels reduce to normal levels at preadolescence.

Harris, Qualter, and Robinson [14] consider that loneliness impacts on sleep functioning in children and has important implications. Sleep disruption reduces endocrine function and decreases immune system functioning. Further, poor sleep quality can lead to hyperactivity in the HPA axis, leading to elevated cortisol levels, and is associated with illnesses such as metabolic syndrome and hypertension. Therefore, they consider that their findings such as lonely children have higher levels of sleep dysfunction may suggest a pathway between loneliness and ill health.

Problems in parent-child relationships increase the probability of vulnerability to loneliness, causing insecurity and very strict standards for relationships [15, 16]. In a study these authors selected two groups, one included adolescents with low parental acceptance (Schaefer's *Children's Reports of Parental Behavior* lowest scores, 25% of the sample) and the other subjects with high parental acceptance (highest scores, 25% of the sample). A multiple analysis of variance (MANOVA) was conducted in order to determine if differences in parental acceptance could explain differences in loneliness. Significant differences between low ($n = 182$) and high ($n = 171$) parental acceptance groups were found: low parental acceptance, Peer Loneliness ($M = 14.4$ $DS = 6.08$) and Parental Loneliness ($M = 17.59$ $DS = 8.02$), and high parental acceptance, Peer Loneliness ($M = 10.89$ $DS = 3.648$) and Parental Loneliness ($M = 9.39$ $DS = 2.12$). This research shows that adolescent loneliness depends on parental styles. Perception of paternal acceptance is important in predicting social and emotional development and functioning of children and adolescents. Family Deficit is crucial since problems in parent-child relationships promote vulnerability toward loneliness.

Weiss [17] made a distinction between loneliness due to emotional isolation and loneliness due to social isolation. Emotional isolation appears in the absence of close emotional attachment, whereas social isolation appears in the absence of an engaging social network. Parental and peer relationships constitute two different social contexts where loneliness develops. Loneliness research in children and adolescents will distinguish between loneliness due to relationship with parents (parent-related loneliness) and loneliness due to relationship with peers (peer-related loneliness) [18].

Although group acceptance is important to children's successful adaptation to peers, it is needed to study children's ability to form and maintain satisfying and supportive specific dyadic friendships as distinct from their ability to gain acceptance in the classroom more generally. Bukowski and Hoza [19] argue that problems in group acceptance do not necessarily preclude satisfactory friendship adjustment, and they point out that concerns about the emotional well-being of low-accepted children might be attenuated if it could be established that low-accepted children have satisfying one-to-one friendships. They consider that children's friendship adjustment has been studied far less frequently and less systematically than children's group acceptance, and the links between these two forms of peer adjustment are poorly understood. For Parker and Asher [20], it seems reasonable that friendship and group acceptance contribute in distinctive ways to children's socialization. They found out that not all highly accepted children had friends.

Goossens [21] suggested that adolescence is a period of life when loneliness is particularly prevalent, and an increase in loneliness is expected when children enter preadolescence.

We present a study with Argentinian adolescents whose objective is to show the relations between loneliness and friendship quality dimensions. The sample comprised 195 adolescents with an age range between 13 and 16 years old ($M = 14.28$ $DS = .76$), from both sexes (110 male and 85 female). The sample (non-probabilistic) was obtained in Formosa, Argentina.

The instrument used to measure loneliness was the *New Loneliness Scale for adolescents* [15, 22], which is based on Rokach and Brock Loneliness Questionnaire [23, 24] and Marcoen and Brumage Loneliness Scale [25, 26]. In this study we used two dimensions of this scale: Peer Loneliness, which includes items such as "I feel excluded by my classmates" *(isolation)* and "When I suggest a game nobody likes to join in" *(rejection)*, and Parental Loneliness dimension (Family Deficit) which includes items such as "My mother and father never had some spare time for me" or "I feel that my parents were generally not supportive of me."

In order to measure friendship, the Adolescent Friendship Quality Scale [5] was administered.

For the fulfillment of the objective of this study, Pearson correlations between friendship quality dimensions and loneliness self-perception were obtained (See Table 31.1).

Significant negative correlations were obtained between Peer Loneliness and Companionship (−.304), Help (−.229), Security (−.148), Closeness (−.152), and Balance (−.157); and a positive significant correlation between Peer Loneliness and Conflict (.193).

On the other hand, Family Deficit or Parental Loneliness correlated significantly in a positive way with Conflict (.209).

Table 31.1 Pearson correlations between loneliness dimensions and friendship dimensions

Friendship scales	Peer loneliness	Parental loneliness
Companionship	−.304**	−.010
Conflict	.193**	.209**
Balance	−.157*	.009
Help	−.229**	.001
Security	−.148*	.053
Closeness	−.152*	.092

*$p \leq 0.05$ **$p \leq 0.01$ $N = 195$

Peer Loneliness is the feeling of being rejected or not loved by friends and mates [15]. This dimension correlated negatively with all the positive dimensions of the quality of friendship (Companionship, Balance, Help, Security, and Closeness). These findings are consistent since with a higher friendship quality, the perception of friends and colleagues' rejection or the fact of not being loved or accepted by them decreases. It should be mentioned that high Conflict is logically correlated to Parental Loneliness.

As regards friendship dimensions, parental rejection or Family Deficit (Parental Loneliness) alters them only when the conflictivity of the bond with peers increases, but not in positive friendship dimensions.

Values, Empathy, and Friendship Quality in Adolescence

To see adolescence from the perspective of *positive development* is not only to consider it from the avoidance of some behaviors such as violence, the consumption of substances, or other risky practices, but it is to consider it also from a generation of skills, abilities, and values that allow the adolescent advance toward adulthood in a successful way [27]. This model of positive vision toward adolescence has emerged in the United States with the works of Catalano et al. [28], among others. It is considered relevant to be able to expand this line of study by generating integrative models that can account for the interaction and the effect that other variables have on friendship as a positive peer relationship. Among these variables, we can mention empathy, which is essential for the bond between peers [29] as well as the values of honesty, integrity, responsibility, and prosociality [27].

Values are an important theme in the positive development of children and adolescents [1] as they have been considered as an important aspect in psychosocial and moral development. They can be defined as non-negotiable ideas, which constitute the basis by which the person acts autonomously, in any situation, in a positive, adequate, and valid way [30]. In their literature review [27],

they have identified values for positive development in adolescents such as the following:

(a) *Social values* which mark a good social relationship and a commitment to society and the community. These social values are *prosociality* (help, collaboration, and care of other people); *social commitment* (participation in community and social activities), and *justice and social equality* (interest in a fair and socially egalitarian world).
(b) *Personal values*, which are related to the personal maturity that leads to consistent behavior with the principles assumed in life. They are *integrity* (behavior based on proper moral principles), *responsibility* (personal responsibility and assumption of one's decisions), and *honesty* (sincerity and communication of truth).
(c) *Individualistic values*, which show individualistic culture. They are *hedonism* (achievement of one's own pleasure over other goals) and *social recognition* (being recognized and admired socially) [27]. It should be noted that these latter values (*individualistic values*) would be counter values for positive adolescent development.

Rodriguez et al. [31] studied values in the Argentinian population, correlating them with act penalization as faults and crimes. They found out that social values are positively correlated with act penalization and that, in particular, prosociality is a good predictor. These authors consider the importance of social values in the social framework and think that an adequate act penalization is necessary to sustain society.

Empathy is another important bond in socialization. Empathy is the capacity to understand the perspective of the other or the feelings and affections of the other, to put oneself in the place of the other [32, 33]. Eisenberg and Strayer [34] consider that empathy implies sharing the emotion that is perceived in the other, feeling with the other. For Davis [35, 36], empathy implies cognitive processes of understanding and adopting perspectives, as well as affective processes of sympathy and experiences of feelings coherent with the experiences of the other.

The approach to empathy has two major paths [29, 37]. The one emphasizes the importance of the cognitive in empathic processes by addressing empathy as a cognitive awareness of the internal states of other people, their thoughts, feelings, perceptions, intentions, etc. The second path focuses on the affective aspect, understanding empathy as an emotional vicarious response to the emotional experience perceived by others. Apart from the two paths mentioned in the study of empathy (cognitive or affective), multidimensional models of empathy have been developed. One of the greatest expert in empathy as a multidimensional variable with cognitive and affective components has been Davis [35, 36]. He considers that empathy implies cognitive processes of understanding and adopting perspectives, as well as sympathy affective processes and experiences of feelings coherent with the experiences of the other. His contribution to the measurement of this variable has been the *Interpersonal Reactivity Index* (IRI). This instrument is one of the most used in the world to measure empathy, providing *cognitive components*: (a) *perspective taking* is understood as the ability of people to put themselves in the place of the other; it implies spontaneous attempts of the person to adopt the perspective of the other in real situations of everyday life. (b) *Fantasy* evaluates the imaginative capacity to identify with fictional characters in films and literature. It also provides *affective components*: (c) *empathic concern* that includes feelings of concern, compassion, and affection toward others when they are in discomfort or in distress, that is to say, they are feelings oriented toward the other, and (d) *personal distress* that refers to the feelings of anxiety and discomfort toward negative facts of the others, that is to say that it implies feelings oriented to the self.

Values and empathy are important variables in peer relationships, particularly in friendship. Bukowski et al. [5] have studied the dimensions of friendship quality: Companionship, Conflict, Balance, Help, Security, and Closeness.

The present study aims to analyze the phenomena to find out if personal, social, and individualistic values and empathy may foster or thwart different dimensions of friendship qual-

ity. The sample comprised 411 adolescents, male and female, 47% boys and 53% girls, with an age range between 12 and 17 years old (M = 14.41 SD = 1.21). The sample was obtained in Entre Ríos, Argentina. The Positive Youth Development Values Scale [27], Interpersonal Reactivity Index [36], and Friendship Quality Scale [5] were used to measure the variables. Hierarchical multiple regression was used to analyze data.

The results of this study have shown that personal values are important predictors for Balance, Help, Security, and Closeness. Perspective taking (empathy) is an important predictor for Help and Security. Empathic concern (empathy) is an important predictor for Balance, Help, Security, and Closeness. In turn, individualistic values negatively predict Balance and Help. These values positively predict Conflict (see Table 31.2). The results have shown that personal values, perspective taking, and empathic concern are important for friendship quality.

In Table 31.3 the values ungrouped can be seen. Honesty is the most important value to predict friendship quality.

The same results can be seen in Table 31.2 where the ungrouped values are found. It can be seen that honesty is one of the most important values that predicts friendship quality.

According to the research by Ciarrochi et al. [38], empathy is related to friendship support. They found out that a high level in empathy is more likely to have highly supportive friends. These results are similar to those in the present study where empathic concern is a good predictor of Companionship, Balance, Help, Security, and Closeness, which can be considered part of friendship support. This shows the importance of empathy in the construction of high-quality friendships, as Hayes and Ciarrochi [39] theorizes. These findings are similar to those of Chow et al. [40] who found out that empathy was positively related to intimacy and conflict management competences.

As it has been said, honesty is the sincerity and communication of truth. It is logical that this value is important in the communication between friends, since friendship is an intimate meeting between peers.

Friendship and Mental Health

Friendship plays a significant role in life and it contributes in many ways to well-lived life.

Friendship is very important for studying the intersection between the personal and the social. Friendship relations [41] offer specific provisions that cannot be obtained in other forms of relationship experience, and they provide unique forms of experience that have immediate effects on well-being. The challenges and opportunities posed by friendship have been well recognized by developmental psychologists.

In children and adolescents, and also in younger and older adults, friendship leads to positive feelings about the self and others and positive psychological health and well-being (e.g., lower levels of anxiety and depression, higher levels of self-esteem). Rubin and Bowker [4] consider that during childhood and adolescence, there is growing evidence that having at least one mutual friend can protect youth from peer victimization and its associated internalizing (e.g., anxiety) and externalizing (e.g., aggression) problems. Further, longitudinal studies found out that the benefits of having friendships during childhood and adolescence persists into adulthood and old age.

Friendless children and adolescents are more vulnerable to peer victimization and abuse. Rubin and Bowker [4] consider that there are also risks associated with friendships when the quality of the friendship is poor or highly conflictive. Individuals who have unsupportive friendships tend to report higher levels of psychological distress and also people with highly conflictive friendships (in which few conflicts are resolved in ways that are positive for both members of the relationship).

Friendship relationships are more important than peer status in terms of their association with adjustment. Popularity is related to children's and adolescent's feelings of belongingness, but friendship is especially related to feelings of loneliness. Although an individual who is considered more popular, is more likely to have friends, and accordingly feels less lonely.

Table 31.2 Hierarchical multiple regression results for Argentinian adolescents (grouped)

	Dimensions of friendship quality											
	Companionship		Conflict		Balance		Help		Security		Closeness	
Variables	$R^2/\Delta R^2$	β	$R^2/\Delta R^2$	β	$R^2/\Delta R^2$	β	$R^2/\Delta R^2$	β	$R^2/\Delta R^2$	β	$R^2/\Delta R^2$	β
Step 1 Social values	.025*	−.013	.034**	−.134*	.085***	.017	.094***	.078	0.075***	.055	.081***	.079
Personal values		.146*		−.025		.253***		.261***		.235***		.237***
Individual values		.058		.122*		−.164**		−.024		−.104*		−.014
Step 2 Social values	.04/.015	−.05	.045/.011	−.116	.116/.031**	−.053	.15/.057***	−.013	.143/.068***	−.042	.152/.072***	−.021
Personal values		.133*		−.010		.214***		.206***		.175**		.185**
Individual values		.07		.103*		−.134**		.014		−.064		.020
Perspective taking		−.046		−.048		.065		.152**		.187**		.098
Fantasy		−.048		.106		−.041		−.025		.012		−.004
Empathic concern		.121		−.042		.177**		.146*		.158**		.176**
Personal distress		.065		−.002		−.023		.035		−.025		.098

*$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$

Table 31.3 Hierarchical multiple regression results for Argentinian adolescents (ungrouped)

	Dimensions of friendship quality											
	Companionship		Conflict		Balance		Help		Security		Closeness	
Variables	$R^2/\Delta R^2$	β	$R^2/\Delta R^2$	β	$R^2/\Delta R^2$	β	$R^2/\Delta R^2$	β	$R^2/\Delta R^2$	β	$R^2/\Delta R^2$	β
Step 1 Prosociality	.04*	.00	.05**	–.04	.10***	–.03	.12***	–.02	.09***	.01	.12***	–.07
Social commitment		.09		–.03		.01		.06		–.002		.12*
Justice and social equality		–.11		–.08		.07		.07		.06		.07
Integrity		.04		.09		.11*		.07		.03		.06
Responsibility		.11		.02		.10		.01		.03		.003
Honesty		.06		–.14*		.09		.26***		.23***		.24***
Hedonism		.05		.08		.000		–.01		–.07		.05
Social recognition		–.004		.05		–.20***		–.03		–.04		–.07
Step 2 Prosociality	.06/.02	–.03	.06/.01	–.04	.13/.03*	–.08	.17/.05***	–.08	.15/.06***	–.05	.19/.07***	–.15**
Social commitment		.09		–.03		–.004		.04		–.02		.11
Justice and social equality		–.12*		–.08		.04		.03		.01		.03
Integrity		.05		.09		.11*		.06		.02		.06
Responsibility		.11		.03		.10		.00		.03		–.004
Honesty		.03		–.13*		.05		.21***		.18**		.18**
Hedonism		.05		.07		.009		.01		–.05		.07
Social recognition		.01		.04		–.18**		–.002		–.004		–.045
Perspective taking		–.05		–.05		.06		.15**		.19**		.09
Fantasy		–.04		.10		–.04		–.03		.01		–.01
Empathic doncern		.13*		–.02		.17**		.14*		.14*		.17**
Personal distress		.06		.003		–.01		.05		–.03		.12*

*$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$

Friendship is a relationship that makes contributions to people development, social adjustment and well-being. The developmental approach to friendship suggests that the effects of friendship are not the same at every age. Friendship relationships are especially important across childhood and adolescence.

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32

Bullying and Cyberbullying in Adolescents: A Meta-analysis on the Effectiveness of Interventions

Santiago Resett and Belén Mesurado

Introduction

Bullying and cyberbullying are considered important risk factors for the mental health of adolescents due to its association with numerous problems of psychosocial adjustment on a personal, interpersonal, and school level [1, 2]. In the last years, many interventions programmes have been developed and implemented with the purpose of reducing bullying and cyberbullying. Although there are several meta-analyses studying the effectiveness of these programmes, no meta-analytic review jointly analyses the effectiveness of interventions on both types of bullying. Consequently, the objective of this chapter is to compare together the effectiveness of the intervention programmes in the prevention of bullying and cyberbullying towards the perpetrator and the victim.

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Bullying: Definitions and Types

First, bullying exists when a person or group repeatedly exposes an individual (or group) to negative actions. Second, such acts must be carried out with the intention of damaging or attacking. Finally, intimidation is based on an inequality of forces between the victim and the aggressor; usually the victim is weaker or has less power than the perpetrator [3–5].

Bullying can be carried out in different ways: verbal (nicknames, insults, etc.), physical (hitting, kicking, etc.), and indirectly, that is, without direct physical or verbal contact with the victim [6, 7], spread rumours, damage the reputation of another student, or exclusion (e.g. leave a student out of a group intentionally, not invite him or her to a party). Nowadays, bullying through the use of cell phones and/or computers – for example, sending rude and hurtful messages with these devices – has given rise to a new type of abuse called cyberbullying or electronic bullying [8, 9].

Although cyberbullying is debated about how to be defined and measured [10] [11], most researchers agree that cyberbullying is a kind of intentional aggression and harmful behaviour that occurs with electronic medium [12], such as computers and mobile phone, resulting in a power of imbalance [13, 14].

Some authors suggest that cyberbullying is merely an extension of bullying executed through new technologies of information and

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communication (NTIC) [9, 15–17]. For instance, Williams and Guerra [18] argue that technological devices only provide an additional medium through which bullying can occur. Considering this argument, one might anticipate that the core characteristics of cyberbullying are no different from bullying. In line with this, some researchers detected a significant overlap between victimization and cybervictimization, specifically among adolescent samples [19, 20]. On the other hand, other authors suggest that cyberbullying differs from bullying in some important aspects or features (e.g. anonymity, massive audience, disinhibition, among others). They suggested that – although they may share features in common – cyberbullying is, in some way, different from bullying [8, 12, 21] and, for example, it is perpetrated by somewhat different groups of adolescents [14, 22, 23]. For instance, Kubiszewsli et al. [23] and Resett and Gámez-Guadix [24] detected little overlap between those who perpetrated bullying and those involved in cyberbullying. Perhaps, the little overlap between bullying and cyberbullying may be due to the fact that, although they are related phenomena, cyberbullying can occur in any time and any place (for instance, outside school), making more difficult for adults to monitor and detect it [25]. It is anonymous, and this fact increases disinhibition [11] and increases potential audience in a larger and huge manner [16].

Nowadays, cyberbullying is very frequent between adolescents, mainly in middle adolescence [26]. For instance, Tokunaga [27] detected that 20–40% of young adolescence has been victims. However, figures vary across researches [8]. In general, the prevalence rates of cybervictimization range across investigations from 10% to 35% [8, 15, 25, 28, 29]. Regarding aggressors, studies indicated that 11–44% are perpetrators of cyberbullying [30]. So, the escalation of cyberbullying has become an issue of increasing concern for teachers, parents, adolescents, and school communities [31]. However, the research on cyberbullying is still in its beginnings compared to bullying [32, 33].

Bullying, Cyberbullying, and Mental Health Problems

Both being a victim of bullying and being a perpetrator are risk factors for the psychopathology of development. Those who are victims present higher levels of emotional problems: greater anxiety, depression, and worse self-esteem than non-involved groups [3, 5, 34, 35]. The aggressors, on the other hand, show higher levels of externalizing problems: antisocial behaviour, substances use, as well as greater impulsivity and attention problems, among others [36–38]. A recent meta-analysis from 18 longitudinal studies with adolescents detected the association between victimization with emotional, behavioural, and interpersonal problems, while finding the association between bullying and externalizing problems, interpersonal issues, and poor school performance [39].

Regarding cybervictimization and psychological functioning, it has been related to a variety of psychological problems, such as depression, anxiety, low self-esteem, low self-concept, and, in some cases, suicide attempts [5, 11]. Regarding cyberbullying some authors argue that cyberaggressors do not present more emotional problems, but they engage in externalizing problems. In fact, cyberbullies score better in mental health compared to non-involved adolescents [37, 40, 41]. However, some recent investigations found that being a cyberperpetrator was linked to poor psychological adjustments [42–45]. For instance, some studies detected that cyberaggressors showed more psychological difficulties and poorer quality of life [46]. In addition, Bauman, Toomey, and Walker [47] demonstrated that cyberbullying was related to suicide attempts in males, while Hinduja and Patchin [48] detected this link in both sexes. Researchers suggested that these results demonstrate a lack of understanding for the perpetrators in their own behaviour, so they possibly engaged in one act of online bullying that quickly escalated, becoming a bigger problem [45].

Bullying, Cyberbullying, and Intervention Programmes

Due to the important risk factor, that is, bullying and cyberbullying, for the mental health of adolescents, a central aspect is to develop effective interventions to reduce such problems. Therefore, many school-based programmes have been devised and implemented in an attempt to reduce bullying and in recent years, to reduce cyberbullying, as well. In 1983, the first large-scale anti-bullying programme was implemented in Bergen, Norway [3, 4, 49]. This programme showed a significant decrease in victimization of about half after the programme [3, 49]. The Olweus Bullying Prevention Programme is recognized by the Center for the Study and Prevention of Violence as 1 of only 11 Blueprints Model Programmes and by the Substance Abuse and Mental Health Services Administration as a model programme in this respect. However, although Olweus programme showed a significant effect in preventing bullying in primary school children, it was less effective in adolescents. Moreover, its effectiveness was less intense in other countries, such as the United States [50, 51]. A recent meta-analysis [52] included 79 studies regarding anti-bullying programme from 2009 until 2018 suggesting similar percentage of reduction for bullying and victimization: 19–20% and 18–19%, respectively.

In recent years, another programme that demonstrated an important reduction of bullying in children and adolescents was the KiVa, an acronym for *Kiusaamista Vastaan*, "against bullying" [53, 54]. KiVa was developed and implemented in Finland and is currently being extrapolated to other countries, even Latin-American countries, such as Argentina, Perú, Colombia, and Chile, among others. KiVa programme was implemented in 8237 children and showed to be effective in reducing school bullying and victimization [54]. However, a drawback of this intervention is that it has been more effective in children and there is much less evidence of its effectiveness in other countries besides Finland.

Compared to bullying, fewer intervention programmes have been developed to reduce cyberbullying, and – even – many anti-bullying programmes were extrapolated to cyberbullying. Many authors [55] suggested that research on effective anti-cyberbullying intervention is lacking. One could suppose that efforts aimed to reduce bullying are expected to also be effective in reducing cyberbullying because of the considerable overlap between cyberbullying and bullying [56–58]. However, other authors argue that because there is not a perfect overlap between bullying and cyberbullying, it is necessary to improve or create evidence-based cyberbullying prevention programme [55, 59]. There is a meta-analysis study [60] that evaluated programme against bullying and cyberbullying that included 17 researches, but its problems were that focus on school-aged children only included studies from the United States, Australia, and North Europe, and research was performed from 2000 until 2013. With rapid changes and development of new technologist of information and communication (NTIC), in only 5 years important changes may have occurred with respect to cyberbullying and its associated behaviours.

One of the most accurate evaluated programmes against cyberbullying is "Media Heroes" (Medienhelden), a German school-based programme that attempts to raise students' awareness about risks associated with new technologies, to increase empathy and social responsibility, and to teach abilities to defend oneself and others from cyberbullying [61]. Two randomized investigations detected that the programme significantly reduced cyberbullying [61, 62].

About the intervention programmes for cyberbullying in Spanish populations, Cyberprogramme 2.0 [63] and ConRed [64, 65] are the best known with some evidence in the reduction of cyberbullying. However, the problem for above-mentioned programmes is that its effectiveness has not been proven in other countries.

This Study

In the last years, different meta-analytic reviews analysed the effectiveness of bullying and cyberbullying intervention programmes [59, 66, 67].

Baldry and Farrington [66] studied the effectiveness of 16 anti-bullying programmes implemented in 11 different nations, and they pointed out that 8 programmes produced desirable results, 2 produced mixed results, 4 produced small or negligible effects, and 2 generated undesirable effects. Although these results were optimistic, Baldry and Farrington [66] affirmed their meta-analysis was not enough evidence to confirm the efficacy of the anti-bullying programmes, because some studies did not have strong research designs and lacked of key information.

Mishna et al. [67] evaluated three programmes on cyberbullying (two implemented in the United States and one in Canada). The programmes were the US-developed I-SAFE curriculum, the Missing Programme, and Help-Assert Yourself-Humor-Avoid-Self-Talk-Own It. In general, Mishna et al. [67] found that the programmes increased Internet safety knowledge but did not impact online behaviour. In other reviews, Van Cleemput et al. [59] examined 15 programmes, and they included 6 programmes in their meta-analysis. The remainders were excluded for different reasons: case studies, problems with study design, and no outcomes for cyberbullying behaviour, among others. Results suggested that the overall effects of cyberbullying programmes were modest, but significant, with some of them yielding greater reductions. These programmes include social skills training, use of peer educators, and information for teachers, staff, and families, among others [68]. Other recent meta-analysis [55] for school-aged children and adolescents included 15 studies that used randomized controlled trials to evaluate programmes against cyberbullying detected. That programme reduced cyberbullying perpetration by approximately 10–15% and cybervictimization by 14%. They pointed out that future investigation needs to address, to develop specific anti-bullying programmes, and to evaluate the overlapping between bullying and cyberbullying.

Although there are several meta-analyses studying the effectiveness of bullying and cyberbullying intervention programmes, no meta-analytic review jointly analyses the effectiveness of interventions on both bullying and cyberbullying in adolescents. Several authors indicated the importance of utilizing evidence based on anti-bullying programmes to better inform cyberbullying intervention [56], such as top-down methods of cyberbullying programmes (e.g. the Barlett and Gentile Cyberbullying Model) [69]. However, effectiveness of anti-cyberbullying programmes is unclear, yet. In addition, something that is not clear is the extent to which cyberbullying interventions are so effective compared to bullying. In line with this, Gaffney et al. [55] asked themself if school-based programmes should target bullying and cyberbullying concurrently or separately. So, the strengths of this chapter are two: (1) conduct jointly a review of scientific studies to reduce bullying, victimization, cyberbullying, and cybervictimization in adolescents and (2) include wide range of studies in Spanish, English, and Portuguese languages.

Based on this background, the objective of this meta-analysis is to analyse the effectiveness of bullying and cyberbullying interventions in adolescents aged 10–19 years, published between 2000 and July 2018 inclusive in English, Spanish, and Portuguese. We also studied whether intervention programmes have any variation in its effectiveness on perpetrator and victim of traditional bullying and cyberbullying.

Method

Inclusion and Exclusion Criteria

The inclusion and exclusion criteria for this meta-analytic review were aspects of population and methodological design of the studies. Concerning the population, we selected interventions on bullying or cyberbullying for adolescents ranging between the ages of 10 and 19 for both sexes. Although adolescence begins at around 11–12 years of age for many authors [70, 71], the age of 10 was taken into consideration because many studies included it and it was very close to the beginning of adolescence. Besides, in some cases, many subjects have already gone through the puberty changes that give rise to it. We also excluded in this meta-analytic review

intervention focusing on children and adults – as, for example, teachers or parents – or intervention focusing on non-community participants (for instance, adolescents with generalized anxiety disorder). Moreover, concerning methodological design, we included interventions that discourage bullying and cyberbullying in adolescents, and that include intervention with and without control groups. The duration of the intervention and follow-up measures were not considered an inclusion criterion. We included interventions with significant and non-significant results in comparison between the treatment and the control group or between the pre- and post-test in the treatment group. We also included interventions carried out inside and outside of the school settings. Finally, we selected publications appearing between 2000 until July 2018 and only those written in English, Spanish, or Portuguese. Studies published in other languages were excluded.

Search Strategy

We used the following databases to do the review of the literature: Dialnet, EBSCO Host, JSTOR, SciELO, ScienceDirect, NCBI, PsycINFO, and Latindex. We performed the search in July 2018, and papers published in English, Spanish, and Portuguese were included. The search keywords used for this meta-analysis were "bullying", "cyberbullying", "victimization", and "harassment"; and the following terms were employed in order to comply with the intervention criteria: "intervention", "outcome", "programme", and "treatment". The search was carried out by combining each of the key terms, for example, bullying and intervention, bullying and programme, etc.

Data Extraction

Three researchers carried out the selection of papers from the database following the inclusion criteria. The selection of studies was made in two stages or steps. In the first step, researchers evaluated the pertinence of the study through the title and abstract of the paper. In the second, researchers evaluated the full text of the paper. Researchers agreed a final list of papers with discrepancies resolved by consensus. Finally, 17 [17] papers of bullying intervention and 11 [11] papers of cyberbullying intervention were left because they fulfilled all of the inclusion criteria, and the data needed to perform the analysis was extracted from them. The information retrieved included author and year of study, intervention (duration, location), sample characteristics (age group and mean, gender ratio), and methodological design.

Statistical Analyses

The Comprehensive Meta-Analysis Programme (version 2) was used to calculate forest plot, funnel plot, and heterogeneity. In the case of dichotomous outcomes, odds ratio was extracted from articles, and in the case of continuous outcomes, mean and standard deviation or *t* test, *F*-statistic, and sample size were extracted from articles. Then, this statistical information was transformed (using Comprehensive Meta-Analysis Programme) to calculate standard differences of mean to allow across study comparisons. When a study had multiple measures for the same outcome, for example, physical and verbal bullying, we calculated an overall effect size averaging the individual effect sizes.

Moreover, we calculated Q statistic and the I^2 statistic to measure statistical heterogeneity between the studies. A non-significant Q statistic and an I^2 statistic smaller than 50 indicates absence of heterogeneity between the studies.

Finally, we used funnel plots and the fail-safe number to study the publication bias. If the fail-safe number is greater than 5 k + 10 (k is the number of articles included in meta-analytic review), it is considered that it is unlikely that the number of unpublished articles with null results would change the significant find-

ings of the meta-analytic review to statistical non-significance.

Results

Figure 32.1 shows the search and selection process of studies included in the meta-analytic review. We identified 5763 studies through databases (see Fig. 32.1). Subsequently, we selected 96 articles for a comprehensive review and finally 16 articles (which include 19 studies) were included in the final traditional bullying meta-analysis. Fourteen articles (which include 17 studies) were included in the final traditional victimization meta-analysis. Eleven articles (which include 14 studies) were included in the final cyberbullying meta-analysis. Finally, eight articles (which include nine studies) were included in the final cybervictimization meta-analysis (see Fig. 32.1).

Meta-analytic Review of Effectiveness of Traditional Bullying and Traditional Victimization Interventions

A total of 23,394 adolescents participated in the studies of traditional bullying intervention (12,964 adolescents were included in the treatment group, the other were included in the control group) and 27,386 adolescents participated in the studies of traditional victimization interventions (13,725 adolescents were included in the treatment group).

Concerning effect sizes of traditional bullying intervention were of standard differences of mean = −0.25 (95% CI = −0.33, −0.17, *Z*-value = −5.88, $p \leq 0.001$) favouring the intervention condition in the prevention of bullying using random model and standard differences of mean = −0.18 using fix model (95% CI = −0.21, −0.15, *Z*-value = −12.59, $p \leq 0.001$). The forest plot results can be seen in Fig. 32.2. Heterogeneity of studies was significant (*Q*-value = 107.31, df = 18, $p = < 0.001$, $I^2 = 83.22\%$).

Besides, effect sizes of traditional victimization intervention were of standard differences of mean = −0.14 (95% CI = −0.23, −0.04, *Z*-value = −2.78, $p \leq 0.01$) favouring the intervention condition in the prevention of victimization using random model and standard differences of mean = −0.11 using fix model (95% CI = −0.14, −0.09, *Z*-value = −8.40, $p \leq 0.001$). The forest plot results can be seen in Fig. 32.3. Heterogeneity of studies was significant (*Q*-value = 163.45, df = 16, $p = < 0.001$, $I^2 = 90.2\%$).

Publication Bias

Finally, we did not detect publication bias, as the funnel plot shown was symmetrical for all analyses (see Fig. 32.4). Moreover, the potential impact of unpublished studies on the analysis was calculated with fail-safe number obtaining a value of 794. We estimated that there is no publication bias because it is unlikely that 794 articles would be found with statistical non-significance.

Meta-analytic Review of Effectiveness of Cyberbullying and Cybervictimization Interventions

A total of 9990 adolescents participated in the studies of cyberbullying intervention (5532 adolescents were included in the treatment group, the other were included in the control group), and 7627 adolescents participated in the studies of cybervictimization interventions (4590 adolescents were included in the treatment group).

Concerning effect sizes of cyberbullying intervention were of standard differences of mean = −0.19 (95% CI = −0.30, −0.08, *Z*-value = −3.31, $p \leq 0.001$) favouring the intervention condition in the prevention of cyberbullying using random model and standard differences of mean = −0.11 using fixed model (95% CI = −0.15, −0.07, *Z*-value = −5.32, $p \leq 0.001$). The forest plot results can be seen in Fig. 32.5. Heterogeneity of studies was signifi-

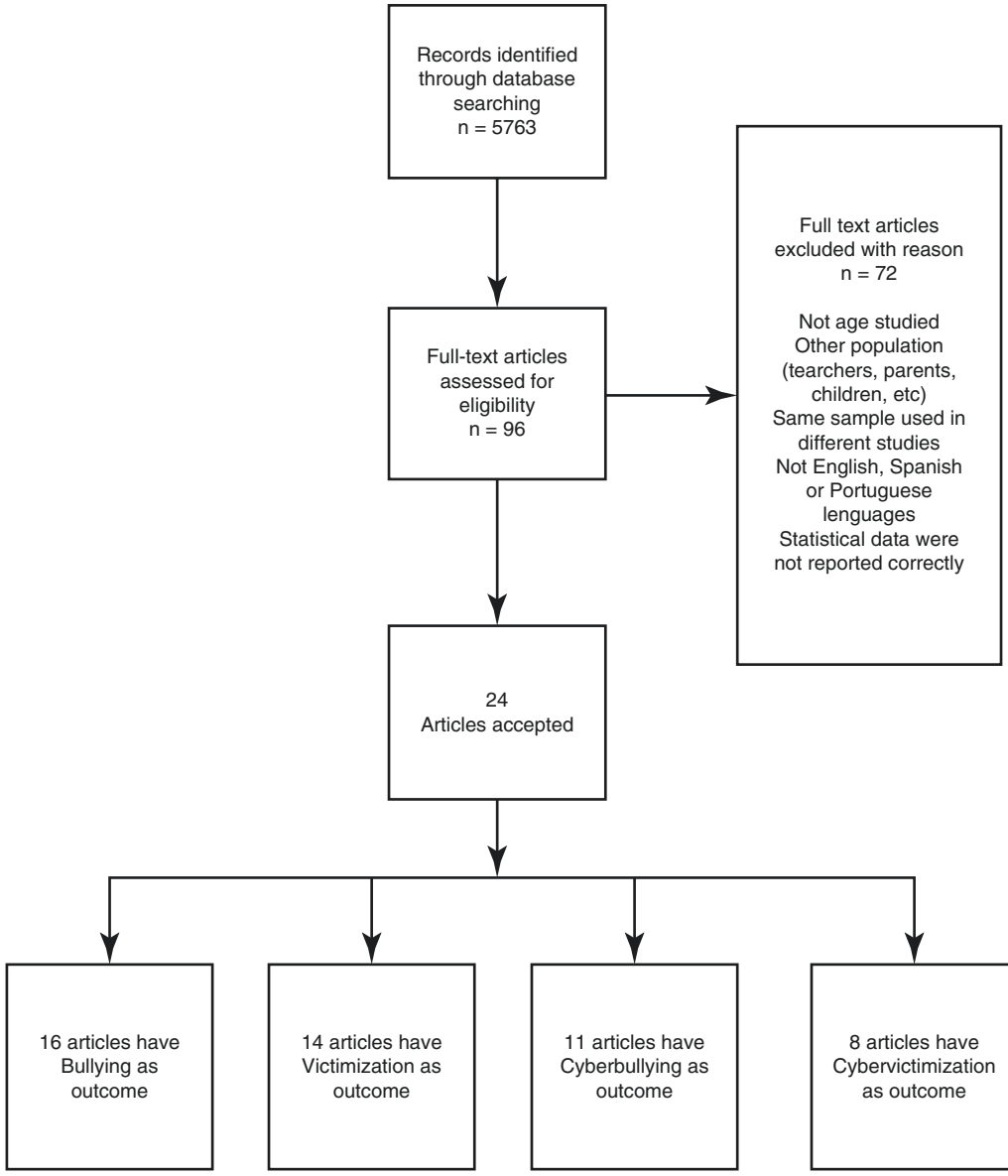


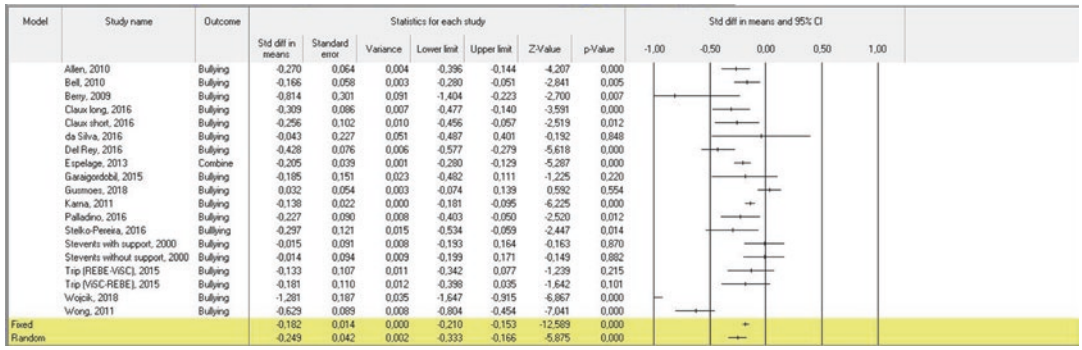
Fig. 32.1 Flowchart of the screening process of 24 articles identified during systematic searches of 5763 search results

cant (Q-value = 77.55, df = 13, p = < 0.001, I^2 = 83.23%).

Moreover, effect sizes of cybervictimization intervention were of standard differences of mean = −0.13 (95% CI = −0.23, −0.04, Z-value = −2.68, $p \leq 0.01$) favouring the intervention condition in the prevention of cybervictimization using random model and standard differences of mean = −0.08 using fix model (95% CI = −0.12, −0.03, Z-value = −3.43, $p \leq 0.001$). The forest plot results can be seen in Fig. 32.6. Heterogeneity of studies was significant (Q-value = 29.85, df = 8, $p \leq 0.001$, I^2 = 73.2%).

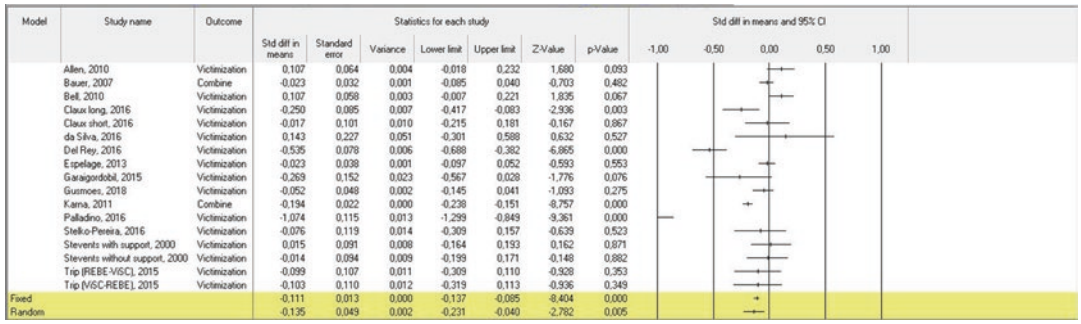
Publication Bias

Finally, the funnel plot does not show a symmetrical distribution for all analyses (see Fig. 32.7). However, the potential impact of unpublished studies on the analysis was calculated with fail-



Model	Study name	Outcome	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
	Allen, 2010	Bullying	-0,270	0,064	0,004	-0,396	-0,144	-4,207	0,000
	Bell, 2010	Bullying	-0,166	0,058	0,003	-0,280	-0,051	-2,841	0,005
	Berry, 2009	Bullying	-0,814	0,301	0,091	-1,404	-0,223	-2,700	0,007
	Claux long, 2016	Bullying	-0,309	0,086	0,007	-0,477	-0,140	-3,591	0,000
	Claux short, 2016	Bullying	-0,256	0,102	0,010	-0,456	-0,057	-2,519	0,012
	da Silva, 2016	Bullying	-0,043	0,227	0,051	-0,487	0,401	-0,192	0,848
	Del Rey, 2016	Bullying	-0,428	0,076	0,006	-0,577	-0,279	-5,618	0,000
	Espelage, 2013	Combine	-0,205	0,039	0,001	-0,280	-0,129	-5,287	0,000
	Garaigordobil, 2015	Bullying	-0,185	0,151	0,023	-0,482	0,111	-1,225	0,220
	Gusmoes, 2018	Bullying	0,032	0,054	0,003	-0,074	0,139	0,592	0,554
	Karna, 2011	Bullying	-0,138	0,022	0,000	-0,181	-0,095	-6,225	0,000
	Palladino, 2016	Bullying	-0,227	0,090	0,008	-0,403	-0,050	-2,520	0,012
	Stelko-Pereira, 2016	Bullying	-0,297	0,121	0,015	-0,534	-0,059	-2,447	0,014
	Stevents with support, 2000	Bullying	-0,015	0,091	0,008	-0,193	0,164	-0,163	0,870
	Stevents without support, 2000	Bullying	-0,014	0,094	0,009	-0,199	0,171	-0,149	0,882
	Trip (REBE-VISC), 2015	Bullying	-0,133	0,107	0,011	-0,342	0,077	-1,239	0,215
	Trip (VISC-REBE), 2015	Bullying	-0,181	0,110	0,012	-0,398	0,035	-1,642	0,101
	Wojcik, 2018	Bullying	-1,281	0,187	0,035	-1,647	-0,915	-6,867	0,000
	Wong, 2011	Bullying	-0,629	0,089	0,008	-0,804	-0,454	-7,041	0,000
Fixed			-0,182	0,014	0,000	-0,210	-0,153	-12,589	0,000
Random			-0,249	0,042	0,002	-0,333	-0,166	-5,875	0,000

Fig. 32.2 Effect sizes for prevention of traditional bullying



Model	Study name	Outcome	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
	Allen, 2010	Victimization	0,107	0,064	0,004	-0,018	0,232	1,680	0,093
	Bauer, 2007	Combine	-0,023	0,032	0,001	-0,085	0,040	-0,703	0,482
	Bell, 2010	Victimization	0,107	0,058	0,003	-0,007	0,221	1,835	0,067
	Claux long, 2016	Victimization	-0,250	0,085	0,007	-0,417	-0,083	-2,936	0,003
	Claux short, 2016	Victimization	-0,017	0,101	0,010	-0,215	0,181	-0,167	0,867
	da Silva, 2016	Victimization	0,143	0,227	0,051	-0,301	0,588	0,632	0,527
	Del Rey, 2016	Victimization	-0,535	0,078	0,006	-0,688	-0,382	-6,865	0,000
	Espelage, 2013	Victimization	-0,023	0,038	0,001	-0,097	0,052	-0,593	0,553
	Garaigordobil, 2015	Victimization	-0,269	0,152	0,023	-0,567	0,028	-1,776	0,076
	Gusmoes, 2018	Victimization	-0,052	0,048	0,002	-0,145	0,041	-1,093	0,275
	Karna, 2011	Combine	-0,194	0,022	0,000	-0,238	-0,151	-8,757	0,000
	Palladino, 2016	Victimization	-1,074	0,115	0,013	-1,299	-0,849	-9,361	0,000
	Stelko-Pereira, 2016	Victimization	-0,076	0,119	0,014	-0,309	0,157	-0,639	0,523
	Stevents with support, 2000	Victimization	0,015	0,091	0,008	-0,164	0,193	0,162	0,871
	Stevents without support, 2000	Victimization	-0,014	0,094	0,009	-0,199	0,171	-0,148	0,882
	Trip (REBE-VISC), 2015	Victimization	-0,099	0,107	0,011	-0,309	0,110	-0,928	0,353
	Trip (VISC-REBE), 2015	Victimization	-0,103	0,110	0,012	-0,319	0,113	-0,936	0,349
Fixed			-0,111	0,013	0,000	-0,137	-0,085	-8,404	0,000
Random			-0,135	0,049	0,002	-0,231	-0,040	-2,782	0,005

Fig. 32.3 Effect sizes for prevention of traditional victimization

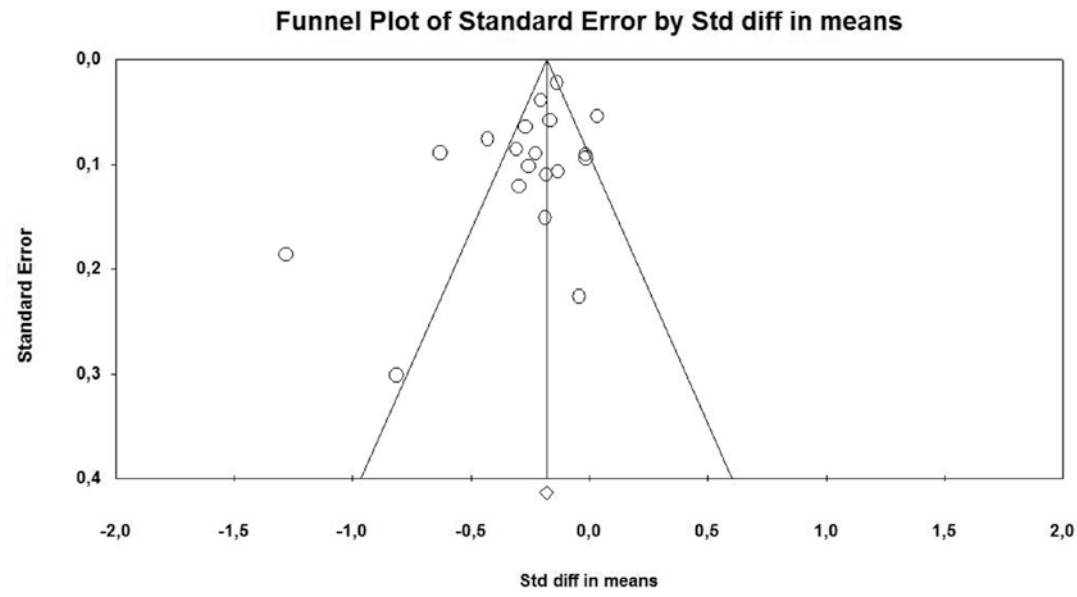
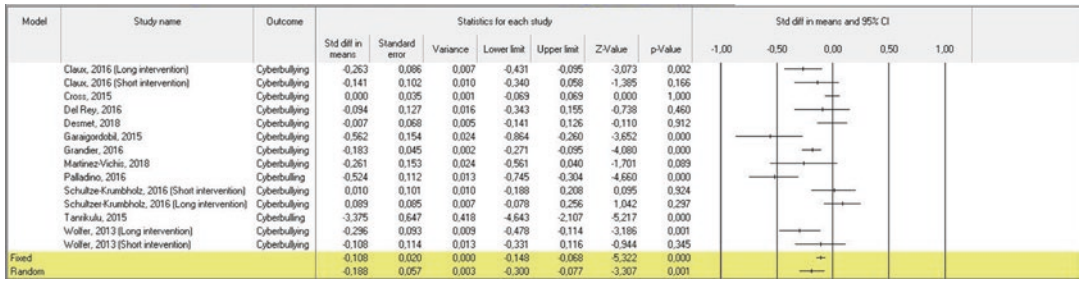


Fig. 32.4 Funnel plot of articles of traditional bullying intervention and traditional victimization intervention



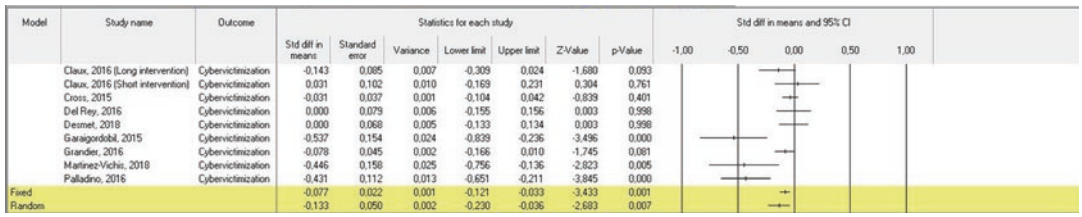
Model	Study name	Outcome	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
	Claux, 2016 (Long intervention)	Cyberbullying	-0,263	0,086	0,007	-0,431	-0,095	-3,073	0,002
	Claux, 2016 (Short intervention)	Cyberbullying	-0,141	0,102	0,010	-0,340	0,058	-1,385	0,166
	Cross, 2015	Cyberbullying	0,000	0,035	0,001	-0,069	0,069	0,000	1,000
	Del Rey, 2016	Cyberbullying	-0,094	0,127	0,016	-0,343	0,155	-0,738	0,460
	Desmet, 2018	Cyberbullying	-0,007	0,068	0,005	-0,141	0,126	-0,110	0,912
	Garaigordobil, 2015	Cyberbullying	-0,562	0,154	0,024	-0,864	-0,260	-3,652	0,000
	Grandier, 2016	Cyberbullying	-0,183	0,045	0,002	-0,271	-0,095	-4,080	0,000
	Martinez-Vichis, 2018	Cyberbullying	-0,261	0,153	0,024	-0,561	0,040	-1,701	0,089
	Palladino, 2016	Cyberbullying	-0,524	0,112	0,013	-0,745	-0,304	-4,660	0,000
	Schultze-Krumbholz, 2016 (Short intervention)	Cyberbullying	0,010	0,101	0,010	-0,188	0,208	0,095	0,924
	Schultze-Krumbholz, 2016 (Long intervention)	Cyberbullying	0,089	0,065	0,007	-0,078	0,256	1,042	0,297
	Tanrikulu, 2015	Cyberbullying	-3,375	0,647	0,418	-4,643	-2,107	-5,217	0,000
	Wolfer, 2013 (Long intervention)	Cyberbullying	-0,296	0,093	0,009	-0,478	-0,114	-3,186	0,001
	Wolfer, 2013 (Short intervention)	Cyberbullying	-0,108	0,114	0,013	-0,331	0,116	-0,944	0,345
Fixed			-0,108	0,020	0,000	-0,148	-0,068	-5,322	0,000
Random			-0,188	0,057	0,003	-0,300	-0,077	-3,307	0,001

Statistics for each study

Std diff in means and 95% CI

-1,00 -0,50 0,00 0,50 1,00

Fig. 32.5 Effect sizes for prevention of cyberbullying



Model	Study name	Outcome	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
	Claux, 2016 (Long intervention)	Cybervictimization	-0,143	0,085	0,007	-0,309	0,024	-1,680	0,093
	Claux, 2016 (Short intervention)	Cybervictimization	0,031	0,102	0,010	-0,169	0,231	0,304	0,761
	Cross, 2015	Cybervictimization	-0,031	0,037	0,001	-0,104	0,042	-0,839	0,401
	Del Rey, 2016	Cybervictimization	0,000	0,079	0,006	-0,155	0,156	0,003	0,998
	Desmet, 2018	Cybervictimization	0,000	0,068	0,005	-0,133	0,134	0,003	0,998
	Garaigordobil, 2015	Cybervictimization	-0,537	0,154	0,024	-0,839	-0,236	-3,496	0,000
	Grandier, 2016	Cybervictimization	-0,078	0,045	0,002	-0,166	0,010	-1,745	0,081
	Martinez-Vichis, 2018	Cybervictimization	-0,446	0,158	0,025	-0,756	-0,136	-2,823	0,005
	Palladino, 2016	Cybervictimization	-0,431	0,112	0,013	-0,651	-0,211	-3,845	0,000
Fixed			-0,077	0,022	0,001	-0,121	-0,033	-3,433	0,001
Random			-0,133	0,050	0,002	-0,230	-0,036	-2,683	0,007

Statistics for each study

Std diff in means and 95% CI

-1,00 -0,50 0,00 0,50 1,00

Fig. 32.6 Effect sizes for prevention of cybervictimization

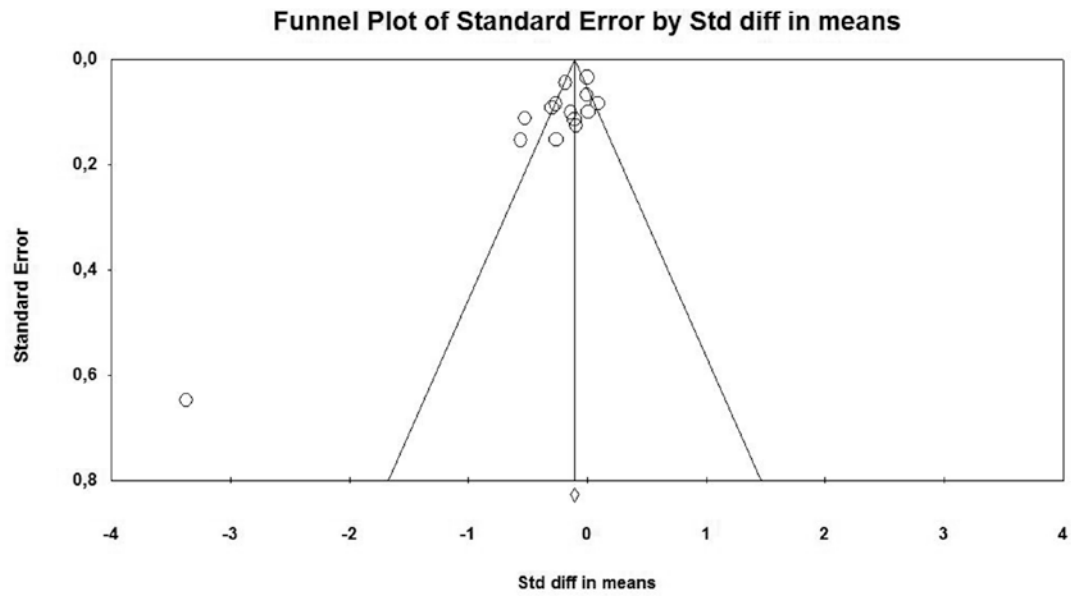


Fig. 32.7 Funnel plot of articles of cyberbullying intervention

safe number obtaining a value of 185. We estimated that there is no publication bias because it is unlikely that 185 articles would be found with statistical non-significance.

Conclusions

Bullying and cyberbullying are important risk factors for mental health of both perpetrator and victims. For example, in extreme cases the victims of both types of aggression can commit suicide or perpetrate school massacres. On the other hand, bullying is an ethical problem that violates human rights, especially the rights of the child and adolescent [72]. Consequently, it was crucial to analyse the effectiveness of intervention programmes to reduce bullying and cyberbullying especially in adolescence, because it is common for these types of aggression to increase during this stage of life. Moreover, it was also essential to analyse the effectiveness of intervention programmes focusing on adolescence because previous studies have shown that some well-known intervention programmes as Olweus programme [50, 51] and KiVa [54] were less effective in adolescence than in childhood.

Although there are meta-analytical reviews on interventions to reduce bullying [52, 60, 73, 74] and cyberbullying [55, 59] in children and adolescence, the advantage of this study is that it evaluated together the effectiveness of interventions to reduce bullying, victimization, cyberbullying, and cybervictimization, and it also included scientific works on three different languages: English, Portuguese, and Spanish. After our review, 17 [17] papers of bullying intervention and 11 [11] papers of cyberbullying intervention between the years 2000 and 2018 were selected. Thus, a sample of 23,394 adolescents was constituted in the studies of traditional bullying, and 27,386 adolescents participated in the studies of traditional victimization.

Concerning bullying, results indicated that there was a significant effect of −0.24 favouring intervention condition (with a significant heterogeneity of 83.22), whereas for victimization effect was −0.14 favouring intervention condition (with a significant heterogeneity of 90.2). Regarding fixed model, effects were −0.18 and −0.11, respectively. In the case of cyberbullying and cybervictimization, effects were −0.19 (with a significant heterogeneity of 83.23) and −0.13 (with a significant heterogeneity of 73.2), respectively. For fixed model, effects were −0.11 and −0.08. These findings are in line with other meta-analyses regarding bullying [74] and cyberbullying [59], which also detected a significant reduction, although modest, of these problems.

As noted, for both bullying and cyberbullying, interventions were more effective to prevent the aggression of the perpetrator than reducing victimized, as was detected in other studies [74]. On the other hand, other meta-analyses found that anti-cyberbullying programmes were more effective in reducing cybervictimization compared to cyberbullying perpetration [55]. Comparing bullying and cyberbullying, the results of the present study indicated that bullying programmes were a bit more effective than cyberbullying programmes, while in victimization and cybervictimization the effect size of the intervention programme was similar.

No publication biases were detected for bullying, victimization, cyberbullying, and cybervictimization. That interventions were less effective for cyberbullying, compared to bullying, may be due to the fact that many interventions for this problem use a non-whole school approach since many of them try to extrapolate interventions aimed at bullying to a phenomenon with different characteristics, such as anonymity, viralization, disinhibition, and that extends beyond the school. Unlike the vast research on bullying, it is well established that there is no consensus on how to prevent cyberbullying [75], as well as there is not a consensus on how to define and measure it [11, 47]. It is also possible that due to the fact that cyberbullying is a phenomenon that extends beyond the school – through the use of new technologies – this adds a complexity, even to the whole school approach.

This study has several limitations that must be mentioned. One of them is the high level of heterogeneity found among studies. The high het-

erogeneity found may be due to different reasons:

1. The operationalization of the constructs, for instance, using different measurement – mainly in the case of cyberbullying. Another problem is that in all cases bullying, victimization, cyberbullying, and cybervictimization were evaluated with self-report measures. Its limitations are well known: social desirability, bias, lack of honest answers, etc.
2. Heterogeneity of the samples. Countries of different cultural traditions, such as Canada, the United States, Finland, Brazil, or Spain, among others. It generates in each of the regions different challenges to an already complex phenomenon [76]. For example, it is known that many successful interventions in the Nordic countries, for instance, Olweus anti-bullying programme, have less cultural and ethnic heterogeneity compared to other nations like the United States.
3. Samples ages that range from 10 years to 18 years old.
4. Different interventions carry out: curriculum intervention, school whole approach, social, and behavioural skill training, among others, which some are known to vary in their degree of effectiveness.

However, it must be noted that previous research indicated that Q statistic and I^2 have a poor power to detect real heterogeneity when the meta-analytic review includes few studies [77].

Another limitation is that some articles were excluded because they used other languages, such as German or French. In the same way, another drawback is that there are few studies regarding cyberbullying [11] compared to bullying [17] included in the present research. However, that is not only a limitation of our meta-analysis but of the cyberbullying literature, as well. That was pointed out by Gaffney et al. [55], as well. Research on cyberbullying is still new, as we suggested. Another limitation of the present meta-analysis is the exclusion of non-school participants and samples other than adolescents. For example, cyberbullying is quite prevalent in college students [78]. Despite these limitations, this work provides an important contribution to demonstrating that interventions for bullying and cyberbullying in adolescence reduce this problem in a significant way, although its effects are small.

Future studies should examine why interventions are a bit less effective for cyberbullying, on one hand, and develop and test effectiveness of specific programme for the prevention of bullying, on the other. It is hoped that these findings will encourage further interest in bullying and cyberbullying prevention researches. Future work should examine the effectiveness of interventions to reduce bullying and cyberbullying but aimed at teachers and parents of students, on one hand, and evaluate effectiveness of anti-bullying programmes with another measure besides self-reports, such as peer nominations. Future meta-analysis should address what components of the interventions may explain variability in intervention outcomes and what components are more effective for the reduction of bullying and cyberbullying or both. Finally, it would also be desirable to generate local interventions for bullying and cyberbullying in each country. It must be considered that both bullying and cyberbullying are complex constructs that acquire cultural features in each of the regions, as it was detected in the case of bullying in a study with 14 nations and 13 languages [79]. That is, bullying can manifest in different ways in each culture. For instance, in Japan bullying is carried in a relational way, whereas in England it is carried out in a physical way [80].

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A Summary of the Developmental Trajectory of Executive Functions from Birth to Adulthood

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Introduction

Executive functions (EFs) refer to a set of cognitive control abilities involved in the coordination of flexible and goal-directed behavior [1]. They are triggered by a wide variety of situations, especially when they are novel and complex; thus, competence in executive functions is critical for self-regulated behavior [2]. EFs include a set of higher-order cognitive abilities. There is general agreement that there are three core EFs: inhibitory control, working memory, and cognitive flexibility [1, 3–6]. Based on these, skills that are more complex related to EFs are built, such as planning, metacognition, reasoning, and problem-solving [1, 3–6]. The neuroanatomical substrate of EFs involves a set of interconnected neural networks that operate in a coordinated way across an integration zone located in prefrontal areas [1, 7].

These cognitive control abilities are among the most complex human processes. They are essential for cognitive, socio-emotional, and psychological development during our entire lifetime. In addition, they are a critical predictor of school performance [8, 9]. Longitudinal design studies have highlighted that satisfactory EF performance in childhood is associated with academic success, better employment status, improved quality of life, physical and psychological health, well-being, and a lower incidence of disruptive conduct and psychopathology in adolescence and adulthood [1, 4, 10].

Hence, the study of the developmental trajectory of EFs has sparked great interest among theoreticians and researchers. EFs develop over a long time, starting in infancy and continuing until adulthood. This is a sequential and multistage process, where different functions mature at different times. Some theoreticians speak about a pyramidal development of EFs, where the most basic abilities precede and underpin the development of other, more complex capacities [1, 3]. In childhood, it has been documented that inhibitory control plays a key role in the development of later EFs [1, 5, 6]. Later on, the three core EFs – inhibitory control, working memory, and cognitive flexibility – act as the basis for the development of other ones, such as planning, problem-solving, and metacognition [1, 3, 11, 12]. The truth is that EFs evolve over time individually until they form an integrated cognitive control system that involves multiple crosslinking

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abilities that work together toward goal attainment.

Understanding how this system is formed has been a great challenge for neuroscience. Research in the past 20 years has produced valuable knowledge by reporting the developmental trajectories of special EFs; however, only a few studies have integrated these contributions into a wide developmental perspective. This difficulty has been mainly related to the complexity and extension of EF development and to a lack of assessment instruments specifically designed for the performance of comparative analyses over different ages [13].

This paper aims to carry out an updated review of EF development from infancy to adulthood by integrating research reports published in several peer-reviewed journals from 2000 to 2019. This summary is expected to update the main achievements of EF development research and identify topics to be addressed by future work.

Frontal Lobe Development and Connections

The emergence and prolonged development of EFs is associated with structural and functional modifications of the prefrontal cortex and its cortical and subcortical connections [14].

Frontal regions develop late. Brain maturation phenomena act first upon projection and then upon association areas [14, 15]. Thus, the prefrontal cortex and the association parietal-temporal areas are the last ones to develop. This pattern reflects the hierarchical organization of the brain [14], where the prefrontal cortex is one of the structures that most heavily connects with the remaining brain regions.

After birth, progressive maturing processes occur – cell proliferation, dendritic arborization, and myelination – and regressive ones, cell death and synapse pruning, that carve and give shape to the brain's anatomy. In particular, myelination and synapse pruning are deemed to have the highest impact on brain maturity.

Myelination helps improve brain functionality as it increases the conduction velocity of nervous impulses. Frontal lobes are one of the last areas to be myelinated. The quantity of white matter in this region increases linearly from 4 to 13 years of age, although this process is not entirely completed until adulthood. It is believed that this maturation phenomenon plays a critical role in EF development during childhood and that it is associated with a higher conduction velocity of nervous impulses between the prefrontal cortex and other brain regions.

On the other hand, a polyneural innervation phenomenon is observed in childhood, i.e., an excess of synapse connections, although not all of them are functional. Therefore, pruning is necessary for the selective elimination of less relevant synapses. On the prefrontal cortex, pruning is continuous from ages 5 to 16, which leads to a decreased synapse density and changes in gray matter during childhood and adolescence. According to Giedd et al. [16], the volume of gray matter in the frontal area increases until adolescence, when it reaches ceiling, and then decreases during post-adolescence and adulthood. The selective elimination of synapse connections is a fundamental process for cognitive development in children. Sowell, Thompson, Tessner, and Toga [17] reported a relation between changes in the frontal gray matter and the evolution in the performance of cognitive tasks from 7 to 16 years of age.

Parallel to these maturing phenomena, changes are observed in cerebral glucose metabolism. The metabolism of the frontal area in the newborn is very low. It is not until 6–8 months of age that an increase in the metabolic rate of the lateral and medial prefrontal cortex is observed [15]. From this time on, the cerebral metabolism continues to increase, reaching adult's value at 2 years of age and its maximum value from ages 3 to 4, when it more than doubles the adult's value. This high metabolic rate is kept unchanged until age 9. Then, it declines and regains adult's value in the second decade of life. Some authors suggest that the metabolism of the frontal lobes increases on a discontinuous basis, with three intense activation peaks at ages 4–8, 10–12, and 16–19 [15]. These peaks coincide with the periods of intense growth of EFs [15, 18–20].

The morphological maturation of the prefrontal cortex is reached at puberty, but quantitative and qualitative changes continue during adolescence. This period is characterized by a linear growth of white matter and a nonlinear decrease in gray matter [16]. Gains in white matter are associated with a faster and more efficient processing of information in the frontostriatal network and a more refined communication between the prefrontal cortex and other cortical regions [21]. Gray matter reduction peaks occur after puberty and during the transition from adolescence to adulthood and are associated with the specialization of the neural networks involved in executive functioning. Baum et al. [22] demonstrated that both modular segregation and global network integration mediated the development of executive function in youth. In addition, changes in interhemispheric connections, cerebral metabolism, and neurotransmission and hormonal mechanisms have been reported. Particularly during this stage, the higher secretion of gonadal hormones has an organizational effect on the neural mechanisms that support cognitive processing [20].

In line with these changes, gains in EFs during adolescence are associated with increased levels of dopamine and serotonin along with a modification in the biosynthesis of neurotransmitters and peptides, which impacts cognitive functioning [23]. All together, these modifications in the neural structure link to significant improvements in inhibitory control [24], working memory [25], decision-making [26], selective attention, and problem-solving [27].

In summary, the structuring of the neural networks that underpin executive functioning has a prolonged maturation process that extends beyond adolescence. Based on this particular feature, EFs have been considered to be a cognitive system that is particularly vulnerable to environmental influence as the slow development of its neural substrate offers maximum opportunities for life experiences to impact brain development [28–30]. Multiple research studies have documented pathways through which the environment models EF development [31–33]. The most significant predictors of the family context are pre- and postnatal stress, the quality of parental care, maternal sensitivity, the parents' educational level, the cognitive stimulation that the child receives at home, and enriched speech interactions between parents and children [33–36]. Variables from the school, neighborhood, and other sociocultural contexts have also been identified as cognitive functioning mediators [37–39]. In brief, the developmental trajectory of EFs should be conceived of as a "development within contexts" [31, 40], originating from a delicate and ongoing interaction between cerebral maturation and environmental influence.

EF Development Along the Vital Cycle

EFs develop across multiple stages. Different functions mature at different times, so it is clarifying to analyze how EFs develop and change along the different vital stages: infancy, preschooling, childhood, adolescence, and adulthood.

Infancy

During infancy, behavioral inhibition and rudimentary forms of problem-solving emerge. The paradigm of object permanence and retrieval is considered to be an indicator of these achievements [41]. The acquisition of this ability assumes that children can create a mental representation of the world around them and keep that information in their mind. This has been studied through delayed response tests, similar to the "A-not-B" task used by Piaget. During these tests, children must look for an attractive object, which is hidden several times in a place (A). After it, the attractive object is hidden in another place (B) and children are expected to look for it there. This task has been suggested to involve the ability to keep information in the working memory and inhibit a dominant response tendency [14, 29, 42, 43]. Babies 5–7 months of age fail in their solving attempts, while it is not until 8 months of age that they succeed at performing this task [29,

42]. The rudimentary ability to inhibit impulsive responses and keep information in memory becomes evident later, from 8 to 12 months of age.

In addition to object permanence, from 8 to 12 months of age, children develop the ability to coordinate means and ends. This ability could be considered another indicator of the emergence of working memory, rudimentary forms of planning, and problem-solving during infancy.

In addition, studies on self-regulation suggest the emergence of executive attention at this stage. Sheese et al. [44] reported that 6- and 7-month-old children who had an anticipatory looking conduct showed higher self-regulation when approaching new toys and less visual attention toward disturbing stimuli. The authors have interpreted these results as early links between the cognitive control system and emotional processing.

Using a longitudinal approach, Johansson [43] found significant improvements in inhibition and working memory between the ages 12 and 36 months. Moreover, she found that simple inhibition and sustained attention were good predictors of later executive functions. These results are in line with the hierarchical framework of EF development, which sustain that inhibition may be considered a core aspect of executive functions [1, 3–6]. Also, these results agree with other researchers who have documented that sustained attention predicted early EFs, advocating early attention as a foundation for the development of cognitive self-regulation [36, 45].

The above cognitive achievements are associated with important maturity changes in children's brains. An increase in the metabolic rate of the prefrontal cortex is recorded at this stage, along with a higher number of synapse connections between the prefrontal cortex and other cortical areas [14].

In short, rudimentary EFs are present at early stages of development, including the ability to willingly regulate and command goal-oriented behavior in response to demands from environmental contingencies [19]. However, these self-control conducts are rudimentary, fragile, variable, and dependent on external stimuli.

Preschoolers

EF development in preschoolers has traditionally been studied through task switching. Children are offered a set of cards that need to be sorted under a certain criterion, such as object colors. In general, the children establish the sorting criterion, and the evaluator just reinforces the right answers. After a number of tasks, the evaluator will say that the criterion used is not correct and children then will have to pay attention to this feedback and find a new sorting criterion. Performing these tasks require working memory, inhibition, and cognitive flexibility.

Studies with preschoolers suggest that 3-year-olds can sort the cards according to one criterion but fail to switch the sorting criterion, i.e., they fail to inhibit the current mental set and refocus their attention on a new set [29, 46–48]. One study [48] reported that this difficulty in 3-year-olds persists even when the examiner explains the new sorting criterion to be used or when the children can verbalize the new applicable rule. The authors believe that a dissociation could exist between what children can think and do, suggesting that the correct resolution of this task involves a conscious reflection upon one's own actions, which would be reached between 4 and 5 years of age. Other authors believe that this difficulty is the expression of attentional system underperformance [47]. The difficulty seen in children to disengage from one mental status and engage into a new one has been called "attentional inertia" and describes the tendency of the cognitive system to keep focused on what it had been focused [47]. From 4 to 5 years of age, children could overcome this attentional inertia and correctly solve this type of task [46–51].

Another way to evaluate attentional control in young children is through their ability for error detection and correction in conflict situations involving spatial locations. Rueda et al. [51] evaluated reaction times in children after errors in the resolution of the Attention Network Test. The authors observed that reaction times increased significantly from 30 to 38 months of age, which suggests that children realized that they had made an error and used that awareness to guide their

performance. The authors concluded that children develop their ability for error detection and correction from 3 to 4 years of age.

In addition to significant changes in the so-called cool EFs, a rapid development of hot executive functions has also been recorded between 3 and 5 years old. Three-year-olds show greater difficulty in delaying immediate gratification and avoiding elections that may be disadvantageous, as compared to 5-year-olds [49]. These changes have been interpreted as a development of the ability to make decisions under circumstances where children need to assess the consequences of their actions and regulate their emotions. On the other hand, relations have been observed between attention control development and emotion regulation. In a series of studies in children 2–4 years old, [51], consistent relations between the ability of children to solve conflicts and their ability to delay gratification and regulate their negative emotions as reported by their parents through the daily observation of the children's behavior were observed. Based on these relations, the authors concluded that attention control is closely related to the ability for the self-regulation of emotions [50, 51].

Finally, the emergence of metacognitive skills has been recorded at this stage. Metacognition emerges early in life and develops over a long term, becoming more explicit and effective as it is under the voluntary control of subjects [52–54]. Most researchers agree that metacognitive skills can be observed at 3 years of age [48, 53]. At this age, children are aware that they and the others are knowers. They can distinguish between thinking of an object and perceiving the object and use the terms "I think – I know" to refer to their knowledge status [52]. At the age of 4, children understand that the others' behavior can be driven by beliefs and desires that can be different from their own. They start to understand that their thoughts may be wrong. Understanding that knowledge is the outcome of the human activity of knowing is a fundamental point in metacognitive development. These initial skills, however, often present in a relatively undifferentiated way.

In the last years, Coughlin et al. [55] have documented the emergency of the ability to metacognitively differentiating between correct and incorrect performance in children of 3 years old. In line with these results, Kim and colleagues [56] captured children's gestures of uncertainty (i.e., children's head tilting or shaking and shrugging). They found that 4-year-olds showed significantly more nonverbal signs of uncertainty than 3-year-olds. These gestures of uncertainty became more frequent the less knowledge the children had. These findings could indicate a gradual development trajectory in early monitoring skills [54].

In brief, the period from 3 to 5 years of age seems to be a time of important EF-related cognitive achievements. Children develop skills related to keeping more than one thing in mind, having flexibility to change the attention focus, inhibiting a dominant response tendency, and regulating their emotions. From a cognitive standpoint, these gains can be associated with improved attention ability and the coordination of the different components of EFs [20]. In a parallel way, from the point of view of cerebral maturation, they can be associated with increased frontal white matter on the right hemisphere. It raises the number of connections of the prefrontal cortex with other cortical and subcortical areas [14]. Although these skills emerge during infancy and develop quickly in middle childhood, their maturation process continues through adolescence.

Childhood and Adolescence

What characterizes EF development during this stage is that different functions mature at different ages and reach ceiling at specific times. The variability of trajectories has not been associated solely with the function under study but also with evaluation tests [18]. In spite of these discrepancies, a general development pattern can be outlined. Meta-analysis studies [19, 20] have highlighted that the time when EFs develop more extensively appears from 6 to 8 years of age. Some experts note another peak of intense growth from 9 to 12 years of age, although more moderate than the former [15, 18].

A sequence of changes occurs in children's behavior during schooling, which suggests a fundamental reorganization of their attention, executive, and self-reflection processes. Below is a summary of EF trajectories individually to make reading easier; however, the interrelation between them makes it difficult to consider these trajectories one by one, as gains in one ability improve performance in others.

Inhibitory Control

Inhibitory control is a multidimensional construct that attempts to account for a series of mental operations tending to suppress inappropriate behavior or an attention tendency toward nonrelevant or distractor stimuli that can interfere with the deliberate resolution of a problem [57]. This cognitive ability improves significantly over school years. In general, studies report that 4- and 5-year-olds show slower reaction times and less accurate responses in tasks that require the inhibition of a dominant response or involve rule switching, as compared to older children [18, 29, 46, 58]. These abilities improve significantly at ages 6–10. A study reported that the most important step forward in the ability to stop a response appears between the ages 7 and 9 [59]. Most research works reflect the effect of age on inhibition tasks up to 13 years old, when this phenomenon seems to reach ceiling [46, 59].

There are some indicators used to evaluate this progress. This is the case of the effect of spatial incompatibility and the associated costs of switching from one block of tasks to a different one. Thus, it goes from a block that requires consistent responses (say "day" when the image of the sun is seen) to another block that requires an inconsistent response (say, "day" when the image of the moon is seen). These functions show a significant decrease with age [46, 47]. Another indicator is the response time: it has been observed that older children and adults show a tendency to increase their response times as tasks become more difficult in order to preserve performance accuracy. Children from 4 to 6 are more impulsive and do not take the necessary time to evaluate their response, which impairs accuracy.

Differences observed between children and adults have been explored with neuroimaging techniques. Functional magnetic resonance imaging (fMRI) studies have revealed different intensities in cortical activation and differences in recruited brain areas between children and adults when they perform Go/No-Go tasks that require the inhibition of a dominant response and the Stop-Signal tasks that require inhibition of an ongoing response. It has been suggested that the more extensive activation found in children reflects a diffuse activity pattern that may originate in an inefficient recruitment of involved brain regions. The selectivity of specific participant regions seems to increase with age.

As to interference control, a broader development pattern is observed. Children aged 6–7 years old have shown significant improvements at performing tasks that involve the inhibition of distractors. This ability increases gradually during childhood and adolescence, which has been associated with increased brain activation in the dorsolateral prefrontal cortex during performance of the Stroop Test [60].

Cognitive Flexibility

Cognitive flexibility entails the ability to switch attention from one perceptual paradigm to another and adapt mental activity and behavior according to environmental demands [1]. This ability follows a gradual development during middle childhood that stretches until adolescence. It is assessed by means of tasks derived from the "task switching" paradigm such as the Wisconsin Card Sorting Test (WCST) [61]. Most studies indicate that cognitive flexibility shows marked improvements in children aged between 6 and 8 [18, 25, 29]. A number of research studies suggest that this ability would reach ceiling between ages 10 and 12 [13, 60, 62], while others report a more extended development [46, 47].

The contradiction in these findings may be explained by reference to the indicators of the psychometric techniques used to evaluate

performance. For example, one of the indicators used is the absence of perseverative errors, which are common in early childhood but decline significantly during childhood and are rarely found in adolescence. Another indicator is the ability to learn from errors and devise alternative strategies. This ability emerges in early childhood and continues developing in middle childhood [18].

Another aspect used to evaluate cognitive flexibility is shifting attentional focus, which evidences extended development. Davidson et al. [46] noted that 13-year-olds display frequent errors in tasks that involve shifting from one mental set to another, even when working memory demands are reduced. The difficulty for adolescents is not that they fail to remember what rules apply but that they fail to transfer those rules quickly to the appropriate response. If even more sensitive indicators are used, such as reaction times or the manipulation of working memory demands and inhibition in task switching, it can be observed that not even adults get to master this ability in full [47]. This could be taken as another indicator of the long developmental course of cognitive flexibility.

Working Memory

Working memory refers to the ability to maintain information active (online) in the mind, manipulate it, and perform acts based on it [1, 46]. This cognitive capacity shows a gradual developmental course, starting in early childhood and extending across adolescence. One study observed that the basic modular structure of working memory – visuospatial sketchpad, phonological loop, and central executive – is present since age 6 and that each component increases its capacity until adolescence [63]. Different studies conducted in schoolchildren have reported significant improvement in the phonological loop of working memory between ages 7 and 13 [29, 60, 64], with one peak at age 8 and another one at age 12 [59]. Regarding the visuospatial sketchpad, it has been observed that children achieve mastery of the task of keeping visual information in the working memory at around 9 years of age. However, if the task additionally involves information manipulation, development takes longer, reaching ceiling at approximately age 12 [13]. These gains have been associated with the differential development of mechanisms for sequencing, sorting, and retaining information in the working memory [29]. Some authors consider that the development of this ability extends until adulthood, with improvements becoming evident between ages 15 and 19 [25, 62].

Attentional Control

Attention allows an individual to select relevant information, sustain, and manipulate mental representations to modulate the responses to a variety of stimuli [65]. Regarded as a vertical control mechanism, attentional control serves the purpose of deciding to what stimuli cognitive resources will be allocated, by either activating or inhibiting the processes that generate and organize information [15, 66].

Execution in different auditory and visual attention tasks improves with age, with striking changes between ages 5 and 8, and subtler changes between ages 11 and 16 [67]. Using the Attention Network Test, Posner et al. [50] have reported considerable improvement in the ability to shift attentional focus between ages 2 and 5, with sustained improvement until age 7, a time when children seem to attain maximum performance [50, 51]. Other researchers have also found that the ability to shift attentional focus reaches ceiling early, between ages 8 and 10 [18, 62]. Although children display a level of attentional performance similar to that of adults, differences in activation were observed in the anterior attention network. When solving conflict situations involving spatial locations, adults show a more refined and focal activation pattern in the prefrontal cortex, while children display a more diffuse pattern.

Similar results have been found among 13-year-old adolescents [68], suggesting that neural networks keep reorganizing. This maturation pattern involves not only further specialization of the anterior attention network (executive

attention) but also a reduction in the time required by the system to solve the task. This may be related to the concept of attentional efficiency. A study conducted in Argentine schoolchildren reported improvements in the attentional efficiency ability in children aged 7–12 [65, 69].

Planning and Problem-Solving

Planning involves the ability to identify a goal, sequence, and organize the necessary steps to reach it, anticipate consequences, and develop and evoke a mental map to direct actions toward goal attainment [70, 71]. This ability develops quickly between ages 7 and 10 and continues evolving in a more gradual manner during adolescence [18, 72–74]. Young children use simple, often inefficient, random and fragmentary organization and planning strategies, but children aged 7–11 develop more organized and efficient reasoning strategies and skills [62, 72].

Some authors establish a distinction between two types of planning: visuospatial and strategic. The former is usually studied in tasks such as maze-solving. Results show that this ability reaches ceiling at around age 9 [13]. A research study with Argentine schoolchildren aged 7–12 reported that the planning ability, as assessed by the number of successfully completed mazes in the Porteus Maze Test, did not reveal significant changes [60]. Furthermore, strategic planning is associated with the ability to solve problems and is usually assessed by means of tower tests. An analysis of the solutions given to the Mexican Pyramid test revealed that children aged 5 and 6 initially attempt to get a higher number of correct answers, which suggests their inclination to focus on the end result of their execution. Only later do children (ages 7–8) tend to control the number of movements performed to complete the designs, i.e., they pay attention to their planning process. The study detected a ceiling effect in measuring the number of correct designs at ages 7–8. Conversely, control over the number of movements involved is attained less abruptly. This suggests a developmental stage characterized by important changes between ages 5 and 8 that become more gradual as from ages 9 and 10 [74].

Planning and problem-solving skills improve significantly until age 12. A number of studies have shown a plateau in the development of these skills between ages 12 and 13 [18, 74]. Some authors interpret this fact as a preference by adolescents for more cautious and conservative strategies leading to correct task execution [72], while others interpret it as resulting from a difficulty by young people to implement or take advantage of their new skills [62].

It has been observed that only at age 15 would ceiling be reached in solving the Tower of Hanoi puzzle with three disks, paying attention to the number of movements [13]. Consequently, strategic planning and the organization of goal-oriented behavior have been reported to continue developing during adolescence, possibly reaching their peak between ages 20 and 29 [62]. Improvements in these skills have been associated with gains in working memory during adolescence.

In summary, visuospatial planning as assessed by means of maze-solving tasks seems to reach ceiling early during child development. On the contrary, strategic planning, which requires selecting and sequencing problem-solving action schemes, displays sustained evolution during adolescence.

Metacognition

Metacognition refers to the knowledge individuals possess about their own cognitive functioning and their attempts to control these processes [75]. By virtue of this second-order cognition, individuals are able to monitor, self-regulate, and develop strategies that will enhance their own cognition [52].

Major changes in the metacognitive ability have been observed during childhood and adolescence. Between ages 5 and 8, children begin to enunciate statements of certainty about their memories and gradually increase their monitoring skills [53, 54]. With the development of inhibitory control, children begin to improve

metacognitive control skills, such as checking their answers to a school performance test or realizing that they do not understand what they are reading. These behaviors become more frequent during primary schooling. Through daily experiences and learning efforts in school, children may slowly calibrate their monitoring skills (i.e., making more precise performance predictions, by becoming less overconfident). While monitoring skills are found to be relatively accurate by the age of 8, control skills (i.e., withdrawal of errors) are repeatedly found to lag behind [53, 54]. Metacognitive development in later childhood and adolescence can be best described as the fine-tuning of earlier developed monitoring and control skills [53, 54]. In sum, this stage sees rapid development of metacognitive awareness and meta-strategic skills, which continue to evolve during adolescence and adulthood, prompting the development of second-order thinking.

Metacognitive thinking displays prolonged development, and, indeed, significant differences can be observed among adults depending on context variables such as their level of schooling [76]. Finally, it is worth highlighting that most studies on metacognitive development have been designed on theoretical grounds, but few have developed tasks or tests to conduct accurate assessments of the developmental trajectories involved in the different aspects of metacognition [53].

Oral Fluency and Processing Speed

Oral fluency refers to the ability to generate the highest number of words in a given period according to a semantic or phonological criterion [77]. On the other hand, processing speed points to the time taken by an individual to extract and integrate information during problem-solving, as well as the speed at which an individual executes basic cognitive functions, such as object identification, decision-making, or simple discrimination among objects or images [78].

Oral fluency and processing speed have been positively associated with age. With respect to the former, most studies indicate that an increase takes place between ages 3 and 5 and continues to develop during childhood, with significant gains between ages 9–10 and 11–12 [18, 59, 60]. This development stretches into adolescence and may reach ceiling at around age 15 [18]. Processing speed shows a similar pattern, significantly increasing between ages 3 and 4 and displaying a significant gain between ages 6 and 10, but this skill would seem to reach ceiling in adolescence or early adulthood [62].

The studies reviewed allow for some conclusions. EFs follow different trajectories, but almost all of them experience a growth peak between ages 6 and 10, displaying a more gradual development from this point onward. Some functions, such as response inhibition and attention focusing, mature early, while others, such as working memory, control flexibility, planning, metacognition, problem-solving, interference control, and oral fluency evidence improvements in adolescence and adulthood, reaching their growth peak between ages 15 and 19. These gains may reflect coordination behind the different cognitive processes involved, which, in turn, may lead to improved performance. These achievements are parallel to structural, functional, and neurochemical changes in the neural networks supporting EFs [16, 21].

Executive gains at this vital stage allow children to mentally process and manipulate greater amounts of information, build mental schemas, and understand the most relevant conditions of a given task or problem [13]. They enable an efficient use of memory strategies, in addition to promoting learning, the development of various problem-solving hypotheses or alternative solutions, abstract thinking, and progress in activity organization and planning [13]. These achievements will have a substantial impact on the children's school, social, and emotional spheres.

Adulthood

It has been observed that some EFs may reach ceiling in early adulthood. In a study evaluating the development of EFs in a large age sample,

functional gains were found in the efficiency of visuospatial working memory, planning, and problem-solving between ages 20 and 29 [62]. It has been reported that EFs involving perceptual and motor components peak around age 20, while EFs involving verbal processing seem to do so later, around age 40 [79, 80].

Generally, EF performance tends to remain constant until it declines significantly between ages 50 and 65. In line with these results, another study in adults aged 20–75 found a significant reduction in the capacity to inhibit a dominant response, cognitive flexibility, divided attention, and processing speed starting at age 60 [81]. Divided attention problems were recorded in younger adults (aged 46 and 59), which may suggest that decline can become manifest earlier in tasks involving greater cognitive complexity.

Interindividual EF differences have been reported to increase with age, in association with factors that could mediate the relationship between age and EF, such as the activation of other cortical areas during the execution of EF tasks, educational level, depression, family support, and the performance of cognitively stimulating tasks in old age. In brief, a significant decrease in EFs is observed between ages 50 and 60, which corroborates that EFs are age-sensitive. Cognitive decline has been associated with neurodegenerative processes, such as a reduction of gray matter from age 65 onward and a decreasing brain activation pattern in the prefrontal cortex [81].

Gender Differences in the Development of Executive Functioning

Previous studies into the development of EFs have not provided consistent data with respect to sex-dependent effects. Some studies found no effects caused by the variable sex or of the sex-age interaction in measurements of executive functioning, indicating that boys and girls develop their cognitive control abilities in similar ways [29, 46]. Other studies, instead, did report differences, which tend to be consistent with the traditional view that girls perform better in verbal skills, while boys do so in spatial skills. Thus, it has been observed that girls show better performance in oral fluency and information organization and processing as compared to boys [18, 72]. In contrast, boys outperform girls in working memory and spatial reasoning. The study by De Luca et al. [62] showed that boys performed better in executive tasks involving visuospatial components, with these differences persisting over an extended period of development (ages 8–75). However, these authors found no interaction between age and sex, which reveals that these executive abilities emerge and develop in similar ways in males and females.

In addition, research has shown consistent gender differences in aspects regarding attention regulation, inhibitory control, and the use of selection strategies for the performance of cognitive tasks in favor of girls, as assessed by various test batteries [36, 67, 82, 83]. One meta-analysis claims that girls outperform boys in various dimensions of the attention domain between ages 3 and 13 [84]. Other studies have also reported advantages in attention, working memory, and inhibitory control for girls, both in direct evaluation tests and through the perception of parents and teachers [59, 83].

Studies comparing teacher reports regarding executive functioning in children point out that educators perceive better performance in inhibitory control, attention, working memory, organization, and planning skills in girls [41, 82, 83]. Greater self-regulation resources among girls may be a factor contributing to better academic and socio-emotional performance in school contexts. Along these lines, teachers report improved work habits, greater focus on school tasks, and better grades in girls than in boys [83]. Finally, it is interesting to note that differences in inhibitory control seem to persist in the course of development. Indeed, a study of 188 adults aged 17–60 found that females presented higher levels of inhibitory control and empathy than males [85].

In a study in Mexican children, girls outperformed boys in attention and memory tests but showed variations according to age. Girls aged

5–8 outperformed their male peers in auditory verbal recognition, encoding a list of shapes and cued visual recall tasks, while girls aged 9–16 outperformed males in letter cancellation tasks and story recall tests. These data match those of previous studies reporting that girls outperform boys in tasks involving memory and verbal learning [18]. Girls showed better performance in tasks involving verbal information or requiring verbal strategies. This could be related to the earlier development of language among girls, which has been associated to a faster increase in vocabulary and the production of complex linguistic constructions at earlier ages or with the greater ability of girls to identify, express, and describe emotions [67].

A recent large-scale cross-sectional study [86] investigated whether adolescent males and females differ in self-perceived self-regulation. Results revealed that females evaluated their attention higher than males, and they reported higher levels of self-control and self-monitoring in middle adolescence.

Gender differences in EFs may be mediated by hormonal factors, gender-related differences in brain architecture [87], and sociocultural factors or, most probably, the interaction among them. Few studies have identified an effect of the interaction between age and sex, and thus it is questionable to affirm that there are differences in the developmental trajectories of males and females. However, it may be interesting to explore whether gender differences persist or change over time, whether they become more marked at certain developmental stages instead of others, and what factors could mediate the relationship between EFs and gender differences.

Conclusions

Based on this research review, it is possible to trace the developmental profile of EFs during the vital cycle.

First, reviewed studies agree that EFs present a prolonged, multistage, sequential development, which is more intense during childhood and then decreases its pace during early adolescence. Most EFs reach ceiling in late childhood or early adolescence, while others, like strategic planning and cognitive flexibility, continue evolving in adulthood. Even when different trajectories are described, meta-analytical studies agree on the existence of a period of intense development between ages 6 and 8. Along these years, children exhibit a significant, accelerated increase in their attentional ability, working memory, inhibitory control, cognitive flexibility, processing speed, oral fluency, planning abilities, and problem-solving. Other two periods of rapid development take place between ages 10–12 and 15–19, although these are less accelerated than the first one. The identification of these sensitive periods represents an important achievement in current research, as it signals the specific times during which EFs are particularly vulnerable to environmental experience and, consequently, become optimal times for the application of cognitive interventions.

Another interesting feature is that, along their course of development, EFs progress from a state of greater undifferentiation to one of greater differentiation. Cognitive control processes emerge jointly, and it becomes virtually impossible to identify their individual components in early childhood. The truth is that executive functions develop and become differentiated gradually until they form a cognitive control system in which the multiplicity of EFs contribute in specific and interrelated manners toward the achievement of an aim or goal. Such differentiation results in stronger hierarchical control on cognition and greater cognitive flexibility [1, 13] and is consistent with the reorganization and greater selectivity of neural networks supporting executive functioning.

Along these lines, it has been observed that the development of EFs has an additive, systematic effect on cognition control. Studies have consistently documented that the cognitive control system gradually progresses toward greater selectivity and regulation of cognitive processes. As a result, a growing capacity to create mental schemas, improved mental flexibility, increased recourse to and complexity of memory strategies,

greater organization and planning of cognitive activity, enhanced categorization ability, and oral fluency are observed, leading to more efficient and abstract thinking, which is also more complex from a psycho-linguistic perspective [13]. Similarly, these cognitive gains contribute to an improved control of emotions, impulses, and conducts, promoting self-regulated behavior and an adequate socio-emotional and psychological development.

Finally, it is important to highlight that the acquisition and development of the cognitive control system is tightly connected to the maturation of the prefrontal cortex and its cortical and subcortical connections. The neural networks forming these connections have an extensive maturation process, during which a number of stages can be identified. Along these stages, brain plasticity increases, and experience has maximal impact on brain development. This particular feature has led researchers to consider executive functions to be a cognitive system that is particularly vulnerable to environmental stimulation. Enriched and varied quality learning experiences will promote EFs, while socially vulnerable and socioculturally impoverished contexts will offer limited possibilities for the promotion of these abilities.

Therefore, we may conclude that the study of the developmental trajectory of EFs and their predictors continues to be an area of great interest for cognitive neuroscience. Knowledge unveiled in the past decades has meant a big step forward in the understanding of EF development. However, some topics remain unexplored: gender differences have not been thoroughly studied, yet few studies have described the decline of EFs in normal and pathological development and cognitive development predictors at different stages of the vital cycle need to be further examined.

Although progress has been achieved in the identification of development precursors in childhood, little is known about the factors promoting these abilities in adolescence, adulthood, and old age. New longitudinal studies of large samples that are well characterized in sociocultural terms and measure EFs with tools permitting comparative analyses at different ages will certainly be of outstanding value. In the same way, neuroimaging studies represent a valuable tool for the identification of changes in neural networks that are connected to EF growth and decline peaks, as well as with neural modeling that is connected with the exposure to favorable or unfavorable environmental conditions. Once these studies are available, it will be possible to design effective interventions more targeted to promoting cognitive control abilities during the full vital cycle with a view to improving full people's development.

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Approach to Learning in the University: Reference to Learning to Learn

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The Reform of Bologna: Teaching, Learning and Research

The new paradigm of European Higher Education, emerged as a result of the application of harmonization mechanisms, is oriented toward a methodology based on learning and focused on the active role of the student, which modifies the traditional function of the university (teaching and research), and a third element comes into play, learning, which should and will be nuclear in the new university model.

This also implies that the student assumes one of the basic principles of continuing education: being lifelong learners. The transfer of their own construction implies the endowment to the student of the most suitable tools for this and, especially, of the knowledge of their own processes to always be able to choose the most optimal and, if this is not done, be able to rectify and change it [1].

It is evident that from the Humboldt University of Berlin, where research was before teaching, the current one in Bologna a stretch is present. Closing the eyes at a distance is simply losing horizons and denying futures.

Learning to Learn

Definition

The European Union (EU) [2] defines competencies as a combination of knowledge, skills, and attitudes appropriate to the context and key competencies as those that all individuals need for their realization and personal development, active citizenship, social inclusion, and employment.

Learning to learn is one of the eight key competences adopted by the European Parliament and the Council of the European Union in December 2006 [2]. Due to its generic nature and appllicability, it has been considered as a transversal competence, which indicates its generic

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nature and applicability in different subjects and activities, or as metacompetence [3]. However, as indicated by Čepić et al. [4], it could be more appropriately qualified as metalearning since the term metacompetence would imply competence to *develop competencies* and describe most of the professional work of teachers.

The EU working group [2] on key competencies identified the concept of learning to learn as follows: *ability to pursue and persist in learning and organize one's own learning through effective time and information management, both individually as in groups*. This competence includes conscience of the learning process and needs, the identification of available opportunities, and the ability to overcome obstacles to learn successfully and means obtaining, processing, and assimilating new knowledge and skills, as well as seeking and making use of guidance.

Exploration of Learning to Learn

The approach of our group to learning and, therefore, when learning to learn has been done through the management of three concepts and their exploration:

1. Learning styles
2. Learning strategies
3. Learn to learn from self-regulated learning

Learning Styles: Learning in Any Context

We began working with learning styles and an objective, to provide our students with a high score in each of the constructs – styles – that determined the tools for their study, with what was expected to be good learners in all contexts (master classes, dissection rooms or laboratories, practices in small groups or next to the patient's bed, etc.).

The concept of pedagogical language style is often used to indicate a series of different behaviors gathered under a single label (management styles, learning styles, etc.). Styles are something like conclusions we reach about the way people act. For Keefe [5], learning styles are cognitive, affective, and physiological traits that serve as relatively stable indicators of how students perceive, interact, and respond to their learning environments.

Consequently, learning styles (cognitive traits) have to do with the way students structure content, form and use concepts, interpret information, solve problems, select means of representation (visual, auditory, kinesthetic), etc. The affective traits are linked to the motivations and expectations that influence learning, while the physiological traits are related to the gender and biological rhythms and biotype and biorhythm of each subject, such as sleep-wakefulness, of the student.

Different types of learning styles have been developed in reference to different theoretical and conceptual frameworks. The one developed by Kolb [6–9], in the USA, is original in the sense that it has been the first to rely on a conception of learning described by the same author, experiential learning (concrete experience, reflexive observation, abstract conceptualization, and active experimentation). Kolb defines learning as the process by which knowledge is created through the transformation of experience. He identifies two main dimensions of learning: perception and processing. He points out that learning is the result of the way people perceive and then process what they have perceived. The two types, dialectically opposed, of perception are concrete experience and abstract conceptualization (and generalizations), and in processing, the extremes are active experimentation (implementation of the implications of concepts in new situations) and observation reflexive. The juxtaposition of the two ways of perceiving and the two ways of processing is what led Kolb to describe a four-quadrant model to explain learning styles. Each quadrant responds to a different style.

Learning styles have been integrated, as individual characteristics, into theoretical constructs seeking to give a global account of the activities of a student who is committed to the progress of learning. All studies involve 72 models of

different learning styles, several of them in frank contradiction and almost a hundred different tools.

Curry [10] emphasizes that the counterparts of this abundance imply an excessive conceptual division, a poorly stabilized plural terminology and, from the operational point of view, a multiplication of measurement scales, whose psychometric validation has sometimes been developed in reference to several different constructs, so that the indicators of validity and reliability are frequently variable, which makes the predictive feasibility of the measures extremely difficult and makes the interpretations and arguments developed from them more fragile.

Usefulness of Styles: With High Scores in All Styles, Learning Contexts Do Not Matter

The application of the styles provides a profile of the students that serves to act in two directions [11]. On the one hand, trying to improve lower scoring styles with the objective that students have high scores in all of them allows them to be efficient learners in all contexts. On the other hand, facilitating knowledge allows the best and most profitable methodological interaction for learning. In both cases it is necessary to determine, first, the learning style of the students.

In relation to the second aspect, to favor the methodological interaction for learning and despite some successful contributions such as the 4MAT system [12, 13], in which the teacher must plan and design activities focused on the integration of all pedagogical styles, reality indicates that it is applied very sparingly.

When the approach to learning styles began, we went to the first aspect indicated above: the diagnosis and improvement of the constructs of students with low scores, that is, to detect those styles that had a score that seemed inappropriate to us. The aim was improving it in order for a given student to have high scores in all of them, which meant that he was able to learn in any context (theoretical, practical classes, dissection room, or next to the patient's bed, to name a few), since, as indicated in the characteristics of the styles, each one has a preference for some specific activities.

The Problem

As indicated above, the first action consists in determining the style for which there are numerous tools or questionnaires. When we worked with different tools in order to see which one best suited our interests, the problem we summarized in the following sentence arose: different tools, different diagnoses (styles) for the same subject, as we have shown in recent publications [14–17] in which two tools have been applied, the Honey-Alonso questionnaire of learning styles (CHAEA, [18]) – Spanish version of the Honey and Munford tool [19] – and that of Felder and Silverman [20].

The results are opposite regarding the active/reflexive styles, so that the possible improvements obtained from the students' learning style knowledge are at least debatable. One of the reasons that have been pointed out [16] to explain these differences, apart from the structural ones, is based on the overvaluation of impulsivity to the detriment of the activity carried out by the CHAEA, and, consequently, when this tool is applied, the active style is undervalued.

In order to correct this dilemma, a new questionnaire [16, 17] was developed based on the previous ones in Kolb's experiential learning [7–9], and the results were compared with those provided by the CHAEA in the same student population.

The CESEA [16, 17], acronym in Spanish of the Questionnaire of Escanero and Soria of Learning Styles, as the new questionnaire is called, assumes the axes of perception and processing. Perception, confrontation with learning, presents in one extreme/pole all the senses that can be used to capture something new (know, read, listen, see), with the exception of the kinesthetic, which is at the other end (try, check, do). To designate the extremes, the *theoretical* and *dynamic* terms have been used. The processing includes from the thoughtful, critical, and argumentative (think) to the application and resolutive (solve, solve) reflection. The terms designated for the ends or poles are *reflective* and *operational*.

The interaction of the vertical and horizontal axes determines and defines four learning styles (quadrants): theoretical-reflective, dynamic-reflective, theoretical-operational, and dynamic-operational. In turn, the two upper quadrants determine, as indicated in the previous section, the analytical cognitive region (theoretical-reflective and theoretical-operational styles), and the lower quadrants, the intuitive cognitive region (dynamic-reflective and dynamic styles-operative).

The descriptive analysis showed that the theoretical learning pole was the preferred one, with a higher average score than the rest, followed by the reflexive one. The one with the lowest score was the dynamic one. In general, the results for the four poles have a high homogeneity (34.4 versus 37.5).

The results of the consistency of the reliability test show that the scale used to measure the 48 items or issues has a good Cronbach's α (0.822). The scales used to measure each of the four learning poles were analyzed individually and the values shown are satisfactory (0.6–0.8). According to Pardo and San Martín [21, 22], the acceptable values for this parameter are from 0.6.

Regarding the CHAEA, the results show differences mainly in regard to the active style, being more homogeneous and high in the new questionnaire, which was intended to correct.

Performance for Improvement

Once the diagnosis of the preferred style has been made, it could happen that: a) in one of the other styles he has obtained a low score (deficit score) in absolute values, or b) rather than, compared to those of their group mates in some styles. Although it does not have a poor score, it is one of the highest or lowest of the group.

Our recommendation, in case of improvement, acts in two ways:

- I *Improvement of the questions that the subject has answered with low scores* [18].

Once the survey is carried out to know the style, the defining questions of each pole are analyzed and discussed with the student. Taking into account the pole that we are going to improve, if we have worked with CESEA, we analyze the poor scores in the answers answered that may go from very marked or notorious to others where it sometimes approximates the cutting notes. It is recommended to start the improvement for the least expensive, that is, for those who almost do it already, or perform but have not acquired the required score, that is, for those who have the highest marks within the deficiency. Alonso et al. [18] recommend starting with those between yes and no, that is, those that have somehow had more doubts in answering.

The improvement strategy goes through the following points:

1. Notebook for improvement. Booklet where all the tasks to be carried out for improvement are recorded in this first analysis visit, together with the tutor or responsible teacher. There is also a tentative distribution over time. They are usually grouped in two or three in three.
2. It begins with the first couple or trio. For example:

   The way of acting described in the questions will be improved:

   - 10A: When I learn I proceed in a structured manner.
   - 19B: Faced with a problem, I seek to dominate the issue-draw conclusions that have qualified with the minimum.

   For the first one, we have agreed on two things: (a) That before starting any activity, a forecast of the points to be addressed must be carried out and that at the end it should be verified that it has been carried out and (b) a summary of the subjects studied must begin every day, sequentially indicating the points to be treated. In the notebook for improvement, daily incidents will be collected every day.

   The second has been approached in a similar way. From the subjects that study every day, it will be prepared a summary drawing the conclusions of it. Each day the incidents of this task will be noted in the booklet, in a different chapter from the previous one, in how many subjects have

been agreed or entrusted and in how many not.
3. After the time agreed with the tutor or teacher in charge, which we recommend 2 or 3 weeks, the progress of the booklet will be analyzed according to the annotations made, observing the level of compliance with them.

If the result has been in accordance with the agreement, a new planning will be carried out, but if not the planned task will be repeated until a new meeting, analyzing the reasons for the breach and trying to solve them. It is ultimately intended that the student unconsciously develop what at first implies or may involve a voluntary and expensive activity.

II *Modeling deficit styles*.

Each style is defined by certain characteristics. In the case of CESEA, the main characteristics are the following described. The characteristics of the poles are presented, for which we recommend starting the improvement, remembering that the styles are the joint of the two limiting poles of the quadrant:

(a) Theoretical (know).
Methodical, logical, objective, critical, rigorous, organized, precise, sequential, formal, deductive, and associative
(b) Dynamic (try)
Experienced, explorer, discoverer, creator, restorative, synthetic, informal, spontaneous, adventurous, impulsive, and inductive
(c) Reflective (think)
Conscientious, receptive, exhaustive, compiler, meditator, collaborative, cautious, and sensitive
(d) Operational (do)
Productive, practical, effective, decisive, realistic, solver, direct, and common sense.

It is also recommended to be quite free with a broad view in modeling.

Next, the defining questions of each pole are analyzed and discussed with the student. Taking into account the deficiencies according to the answers answered and the characteristics of the different poles, the strategy of the improvement of some characteristic of the style is elaborated, which may not coincide exactly with a certain question and the improvement objectives may be broader than in the previous section, since they not only respond to the improvement of the poorly punctuated questions but to those qualities of the style that in common agreement are estimated to be susceptible to improvement. The objective from the first moment must be very clear on both sides: the student must be an efficient learner in any context. The treatment, in essence, is as in the previous section.

Learning Strategies: Learning from the Best

The approach to the topic of strategies requires, apart from the precise conceptual location, a certain knowledge of the different denominations of them that have been used by the various authors who have studied the subject, since there is a real jungle of names that can discourage who approaches the subject ignoring this problem.

There are a lot of definitions. Here we will mention only two of them. For Álvarez et al. [23], they are *intentional guides of action with which it is a question of putting into practice the skills that establish the learning objectives*. Monereo [24] defines them as *a set of actions that are carried out to obtain a learning objective*. These actions correspond to a series of cognitive processes in which, according to the author, it would be possible to identify cognitive abilities and skills, but also techniques and methods for the study.

At present, as Fernández-Borrás et al. [25] says, the learning strategies are located at the same hierarchical level as the specific thematic knowledge of each discipline. From this point of view, knowledge of these strategies is shown as a prevalent action in institutions dedicated to higher education. In this regard, Coffield et al. [26] write: *learners become more effective as learners if they are aware of the important qualities they and other learners possess*.

Classification

Although there are numerous classifications of strategies, in this chapter we will focus on metacognitive and cognitive.

Metacognitive Strategies

The metacognition construct has been developed based on contributions from leading scholars from various research areas [27–30] and implies not only the person's knowledge about the factors involved in their understanding process but also knowledge of how these factors act and interact to contribute to the realization and results of their cognitive actions. Metacognitive knowledge develops throughout life and is closely related to the frequency of use of high-level processes that involve two types of activities: on the one hand, being aware of what is known about the material that should be learned and the processes involved in its acquisition and, on the other, be able to regulate the activities that must be carried out for learning to be successful [28]. In this way, metacognitive knowledge allows the student to reflect on their own thinking in order to promote autonomous learning and academic success [31–34]. In this sense, the metacognitive knowledge that an individual possesses allows him to feel more motivated, taking into account that as he becomes more aware of the self-regulation mechanisms involved in carrying out academic activities, he becomes a better learner [33].

For us [14], the planning (first of the two explored components of the metacognition) and the grade obtained are significantly correlated ($p < 0.05$). The theoretical style is positively correlated with the planning strategies ($p < 0.05$) and evaluation (second of the metacognition components explored) ($p < 0.01$) and while the reflexive one only does so with the evaluation ($p < 0.05$).

On the other hand, the relationship between metacognition levels and academic performance seems clear. In this sense, in a work carried out by Bernad-Mainar et al. [35] – next section – it is indicated that the correlation between academic results and cognitive awareness reaches significance ($p = 0.001$) regardless of the level of academic performance and the statistic used (correlation and comparison of means). It is curious that this correlation is fulfilled regardless of the type of studies taken. This work also shows that the students of the last courses (final level) reflect having a greater cognitive consciousness than those of the first, which implies that the passage through the race not only provides more knowledge but a greater control of the learning process itself. In this line, two complementary data were also highly curious: (a) in the first courses, the level of cognitive consciousness of the students does not keep parallels with the academic performance (70% of underperforming students are among the students of higher cognitive consciousness) and (b) in the students of the last courses it happens that as academic performance decreases, the level of cognitive consciousness also decreases.

Cognitive Strategies: Learn with the Best Strategies in Any Environment

A First Experience

In 1995, the ESEAC scale that had been prepared by Bernad [37] and implemented at the University of Zaragoza by different collaborators [35] was presented at the Meeting of the AMEE (Association for Medical Education), held in Zaragoza [36], and, subsequently, applied to the students of Physiology of the Faculty of Medicine of Zaragoza [38]. This scale, unlike the rest of the instruments, measures behaviors and not so much the *opinions* of the student. In this sense, this scale wants to avoid two serious inconveniences of this type of tools. In the first place, the items of the other instruments are elaborated based on the opinion of the student – what he says to do, what he thinks – and they forget about aspects of behavior that by their very nature are directly observable and, therefore, truly objectionable – aspects shown in the *execution* of concrete tasks. The second drawback, which ESEAC does not have, is that the items of the commented scales are formulated in a general or decontextualized way (*I take notes in class; I seek to have prestige among my classmates, friends, and family; when I read, I differentiate the aspects and important or main contents of the accessories or secondary; etc.*). The inclusion of abundant items such

as these in the aforementioned scales invites us to think that the learner's behavior would have as a reference a unique and undifferentiated framework in which the behavioral differences derived from the variety of factors that intervene within the precise context in the context are not contemplated. That learning really occurs (different disciplinary structures, different types of relationships within the group, different personality and teacher training, etc.), that is, everything that is included today under the label of *contextualized* learning.

The ESEAC scale, according to Bernad et al. [35], is defined by four characteristics:

(a) A holistic or global conception of learning. What is attempted with this scale is not so much to study the influence of each variable, one by one, in the process of learning from students. The aim is to take note of the set of a representative sample of them with the different steps and nuances that can be distinguished in the said global process when learning is analyzed in its strategic version.
(b) An ecological approach to learning (ESEAC as an instrument aimed at facilitating and enriching the activity of teachers in their task of evaluating students within the framework of their usual work).
(c) Attention focused on student activity.
(d) Generability of the analyzed processes.

Other Tools

As with the exploration of metacognition, the tools used for the exploration of learning strategies offer a lot of possibilities [16], although in this chapter only reference is made to what we usually use.

The ACRA (Spanish acronym for Acquisition, Coding, and Recovery and Processing Support) [39] based on learning theories includes cognitive, metacognitive, and socio-affective strategies. From our point of view, the structuring of the first two scales (acquisition and coding/storage) is debatable, since the storage strategies are located both in the first scale, in Acquisition of Information, and in the second, in Coding (called Coding or Storage, which is still questionable). On the other hand, the fourth scale, of Support, in the group of Socio-affective Strategies includes together in the same strategy the Intrinsic and Extrinsic Motivation, both punctuated in the same direction, which is still striking. This can give the impression that the intrinsic motivation is the same as the extrinsic one. In addition, one does not include a type of fundamental strategies, such as those of Search, Collection, and Selection of Information, and other important types of other types are contemplated. Thus, the Processing Strategies do not include Personalization, Creativity, and Transfer. Nor do they include the Value of the task, the Self-efficacy, and the Control of the Context among the Support Strategies. On the other hand, the questionnaire includes excessively long items and of doubtful intelligibility given the population to which it is addressed.

Deciding on this tool, in the end, focused on the ease of application and interpretation and why our work is supported either by the tutor or the teacher who deals with this competence.

Acting for Improvement

1. Once the strategy survey (ACRA) has been carried out, the answers given by the students together under the idea of coverage that the students with good strategies are the students with high performance are discussed by practice sections. The strategies of each one of the explored processes are discussed separately, which are the three basic cognitive processes of brain functioning: (a) acquisition, (b) coding or storage, and (c) recovery or evocation of information, leaving for last place other processes of metacognitive, affective, and social nature that are addressed by the support strategies that are also necessary.

   From the knowledge of cognitive processes can be deduced mental procedures or management strategies, which are called "micro strategies," learning tactics, or study strategies. The construction process of the ACRA was based on this theoretical framework [39].

   Each cognitive process has a certain number of strategies: (I) 7 information acquisition strategies; (II) 13 information coding strate-

gies; (III) 4 recovery strategies; and (IV) 9 processing support strategies.

2. It is about students getting to know each other and seeing the strategies they use, for those who need to incorporate them into their collection. It is, as we insist, to know well and to have previously explored the metacognition, since the second component according to Flavell [28] is the control or domain over them. This indicates the control that the student exerts on the strategies used to learn a certain subject, which implies that if a strategy is not adequate or does not work as it should eliminate it from his arsenal.

In this regard one of us occurred the following fact:

We were talking about acquisition strategies (Exploration, Linear Underline, Idiosyncratic Underline, Epigraph, Overhaul, Mental Review, Repeated Review) and, specifically, the underline, which everyone did, as was the case with the idiosyncratic they did. All but one student, male, said that he had no case with markers of different colors. The rest were women students. He assumed that this was the Acquisition strategy he was going to adopt. After 2 or 3 weeks, he met again with the professor in one of the halls of the faculty, and after the timely greetings and inquiries he said that underlining the page with so many colors he became dizzy and could not study. That meeting ended with a memory of metacognitive strategies. The professor suggested – Flavell, second part of the definition of metacognition, to which the student responded without hesitation – I have already given the markers.

One issue is metacognition and another is what we are trying to achieve: provide students with fewer strategies with all the luggage available to students with better performance.

3. With the strategies incorporated in a particular student, they will be followed up as has been done with the learning styles, writing down in a booklet the incidents of each day to be analyzed in the meetings scheduled with the tutor.

Learning to Learn Through Self-Regulated Learning

Lifelong learning, an essential skill of the twenty-first century, has been driven by the constant need for information experienced by today's professionals, enhanced by the growing access to information and communication technologies (ICT). The citizens of the twenty-first century are experiencing the transition from a training model focused on the figure of the school – and university – teacher to a flexible and autonomous model that complements the formal education established with autonomous practices.

Self-regulation of learning (SRL) is a central issue of relevance and validity in the educational sciences. The SRL was originally raised by Zimmerman [40], in 1986, within the framework of the theory of social learning proposed by Bandura [41]. The SRL process consists of the deliberate organization of cognitive, behavioral, and environmental activities that lead to success in learning. The ARA can be conceptualized as a psychological construct that refers to the process by which the student configures his activity and organizes his environment in order to achieve the objectives that are imposed, or that is imposed, in front of an academic activity, autonomously and motivated.

Its relevance is that it constitutes one of the best predictors of academic performance [42, 43]. The characteristics attributed to self-regulated persons coincide with those attributed to high-performance and high-capacity students, compared to those with low performance (or learning difficulties) who have deficits in these variables.

Exploration of the Learning to Learn

The need to establish indicators to measure the competence of learning to learn had a fundamental thrust with the creation of the CRELL (*Center for Research on Lifelong Learning*) in 2005. This center, together with the European Network of Policy Makers for the Evaluation of Education System, proposed to develop an instrument to

measure competence [44]. For this, in addition to the conceptual analysis, they considered four evaluation instruments [45]:

(a) The tests of learning to learn, from the University of Helsinki within the project *Life as learning* (LEARN) [46]
(b) The Effective Lifelong Learning Inventory-ELLI, from the University of Bristol [47]
(c) The cross-curricular skills test developed by the University of Amsterdam (cross-curricular skills test-CCST) [48]
(d) The metacognition test developed by the Autonomous University of Madrid [49]

Two of these instruments directly did not measure the competence of learning to learn but were analyzed for their great relationship with the construct.

From these studies the competence was defined, and a pre-pilot measurement test was developed [50] that was applied in several countries, including Spain [51]. In this way three large dimensions were defined: affective, cognitive, and metacognitive. Subsequently, as indicated by Muñoz-San Roque et al. [52], various Spanish authors have defined the construction of the competence learning to learn from different perspectives in order to build mechanisms that allow their evaluation. The previous authors made a selection of those dimensions that were common to all of them and that defined the perception of the competence of learning to learn from the perspective of self-regulation. From this selection a theoretical structure based on three dimensions arises: management of the learning process, self-evaluation of the process, and self-knowledge as an apprentice. They collect their exploration in a measuring instrument to know the level of development of the competence of learning to learn. This instrument is called EADCAA, the Spanish acronym for the Self-Perception Scale of the Level of Development of the Learning to Learn Competition (Escala de Autopercepción del nivel de Desarrollo de la Competencia de Aprender a Aprender). It consists of nine items grouped into the three dimensions that maintain the different conceptual nuances:

1. Learning management, four items
2. Self-evaluation of the process, two items
3. Self-knowledge as an apprentice, three items

Results

The results obtained for the first time with this tool in students of the Faculty of Medicine are presented worldwide in this publication. Seventy eight students in the first year of Medicine, Physiology subject, participated in the work, of which 19 (25%) were men and 57 (75%) women. All students enrolled (175) were informed of the purpose of the practice and were invited to fill in the questionnaires. Only students who entered their data informatically have been treated for this study.

The following table represents the averages for the three dimensions that make up the EADCAA tool. The items were valued with a Likert from 1 to 6 (Table 34.1).

As can be seen, all these means are found in decimal basis in figures greater than 7.

In order to show if any student needed special treatment, the following range was established:

- From 1,00 a 2,49
- From 2,50 a 4,49
- From 4,50 a 6,00

The values obtained in each range are presented in Table 34.2.

Table 34.1 Average values for each of the three dimensions

	Group values		
	Minimum	Mean	Maximum
Learning management	2,25	4,38	5,75
Process self-assessment	3,00	4,43	6,00
Self-knowledge as an apprentice	1,33	4,62	6.00

Table 34.2 Number of scores in the defined range

	Scores in the range of		
	1,0–2,49	2,50–4,49	4,50–6,0
Learning management	1	39	8
Process self-assessment	0	35	43
Self-knowledge as an apprentice	1	29	48

The student who presented a low score in the learning management dimension turned out to be the same with a low score in the self-knowledge dimension as an apprentice. The average was 2.11 and had low scores in all dimensions. No more students of these characteristics were found. Some cases of the following rank in any of the three dimensions presented deficit scores. For example, in two cases, learning management had scores of 2.5 and 2.6, respectively. In contrast, in the other dimensions, the view changed completely.

In order to analyze if any item had any special difficulty or was not on the same frequency as the remaining ones, the following figure was prepared, where it is stated that items 2 and 3, especially the latter, do not follow the same behavior as the rest (Fig. 34.1). It can be seen that the values of items 2 and 3 are the ones with the lowest scores. Both values correspond to learning management, and its statement is as follows:

1. I organize my study by setting realistic goals (before I begin I am able to organize the study time necessary to achieve the objectives I have set).
2. I set times for study.

These results put us in the consideration that, together, our students are good students and master learning to learn. They also specify on this competence that time management is the domain where some may have certain problems.

Finally, we must indicate that we assume as Muñoz San Roque et al. [52] that the use of these and other tools, especially those aimed at determining approaches, allows to know the dimensions and indicators that define the competence. It will or should facilitate teachers to design and use appropriate teaching methodologies for their development and evaluation according to what is marked in the course schedules.

Action for Improvement

Once the questionnaire has been completed, students analyze the global and domain scores, in the case that one is susceptible to improvement. If so, the items are analyzed in the case that one in particular deserves preferential treatment. For example: let's focus on domain 1: Learning process management. Here, the item two is: "I organize my study with realistic goals (before starting I am able to organize the study time

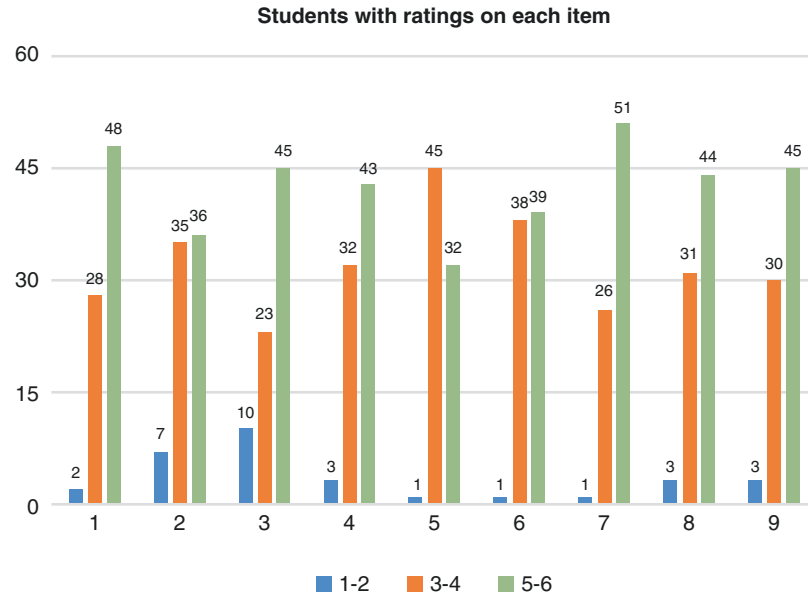

Fig. 34.1 Number of students scoring in each item

necessary to achieve the objectives I have set)". It is qualified with a one. The other three items have acceptable scores.

It would be possible to start working on this item by making aware the steps followed in an activity, for example, the study of the subjects, the times planned for each session and the times actually invested, analyzing the greatest mismatches, and, despite the success that can be achieved, if the planning has been correct. If it has not been, this planning is modeled, reflecting on the different steps, inviting the student to start the task on the same day in an appropriate manner. The process from here would be identical to that indicated above for the improvement of styles and strategies.

This concludes some of the actions that we have carried out as teachers regarding learning. We have been not renounced to teaching and aspects related to its improvement and, of course, research. To conclude, it should be noted that everything indicated here is a part of the tools and procedures that are introduced on the Internet, DEMETIC database (Spanish acronym of Law, Medicine, Education and Information and Communications Technologies (TICs)), for educational experiences.

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35 Comparative Cohort Study of Burnout Syndrome in State Schools' Teachers

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Introduction

More than 40 years have passed since Maslach [1] raised burnout syndrome as a psychopathology resulting from performing a work activity.

Along that time, there have been many contributions to that construct, conceptual adjustments, and technical changes that the same authors made. It is the case of Maslach and Leiter [2, 3] just to point out some. In this development of knowledge production, burnout suffered from different destinations, which biology (or its related studies) intended to appropriate.

The burnout association with different medical symptomatology is, at least, to an extent, the result of the different ways of trying to understand psychology, its history, and scholastic struggles.

In view of what has been said, we must indicate at the beginning that our tour about the topic, which lasted more than 10 years, will be presented with a psychosocial approach, and in that perspective, we will make some observations about the theoretical positioning we have adopted, since the several contributions that occur day by day can be capitalized into a syndrome breakthrough.

As we have said in some other publication, our journey about the topic since 2013 was covered in three different research projects rooted at the Faculty of Psychology of the National University of Rosario.

We are ending this chapter with a group of considerations which are hand in hand with our current research project planning which, fortunately, is taking a perspective at a national level.

Some General Considerations

As it is well recognized in the literature about this topic, education workers are one of the main groups at risk of the psychopathology known as burnout.

Together with health and social workers, it is one of the most studied and quoted groups at risk in specific literature. This phenomenon takes place because burnout is suffered among workers who deal with people steadily (students, colleagues, patients, customers, etc.).

This specific event (which many times is overlooked in amateur literature) leads to typifying the work environment in a precise way, having Work Overload (Overwork) as one of the main variables related to the syndrome, and introduced to account for it.

In our case, the school environment is well-known, since it has been studied and described in various opportunities in the last 50 years by psychologists, educators, anthropologists, and sociologists, among others [4, 5]. Such work

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environment descriptions have been extremely useful to describe the specific setting where we have studied burnout syndrome.

The publications of Burke and Greenglass [6], Manassero et al. [7], and Guerrero Barona and Rubio Jiménez [8] in depth emphasize labor stressors at school environments. We highlight these examples as the most complete, best achieved, and most quoted in the studies about the topic.

On the other hand, the concept of Resources (relationship Demand/Resource) quoted from the studies of Karasek [9] and Karasek and Theorell [10] are incorporated in the social psychology studies when it is attempted to specify the intervening (or modulating) variables in the different work environments.

To the previously highlighted variables (Work Overload and Demand/Resources), we must add the variables of Social Support, Role Conflict, and Locus of Control. These variables synthetized well-known models in studies about Work Stress, which nowadays still maintain full validity and which several authors about burnout syndrome incorporate in their studies.

These fundamental variables in field studies of social psychology are rarely studied or taken into account, thus weakening possible links and reinforcements between these qualitative and quantitative strategies.

Separately, a three-factor analysis of burnout assessed with Maslach Burnout Inventory (MBI) has been discussed very few times. This fact, which can be taken into account as something positive or negative when it is intended to make an assessment of the syndrome development, encouraged the MBI (in its different versions) to be the almost exclusive method to make studies and evaluations of burnout syndrome.

The result that we can recognize today (and delimit) from burnout is due to the fact that the burnout definition and what the MBI measures have become a kind of revolving door, where the MBI measures burnout and burnout is what measures MBI.

Apart from the tautological fact and the rest, burnout syndrome research has not stopped. Rather, it seems that the fact that burnout is measured by MBI made (and causes) researchers to have a precise tool to start new research.

With this theoretical conceptual and historical framework as scaffolding (presented here in a brief way), our project is framed within social psychology, which we have pointed to in many opportunities by our workgroup [11–13], among others, since we basically give credit to theories which pose the individual as the center of a multiplicity of social crossings.

Since the beginning of our research work, the references which did more justice to the field observations were theories related to social constructions. Thus, for example, the works within the theories of social exchange show that the individuals most identified with the work group and organization are less affected by stress. The achieved identification is directly related to a person's construction of identity (shared social identity). As a result the person has a higher reception of social support, and, in addition, they are less affected by work stressors. In this sense, social support has a fundamental role to prevent work stressors as pointed out.

The studies of Ashforth and Mael [14] and Lee and Ashforth [15] hold that the variable known as Emotional Fatigue influences and conditions negatively Depersonalization and Low Work and Personal Performance. Thus, the three dimensions assessed in the syndrome remain involved.

In this theoretical perspective, we also find the works of Schaufeli and Dierendonck [16], Dierendonck, Schaufeli, and Sixma [17], and Schaufeli and Enzmann [18], which deepen the approach, pointing out as main issue the social and organizational identification and identity which the worker can build with that basis. It is a fundamental element from which they can perceive the received social support and in this way obtain resources to avoid the syndrome.

As we advanced in the different projects along the last decade, our vision of the field had to be adjusted. The experience of transiting different organizations led us to rethink burnout like a result of the organization of the work within the organizations, of the ways of managing, the definition of roles and responsibilities, and the possibility (sometimes remote or idealistic) that

workers should participate in the decisions of the tasks involving them.

Work Overload and Role Conflict as main stressors highlighted along the interviews in the different schools gave us the sign to think about how the organizational bureaucratic system works in the group of education workers. It allows also to trace relevant bibliography. Of course, the theory of Demand/Resource at work [19, 20] was also useful when the time came to determine what we wanted to go in depth in our view upon burnout.

Although burnout can be defined as an individual phenomenon as pointed out by Maslach et al. [21] and Halbesleben and Buckley [22], some researchers observe that it can be either an individual or group phenomenon [23] and that it is produced in a specific work environment, with a particular way of working and in an organization model.

Group, work team, organization model, and work environment or atmosphere make a group of several studies that along time assembled and made burnout studies progress, redefining or enlarging the vision that we had about it. As it may be imagined, each of these observations gave place to a new conceptualization and at the same time a lot of discussions. In this way we are conducting our reasoning.

The first is a difference between work team and group as understood by Jaques [24], just to mention one of the most well-known theorists. In this form the studies of work in groups is located in the center, and it constitutes the place where depressive and paranoid anxieties come into play.

In this sense, we cannot ignore the existence of different levels of personal commitment which originate in a work team and in a group, even in a group within an organization.

When we talk about topics which imply sensitive levels of individuals' lives, we include empathy, trust, cooperation, and solidarity, among others. They have been related topics of the group studies in social psychology for decades. They have not been incorporated with the necessary importance. Due to this reason, it is not strange, as we will see soon, that many of these topics were incorporated in one or other way within the AWS. In addition, regarding to these distinctions' approach, we want to highlight a concept we have been using since the use of AWS [25]: the idea of justice within the organizations.

This idea of justice makes the work group or team solid, helping to create supportive trustworthy bonds, which are necessary to sustain the resources on a daily basis. Interaction and communication among work partners is more honest when the sense of justice is steady in the group and in some way all (or at least most) individuals feel that the participants in the same topic or situation enjoy equal conditions and opportunities.

Justice was associated to burnout in several works that look for some positive association. In this respect the work of Moliner et al. [26] considers that the role of justice in the work atmosphere is fundamental, and it is directly associated with low burnout. The way to approach the issues, the way of making decisions, and the confidence in the supervisor are the main points to assess when it comes to justice in a labor research on burnout syndrome.

On the other hand, and as counterpart, competition in work teams has a high impact with burnout measurements. In this sense, we can see how competition generates anxiety and the worker becomes defensive. As a consequence, little collaboration may be observed, not only with the team but also with the organization. On the other hand, many organizations manage the meeting points for the workers, thus trying to dissipate negative feelings generated by the competition. So, this individual energy generated by competition is neutralized.

The decrease of competition and anxiety generated by this procedure has the result of a decrease in the two main burnout variables (Emotional Fatigue and Cynicism). It generates a higher group participation, an honest disclosure of feelings of the team participants with the other members, and a channel to help to make decisions at an organizational level. The works of Le Blanc et al. [27], Bono et al. [28], and Halbesleben et al. [29], among others, take this approach.

As we have said in our earlier works that the idea of Value is the central concept of AWS. It is also the concept that we find more often in

interviews, since the ideas and feelings of the individual's history are synthesized in it, with the values which must come into play in the several situations of work dynamics.

On their behalf, the works of Golembiewski, Munzenrider, and Carter [30] highlight and describe the work process, dynamics in which the teacher starts losing enthusiasm and commitment with the task they had when they started to work. Work tensions and stress provoke an answer which can be understood as a first opportunity for the arrival of burnout syndrome.

Authors go a little beyond that and point out that the first dimension affected is Depersonalization. When the worker begins to feel the weight of his job, he responds by adopting cynical and distant attitudes with students and colleagues, moving on to a second stage where the worker, overwhelmed by work, is overloaded. In this condition, he perceives the Role Impoverishment and begins to accuse ideas of Low Personal Performance and, in the long term, Emotional Fatigue.

The feeling of loss of Self-control and Autonomy deteriorates their image as a worker and many other times as a person. Fatigue, tiredness (physical), and irritability take place more frequently.

Among the differences we want to highlight is research framed in the line of psychology known as Positive Psychology. As said by Duran et al. [31, 32], this new line stresses and opposes Burnout to Engagement. Such author includes vital and work satisfaction, as measures of general well-being and well-being in the context of work. On our part, we intend to include the relationships produced within and out of the work field, and the possibility of using peer and reference groups, as a way of performing a more complete and comprehensive diagnosis assessment.

Our Approach, Sample, and Analysis

We made the descriptive analysis based on nine cohorts which comprise from the year 2008 to 2016. Each cohort is made up of different schools from different areas of Rosario City. In all of them, we have used the MBI-ES as a tool for epidemiological and approximation assessment to the conundrum in the different tested groups, with a total of 904 valid questionnaires (and with a rate of average answer of 79%).

We accompanied the year segmentations with in-depth interviews in the different education centers, as the main tool to collect data for qualitative assessment.

The most relevant results tell us that the average age of the tested teachers is 42 years old. A little more than 86% are women, 75% has regular couple, and 19% does not have a couple.
The average of work experience is more than 16 years and an average of a little more than 10 years working in the same institution and/or in the same place of work. Seventy-seven percent of the teachers have a regular job, 38% work in the morning shift, almost 30% in the afternoon shift, and a group of almost 24% has double shift.

The average of the three dimensions of burnout syndrome are the following: Emotional Fatigue is 2.5%, Depersonalization is 1.16%, and Low Personal Performance is 3.78%.

In the analysis of reliability according to scores of Cronbach's alpha, the following results have been obtained: 0.864 for Emotional Fatigue, 0.602 in Depersonalization, and 0.774 in the Low Performance scale.

Table 35.1 shows the behavior of the three main dimensions of burnout syndrome according to the measurements obtained along the nine cohorts.

Results of the reliability tests done with Cronbach's alpha are shown in Tables 35.2, 35.3, 35.4, 35.5, 35.6, and 35.7.

Qualitative Analysis

The qualitative analysis of data was performed in three simultaneous ways. On the one hand, firstly we took into account the variables of Areas of Worklife Survey of Leiter and Maslach [25]. Secondly, on the other hand, we made an analysis of content. Thirdly and finally we traced hints that take us to the origin of the syndrome and a

Table 35.1 Behavior of the three main dimensions of burnout syndrome according to the measurements obtained along the nine cohorts

	Emotional Fatigue	Depersonalization	Low fulfillment
2008 ($N = 95$)	2.7	1.95	3.65
2009 ($N = 103$)	2.61	1.76	3.79
2010 ($N = 94$)	2.48	1.71	3.75
2011 ($N = 101$)	2.73	1.76	3.53
2012 ($N = 127$)	2.28	1.46	3.84
2013 ($N = 163$)	2.34	1.44	3.92
2014 ($N = 106$)	2.55	1.57	3.79
2015 ($N = 74$)	2.39	1.45	3.93
2016 ($N = 41$)	2.61	1.37	3.83
TOTAL ($N = 904$)	2.5	1.61	3.78

Table 35.2 Emotional Fatigue: reliability statistics

Cronbach's alpha	Cronbach's alpha based on standardized elements	Number of elements
.864	.867	9

Table 35.3 Emotional Fatigue: statistics of summary element

	Average	Minimum	Maximum	Range	Maximum/ minimum	Variance	Number of elements
Averages of element	2505	1655	3325	1670	2009	.278	9
Variance of element	1078	.846	1537	.690	1816	.049	9
Correlations among elements	.421	.217	.619	.402	2857	.009	9

Table 35.4 Depersonalization: reliability statistics

Cronbach's alpha	Cronbach's alpha based on standardized elements	Number of elements
.602	.623	5

Table 35.5 Depersonalization: statistics of summary element

	Average	Minimum	Maximum	Range	Maximum/ minimum	Variance	Number of elements
Averages of element	1610	1280	1759	.479	1374	.037	5
Variance of element	.896	.352	1423	1071	4039	.158	5
Correlations among elements	.249	.125	.543	.418	4346	.013	5

Table 35.6 Low Personal Performance: reliability statistics

Cronbach's alpha	Cronbach's alpha based on standardized elements	Number of elements
.774	.773	8

Table 35.7 Low Personal Performance: statistics of summary element

	Average	Minimum	Maximum	Range	Maximum/ minimum	Variance	Number of elements
Averages of element	3789	3662	3998	.336	1092	.015	8
Variance of element	.647	.463	.779	.316	1682	.011	8
Correlations among elements	.298	.133	.438	.305	3292	.005	8

possible relationship with diseases known as psychological (basically anxiety and depression).

Following the AWS in each of its six areas, we will show some contents as a way of example according to the correspondent area.

Work Overload is present in almost every interview, in phrases such as:

– *Every time we have more and more things to do and we don't have enough time.*

Or:

– *With the time it became complicated, I had to go back to study to be updated.*

These expressions are the most characteristic.

Lack of control could be observed in more than the half of the interviews clearly, in expressions like:

– *The head teacher says and leaves.*
– *We are told that supervision is going to come but not when.*

The insufficient reward relates basically to the *low received payment (salary)* for the work performed (as effort), a constant complaint which involves the heads of institutions. Also in this respect some teachers said that they had to spend part of their income on material for the students, to organize some activity in class.

When we analyzed social bonds, the analysis was very dissimilar, since it included lack of time for the family or the children and *feeling tired (or not in the mood) to leave.* In the analysis of the material, this appears repeatedly as claim of the other, which is interpreted as fatigue or lack of interest. In some cases it was speculated that this fact can bring them consequences in short or medium term. In this way the couple relationship was the most quoted.

The lack of equity was present at interviews in a diffuse way. In some cases we found comments such as *we are all similar, but..,* (ironically). In this point the most relevant accusations aimed at the possibility of training, time, duration, and distance from which courses are offered.

On the other hand, the conflict of values was reflected in the part-time staff at work, since discontent and the consequent reconsideration of how to continue was a topic they frequently mentioned. Regarding this last area of analysis, which is the most important for us, we refer to the work of Leiter and Shaughnessy in 2006 [33]. In this study they posit that the two main links in Work Overload are in Control and Value. The variable of Work Overload acts directly on Emotional Fatigue.

At the end of the work environment analysis process, the variable Value acquires a fundamental importance, since it is the last frontier that the work overload demand must overcome to reach the burnout syndrome. Although the quoted work was performed on nurses, we must get ready to analyze this hypothesis of work, basically if we think that workers with a type of idealist personality have in their value scale (Value) a source of support and an (existential) basis in their lives.

Some common denominators were observed along the interviews; these could be synthesized in three fundamental axes.

The first axis highlights the way in which work has increased in the last years, not only in quantity but also in complexity and in a good number of interviews (in different ways). This comment was followed by a complaint (and/or discomfort) because of the lack of possibilities to accompany this complexity, lack of specific training proposals, and (in some cases) disparate interest in the education community. Lack of interest may be observed in some occasions. It may be accompanied by discomforts or inconveniences even expressions of annoyance.

The second common axis is centered on a lack of health prevention and care of workers in general. It may be perceived as coming from the institution, competent authorities, and users of the service. In this respect, episodes of violence of all kinds are highlighted. A general acceptance and the fact that they may be seen as natural contribute in this way. Some interviews manifest this situation (lack of care and protection of the workers at work) basically from the authorities and people in charge of the institution. An attitude of

resignation and acceptance of the different violent episodes that occur in the place of work, as well as an intention of forget them, completes this vision.

The third axis refers to the poor training received to handle situations related to the affective realm, which necessarily comes into play when working with people. This issue is directly related to the dimension of Emotional Fatigue and must be mostly emphasized when designing curriculums and planning of education workers' health prevention programs. Many interviewees relate this fact to the poor practice obtained at the teaching training to obtain the degree. It prepares them for professional exercise, but very little to deal with situations that have scarcely to do with education practice. In that way they end up spending their time in a practice that has a lot to do with social assistance and solidarity. When we point to Work Overload as well as the relationship between Demand and Resources, we clearly highlight these points.

Two general observations emerged when relating field observations, informal conversations, and certain information that was made public through the media and also when examining interview material.

The first is the role played by the organizational structure and its overall performance, as well as the context in which burnout is developed. This appeared in interviews conducted in several ways. In general, the interviewees referred to the self-perception of the performance of roles within the organization. In this sense, it is reluctantly accepted that the organization does not appreciate or value all the work done by the teacher, although very few point to the institution as direct responsible, or its structure and operation. They see the institution as another victim of the economic, political, and social situation we are going through.

The second, on the other hand, arises from the analysis of the interviews as a whole. In them it was not observed that workers give fundamental importance to the work process or to the task they perform as conditions for the development of burnout syndrome. We should clarify that most of them seem to have no more than vague ideas about burnout syndrome. Many times this syndrome was confused with work stress. For this reason, in some interviews the idea of work stress came into play, relating it to different attitudes and symptoms such as sleep and digestive disorders. They can also fall into self-medication and, eventually, the consumption of coffee, mate, and cigarettes, irritation or mood swings, and problems in the couple or family.

The possibility of relating the work problem with the symptoms found in the various groups of teachers was interpreted as an advance before the individual or collective defense mechanisms described by Dejours [34] and the lack of information about the syndrome. In many interviews it was noted that the staff handles the forms and the way to access the so-called absence medical leave or sick leave, which does not appeal to them, and in a general and very vague sense, they see it as a personal weakness. With few exceptions it is not an option that they consider valuable.

Pending Lines

In the following way, we are going to point out some problems that can be addressed through the studies we have been carrying out and the possibility of generalizing and socializing them.

In the methodological order, one of the big problems is the comparison between them. Comparison of quantitative studies and the application of the MBI lead to difficulties. The Likert scales used are not always the same. It is a fact that each researcher opts for one in particular. The comparison of results produced by the same tool, but with different Likert scales, produces a variation of the results, making difficult the comparison.

In this sense, some authors propose the numerical scale as semantic differential. The difficulties pointed out by the researchers about the possibility of making a theoretical proposal about burnout syndrome are pending (among others), before the inventory (MBI) crushed the definition and, with that, the possibilities of new theoretical proposals.

As a consequence of this (and other factors, no doubt), many authors prefer the pointed path. Salanova et al. [35], among others, opposes Burnout to Engagement, leaving aside the theoretical debate about the syndrome. It may be observed in Schaufeli et al. [36].

On the other hand, some researchers developed their own tools, a kind of reaffirmation of the syndrome as such, which includes (and expands) their vision, through new variables that come into play in the situation of the worker at work. In this sense and referring to researchers about burnout syndrome in our language, we cannot avoid pointing out Gil-Monte [37, 38], on the one hand, and Moreno-Jimenez, on the other [39].

Other point to be considered is that we cannot avoid to separate the psychological markers from the biological ones on a more analytical than practical basis. Although it is recognized that it is very difficult to direct the studies through a purely psychosocial vision, we cannot fail to notice that many studies end up being a psychological attachment to the biological vision. In those terms they are not taken advantage of a possible deepened study from a psychological perspective. It has much to explore and contribute to the construct. We even dare to speculate that burnout is a psychological syndrome (alteration of the perception of emotions) that has little (or nothing) to do with the medical or biological symptomatology.

Undoubtedly, the aforementioned leads us to pay attention to two important points in burnout studies. One of those points focuses on the clinical aspects of the syndrome, the differences raised (basically through the theory) in terms of the causes and the development of the disease. A great deal of research can be pointed that relates the syndrome with the Big Five Personality Theory. In this regard we highlight the relationship between the basic types with different assessments of (and according to) the labor stressors.

On its part the qualitative analysis of interviews leads us to be able to describe and understand better the interfaces between stressors and the different dimensions of the syndrome's symptoms. In this regard we allow ourselves to use the AWS tool to frame the semantic analysis but not fall into the exclusive quantification of the field, because we believe that at the beginning (at least in this exploratory stage of the syndrome) it reduces vision and prevents the appearance of new contents. The offshore and intercultural developments can be an opportunity to deepen the field.

Our study perspective which will allow to address burnout syndrome through social psychology is a construction process which addresses not only theory but also tools, and in that relationship we focus our work of more than 10 years. We resume and reflect on this first observation where the workers (who suffer from burnout) are in contact with other people, and this immediately led us to think that teachers spend more than 5 daily hours with students and that this contact is mandatory.

This forced and sustained contact exhausts and depletes the workers' resources after a while, and in this repeated situation of dismantling of resources is where the feelings (emotions) arise on the surface and when the time of vulnerability appears.

Similarly to other occupational pathologies already studied (or that continue being studied), the group is the main individual worker's support. The group is the mattress where the teacher's dreams and desires and the institutional rules and obligations are. The group (the groups, both the formal and the informal) is the best health ally and is the great absent in the mental health studies in general and burnout in particular.

The group (the collective) is one of the central aspects when training courses are held, when organization's leaders are prepared, when we talk about motivation, and so on, in all the relevant topics of the organizational field but absent (without prior notice) when studying workers' health.

It is remarkable when health is discussed, an evaluation of a psychological type is performed, but the group factor does not seem to gain that fundamental role. In fact, the importance of groups is almost null when mental health evaluations are performed in general.

Managing emotions (anxieties, fears, sadness, happiness, rage, wrath, anger, helplessness,

angst, etc.) put into play in the work environment appears as individual exits (if they are not individual markers of possible pathologies) and never (not even as mitigating) of the specific group space (and not organizational) where it occurs.

In this precise respect, our studies aim to differentiate peer and reference groups both in the work environment and in the worker's life project and thereby obtain salutogenic health markers for a possible (future) intervention.

Provisional Conclusions

The discoveries have a clear effect on the evaluated group and can contribute to generate changes to solve the problems studied above. They allow for developing mappings which enable the implementation of prevention programs on the target population, considering that the work with identity and reference groups allows to transcend the simple work project, by including it in a life project based on knowledge of health markers.

As mentioned earlier, intervention on this risk group allows improving in the working health conditions and moving toward proactive life projects.

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36 Traumatic Situations and Mental Disorders in Migrants, Refugees and Asylum Seekers

Raúl Gagliardi

Introduction

"My son wakes up afraid in the middle of the night. He wakes up screaming. This is how children have been affected. He has bad dreams and wakes up crying and sometimes runs out onto the street. He has nightmares because of the war and air bombardment. Because of fear. A child was slaughtered in front of him, so he started to dream that someone is coming to slaughter him. When a child witnesses a beheading, how could he not get afraid?" A father in Syria.

"The children are psychologically crushed and tired. When we do activities like singing with them, they don't respond at all. They don't laugh like they would normally. They draw images of children being butchered in the war, or tanks, or the siege and the lack of food". A teacher in the besieged town of Madaya in Syria.

At least 3 million Syrian children under the age of 6 know nothing but war, and millions more have grown up in fear under the shadow of conflict [1].

Mental health problems exist in all populations. Some people are particularly vulnerable to them, for example, those who live in war zones, in dictatorships or suffered from environmental disasters or great famines, those who live in refugee camps and, in general, migrants who are not accepted in the country in which they live. Children and adolescents are particularly vulnerable to the mentioned traumatic situations.

The mentioned groups frequently have difficulties of access to mental health systems, either because these system do not exist, as in many refugee camps, or in urban townships, or because they exist but migrants do not have access to them for lack of information about their location and access mechanisms, for economic problems or for legal problems, when migrants are illegal and do not want to appear in public institutions for fear of being arrested. Other frequent problems are related to communication difficulties with the staff of mental health centres. To the communication difficulties that local patients have, migrants have problems derived from cultural differences, language or conceptions about mental health and the taboos and stigmas attached to them.

There are hundreds of millions of migrants around the world and the number is augmenting. Millions of people from Africa, Asia, the Caribbean Region, Latin America and Europe migrate looking for better living opportunities in Europe, Canada, Australia, Latin American countries or the USA. Hundreds of thousands of Central American and Mexican people migrate to the USA, where many of them live clandestinely. The number of international migrants grows to 49 per cent, from 173 million to 258 million persons from 2000 to 2017 [2]. According

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to the International Organization for Migration, over one million migrants and refugee arrived in Europe through irregular means in 2015, which is the biggest wave of mass migration since the aftermath of the Second World War [3]. Hundreds of millions of people undertake internal migrations, in particular from rural areas to urban areas. In 2012 the number of internal migrants was estimated to be 763 million [4].

The large number of migrants, refugees and asylum seekers in particular in the refugee camps and in townships and the traumatic situations that these people experienced imply a high concentration of people with mental health problems in places where mental health services are crowded or directly do not exist. To solve this problem, a community approach to mental health may be useful in order to inform patients, their families and the community about the most frequent mental illnesses, the symptoms they present and the ways to reduce their impact. This approach should be based in quality researches with the participation, when it is possible, of members of migrant communities in order to ensure the knowledge about the cultural, social and economic issues related with mental health and apply it to diagnosis and treatment of mental diseases [5].

Migration and Health Problems

Migrants can have different health problems than those of the local population. Some frequent diseases in the country of origin and in the countries where they travel are not common in the host country. Newly arrived migrants and refugees can have communicable and non-communicable diseases, such as gastrointestinal and dermatologic infections, respiratory infections, chronic conditions and mental problems. Frequently they have vaccine-preventable diseases [6]. Many authors state that the migrant population is more vulnerable to health issues, especially psychological disorders, along with a higher level of anxiety or greater pessimism about the future, often caused and/or compounded by the loss of one's social support network and isolation due to lack of knowledge of the characteristics of the host countries [7].

In some countries migrants do not have free access to public health system. Frequently they do not have the economic resources for private health care. They have lower rates of health insurance and use less health-care services and receive lower quality of care than local population.

Migration and Stress

Migration can produce different results. It can determine an improvement of economic situation and living conditions. However, in many cases migrants suffer from situations that can expose them to stressful conditions and increase the risk of health problems including mental disorders [8].

Stress is a complex process produced by some factors (stressors) to which the organism must adapt. The responses can provoke changes in the physiological mechanisms as well as in thoughts, feelings and behaviour. If the capacity of the organism is not sufficient for adapt to the stressful condition, a mental disorder can prevail [9]. Stressful situations can have a direct impact on health (e.g. provoking heart and vascular diseases, diabetes 2, etc.) and produce mental disorders, such as depression and post-traumatic stress disorder (PTSD), and augment the risk of suicide [8].

For migrants, the stressful situations can occur before the migration, during the travel and after their arrival to the host country.

Traumatic Situations During the Migration

Many people suffer from traumatic situations during the migration, travelling the sea in small and crowded boats, walking hundreds of kilometres, travelling desert regions in harsh conditions, having the risks of being kidnaped, suffering from sexual abuse and being transformed as slaves. Thousands of migrants drown in the Mediterranean Sea before arriving to European seasides. While this article was being written,

117 migrants travelling in a small boat drowned in the Mediterranean.

Internal migrants should often do long and harsh travel to more safety regions. Frequently they lost everything and should live in refugee camps.

Traumatic Situations in the Host Country

Migrants, in particular illegal migrants, refugees and asylum seekers, often suffer from traumatic situations after their arrival in the host country, when they are not accepted or when they do not have the capacity to adapt to the new situation. They suffer from stigma and marginalization, are discriminated in jobs or have difficulties on having a good housing. They often have low-qualified and low-pay jobs or should undertake informal economic activities. The stressful situations can include urban violence, racial discrimination, detention or reclusion, abuse by law enforcement officers, forced removal or separation from their families and deportation.

Many refugees and asylum seekers live without privacy in overcrowded townships or in tents in refugee camps. Often they suffer from sexual, psychological and physical abuses, in particular children and women. Children and adolescents frequently have not access to school and their future is uncertain. These problems reduce the possibilities to undertake better economic activities, in a vicious circle that maintains poverty and unhealthy living conditions. Difficulties for accessing the health system augment the frequency and relative importance of these problems.

Adaptation to the New Conditions in the Host Country

The adaptation to the new living conditions is a difficult process for migrants, in particular for asylum seekers and refugees. Different factors determine the vulnerability of these populations: different culture, low socioeconomic status and lack of legal status, low proficiency in the local dominant language, bad habitat's characteristics, lack of possibilities for employment, social marginalization, problems with health insurance, lack of knowledge about the characteristics of the local health system and low capacity to access to it, difficulties in the communication with health staff, etc. Young refugees are particularly vulnerable to these kinds of difficulties that can provoke mental problems in them, as well as also problems in physical and psychological development [10].

Migration can provoke a rupture of the communication with the original community. Migrants frequently do not have the framework of support that they had in their original country. This rupture can have negative effects at psychological level, such as loss of identity, reinforcement of a "mythical" identity, low self-esteem, frustration, depression, reject to the culture and the characteristics of the host country. These phenomena are more frequent for women.

The isolation of migrants from the host society, in particular the isolation of migrant women, is a source of problems such as lack of capacity for access to the health system and communication problems with the health staff.

All these factors are present in different degrees and have different impacts on migrants, refugees and asylum seekers. Understanding these factors and having the capacity to deal with them are very important for social workers and health staff in order to help solve the mental health of migrants and improve their capacities for adaptation to their new conditions of life.

One of the biggest problems related to migration and stress is chronicity. In the majority of case, migrants cannot change the stressful situations. In other words, the stress is continuous until they change their living conditions [11, 12].

There are asylum seekers that should wait for years until they obtain apolitical asylum or, with much more frequency, are expulsed from the country. There are also migrants that do not have legal status in the host country and live with the anxiety to being sent to their original countries [13, 14].

Legal status is a major determinant for illegal migrants, refugees and asylum seekers to

have access to social services and health care. Problems in legal status can provoke anxiety, low self-esteem and depression. Children are particularly vulnerable to this stressful situation.

Taboos and Stigma About Mental Health

Attitudes about the mental health of migrants and members of marginalized communities are heavily influenced by their culture. Mental health practitioners must take this phenomenon into account to avoid making mistakes in the diagnosis and treatment.

Mental health is a persistent stigma in many communities around the world. One of the biggest challenges that mental health workers face is to change negative attitudes related to mental issues of individuals and communities. Frequently in immigrant and refugee families, it is taboo to discuss mental health problems because their culture indicates that having such problems is "being crazy". That causes great difficulties in the search for treatment because families are afraid of the shame of having a crazy relative [15].

In the County of Orange, California, USA, experience of practitioners in mental health indicates that there exist several mental health stigmas in Korean, Vietnamese, Latino, Arab, Persian and Chinese communities. A study done in Vietnamese and Korean communities in the County indicates how frequent the families felt the pressure for keep up appearances. This attitude can be an explanation for the stigma on mental health issues. In the Arab community, the stigma is presented in language, because mental illness is descripted using a despicative word, equivalent to "crazy". The analysis of the frequency of people asking for care in mental health centres shows that in the Arab community those who ask for help are new becomers with PTSD symptoms coming from countries in war, such as Syria, Iraq and Afghanistan [16].

In Syria it was found that one in four children has mental health problems. However caregivers frequently do not seek help for them because mental health issues are taboo in their culture. Talking openly frequently about these issues caused embarrassment and suffering in children who had mental problems. This situation improved during the war and there is greater acceptance of children and youth to seek help. Psychosocial activities such as art and drama are the most accepted. A psychosocial assistance center was recognized as very efficient to achieve attitude changes and seek help. However, the negative attitudes and the associated stigma are still a great problem to be solved. In some areas of Syria, the clinics do not publicize their work in mental illnesses, and the possible patients are given word of mouth. Older boys and young men are those who have more resistance to seek help. They are pressured by their family to mature and fix their affairs alone as men. This situation causes an increase in the aggression of those who have mental health problems and do not ask for help.

Cultural Consonance

The adaptation of migrants to the host society implies frequent cultural changes. The theory of "cultural consonance" can provide elements for understanding the impacts of the cultural changes in mental health. Culture is, in this theory, considered as a series of cognitive models that are shared by the members of a social group and that describe the different domains of the life of that group. The aggregation properties that culture determines in a group derive from sharing those models.

Each individual has his own representations of the world that correspond incompletely to the shared cultural model. That correspondence is called the "cultural competence". The relationship between cultural competence and psychological problems is not clear.

On the other hand, each individual can put into practice in his own life the cultural models of his society that he accepts as his own. That is to say that there is a greater or lesser "cultural consonance" between what an individual does and what the culture of his social group

indicates. Cultural consonance is the degree to which each individual approaches, according to their own behaviours and beliefs, the behaviours and beliefs that are encoded in the cultural models to which they subscribe. Low cultural consonance is related to psychological problems [17].

Some researches indicated that there are associations between cultural consonance and some physiological characteristics of an individual, such as arterial blood pressure. There are also association with morphological characteristics, such as body mass [18]. A significant interaction between cultural consonance and a genetic polymorphism for the serotonin 2A receptor in the prediction of depressive symptoms has also been shown. These results suggest the existence of different pathways between the symbolic elements, the culture and the molecular ones, such as the metabolic and physiological results of a genetic polymorphism [19].

A migrant suffers from changes in the social environment in which he lives and his cultural consonance may be low, which, according to the indicated research, can induce health problems and, in particular, depressive symptoms.

Migration and Mourning

Some authors consider that migration is similar to mourning, because the migrant moves away from his loved ones and the conditions that he had in his original country (culture, social status, language, etc.) [8].

Three types of mourning during migration were proposed: the "simple mourning", when the migrant has good conditions in the host country, the "complicate mourning" when the migrant suffer from serious difficulties and the "extreme mourning", when the migrant cannot deal with the conditions of life and cannot adapt to the new situations. In the last type of mourning, the migrant suffers from a chronic and multiple stress that is called "Ulysses syndrome", following the myth of Ulysses. The manifestations of extreme mourning can be very serious, such as depression, anxiety, health problems related with somatization and cognitive problems such as temporal and physical disorientation, memory deficit and attention deficit [20].

Stress and Acculturation

In a fast changing society, as modern society, any individual needs to adapt fast to new situations, such as changes in job market characteristics, language, culture, technology, economy, political situation, etc. In many cases the process of adaptation is smooth and unconscious. However there are individuals that do not have the capacity to adapt their behaviour to some of these changes in the society. The problems in adapting to new conditions are an important cause of mental problems, such as depression and anxiety.

The changes in the social and economic environment for migrants can determine a process of "acculturation" that implies many changes in their lives, including cultural and ethnic identity, language, attitudes and values, social relations, gender roles, roles in the family, eating patterns, artistic expressions, communication rules, etc. Acculturation can provoke family conflict, intergenerational conflict, communication difficulties, solitude and isolation. All these situations can provoke health problems, including mental problems [21]. In some cases the process of acculturation can stimulate alcohol consumption [22].

Acculturation is a conflict between loyalty to the culture of origin and to the new culture. It implies for migrants the coexistence of different cultures, the culture of the host society and the culture of their original society. This coexistence is expressed in many ways, depending of the specific situations. For example, migrants can accept and enjoy local food but eat the same food as in its original country at home. They can speak the local language at work or in public places but talk in his original language at home or with other migrants from the same country [21]. The process of acculturation can have some steps, for example, migrants learn the local language and later start to change their behaviour adopting the local behavioural rules.

The process of acculturation is more difficult for the migrants that have little contact with local

population, such as women from countries where there are dominance of husbands, fathers or brothers over women and where women only communicate with their own family or with friends of the same origin. In Geneva the communication with migrants suggests that the difference in the capacity to speak the local language between men and women in the same family is a signal of this kind of situations. Frequently these women do not work or have low-pay jobs, such as domestic employees, and they have not the opportunity to speak the local language. Depression, low self-esteem and anxiety are frequent in these women isolated from the local community.

Dressing is another signal of difficulties for adapting to the local culture. For example, migrant women in Europe that dress in traditional Muslim religious way, such as long dress and chador, are immediately identified as having a different culture and religion than the local ones. For adolescents and young women, dressing in a religious way provokes problems in their education, when the use of chador is interdict in public schools, as in France. This situation becomes a political issue in some European countries, with big discussion between those that support the laic education without religious symbols and those that defend the right to use them in schools.

Usually men from religious or traditional cultures dress in the same way as the local population. However in some cases they show some signs of religious identification, such as a long beard, a cap and a long dress.

In the process of acculturation, there are two elements, the migrants' will for integrating the local society and the will of local society for accepting migrants. When migrants and local society have both a positive will, the integration is relatively easy. Migrants become part of the country, and the first and next generations are citizen with similar attitudes and values with the local society. In some countries, such as Argentina, second-generation or third-generation migrants speak fluently the local language and consider themselves as citizens of the country.

More conflicts appear when the local society rejects migrants from some ethnic groups, such as, in some European countries, Arabic and South Saharan African or some religious groups such as Muslim or Hebrews. Racism and xenophobia are factors that reduce the possibility of integration and determine the marginalization of migrants.

The "phantom" of the invasion of Europe by Muslim is one argument that is used by far right parties in elections at national level or for the European Parliament. In the same countries, this kind of argument allows these parties to win the national elections. Probably there is a big psychological impact in migrants of these political issues, in particular in asylum seekers that see reduced possibilities to be granted by the political asylum.

There are four possible situations in the process of acculturation: integration, assimilation, separation and marginalization [23]. In the first situation, integration, there is a coexistence of local culture and original culture. Migrants acquired new elements from the local culture but maintain other elements of their original one. The coexistence of both cultures can only be developed in multicultural societies that accept cultural diversity have low level of racism, xenophobia, ethnocentrism and discrimination.

Assimilation means important changes in migrants' culture and values, from the original ones to those of the local society. Migrants want to become part of the local society and be accepted by it.

In the situation of separation, migrants maintain their culture and values and refuse to integrate to the host society.

Marginalization is a situation where migrants do not maintain their own culture and do not acquire the local one. Migrants are separate from local population and there is diffidence between both groups. In this situation, migrants frequently suffer from a sense of alienation and a high level of anxiety. Marginalization can persist for a long time and be present in future generations of migrants. This is a very bad problem for young

migrants from the first or successive generation because they do not have a "referential culture" for constructing their own identity.

Stress during the process of acculturation can cause or reinforce post-traumatic stress disorders (PTSD), anxiety and depression [24].

Temporal Organization and Planning

An important element for adaptation to the new society is adaptation to its temporal organization that is linked to the perception of time as well as perception of planning, organization and achievement of activities. Temporal organization is linked to psychological structures internalized in the first years of life and is essential in the establishment of relationships between the child and the society [25]. Temporal organization is an important issue for preventing diseases, having healthy behaviour and adhering to treatments. It is also an important issue for many jobs where doing the activities in time is important.

Edward Hall, who analysed communication problems between cultures, discusses two main cultural systems: one of them is called "monochronic" system, present in developed societies, with a rigid temporal organization, in which "one thing is done at a time". In that type of societies, great importance is given to time (time is money). The perception of time is precise and the activities are organized using precise temporal measures (days, hours, minutes, etc.). In monochronic societies, meeting the proposed activities is more important than meeting social demands. Planning and complying with the proposed plans is very important. The use of new technologies is also favoured. The advantage of monochronic societies is efficiency.

The other temporal system proposed by Edward Hall is called polychronic temporal system, a characteristic of societies in which more importance is given to social and family relations than to the fulfilment of activities. This system is a characteristic of very convivial societies in which it is accepted to perform several tasks simultaneously and time is not a value in itself. The advantage of polychronic societies is the development of social networks and protective families [26, 27]. Another advantage is creativity and the capacity to deal with the lack of resources for solving problems ("system D"). An example of a polychronic society organization is the "big family" characteristic of many African ethnic groups.

Following the analysis of Oscar Lewis about the population that lives in extreme poverty [28], I will also propose another kind of temporal organization, the "survival temporal organization". The migrants who suffered from traumatic situations in their original country or during the travel could develop a survival strategy, which focuses on solving the most urgent issues. The survival strategy has a temporal organization centred in the present day that allows to solve immediate problems but can provoke difficulties in planning and undertaking long-term activities. Living on a day-to-day basis, solving immediate problems and not losing time planning for future conditions that they do not know are survival strategies also present in other vulnerable populations, such as those living in extreme poverty that do not have stable jobs.

Migrants who have a polychronic system or a survival system can have difficulties to adapt to societies with a monochronic system. Frequently they do not arrive on time, do not organize their activities well and do not comply with the production norms in their jobs. The local population can think that migrants are lazy or are very distracted. This situation provokes that local population often considers these migrants negatively. At the same time, these migrants suffer from stress when they feel required to arrive on time or be more effective in their work.

One of the problems related to temporal organization is that frequently the persons are not aware of it. Individuals think that their way of organizing activities and the value they give to time is the only correct way. People who have a monochronic system think that those that have

different temporal organization "do not like" to word and do not have an idea of the importance of punctuality. The migrants that have a polychronic system think that the monochronic host society is "cold" and that local people only want to work and have money.

The Socio-Cognitive Niche

The analysis of the problems encountered by migrants can be organized into three major domains: the traumas suffered in their country of origin during the trip or in the host country, the adaptation to the new society and the conflict to decide between maintaining the original culture and adapting the culture of the host country. Neuroanthropology developed the concept of socio-cognitive niche [29] that can be useful for understanding the migrants' problems for adaptation.

Every person has a very large and very complex set of relationships with the social and ecological environment in which he lives. Smell, noise, number of people on the street, light, temperature, colour of the sky, hours of daily light, type of food and type of housing are some of the environmental factors to which the individual must adapt. There are also social and cultural factors related to the life of any individual such as language, habits, rules of communication, structure of the family, technologies, music, social hierarchy, etc. Some of the environmental and social factors are pleasant, others are negative, but all determine the conditions to which each individual is accustomed. Without being aware of many of these factors, a person gets used to them, develops according to them, builds their identity on their behalf and often makes decisions based on them. Many of these factors change for migrants. There are not only changes in isolated factors but in all the system of relationships between migrants and their conditions of life in the host country.

The concept of niche was developed in ecology. The "ecological niche" is the ensemble of environmental variables that determine the life of a population, animal or vegetal in an ecosystem. It includes all the relationships between the population and all the elements of the ecosystems such as predators, preys, characteristics of the soil, light, weather, water, nutrients, etc. [30–32].

The socio-cognitive niche is constructed by the integration of the perceptions that an individual has about their relationships with environmental, social and cultural factors. The socio-cognitive niche includes symbolic constructions and rites [33].

It is important to clarify that the socio-cognitive niche is not the set of relationships of an individual with its social, cultural and environmental environment but the set of relationships that the individual perceives, giving them a precise meaning and specific responses. That is, in the same community, different individuals may have different socio-cognitive niches (in the same way that different animal populations in the same ecosystem have different ecological niches).

An interesting problem appears here, to what extent changes that an individual has during his life (e.g. changes in knowledge, in professional activities, in the habitat, etc.) imply changes in the socio-cognitive niche? Another problem to solve is whether the problems of adaptation to the socio-cognitive niche can generate mental problems.

Migration implies a change in the socio-cognitive niche of the migrant, a new system of relationships with new environmental, cultural, social and professional factors. The migrant must adapt to the new socio-cognitive niche, which may imply that he must change some of its attitudes, values and behaviours.

Another problem appears here, to what extent the migrant perceives the new factors in the host country, the new characteristics for him of the local community. It will be difficult to adapt to factors that he does not perceive or to which he gives a different meaning than the local population. He must learn to perceive factors that are important to the local community but were not important in his home community.

On the one hand, there are changes in the environmental and social stimuli that the migrant perceives. On the other hand, there are problems that must be solved, such as finding a job, being accepted by the local community, access-

ing health services, being able to communicate with members of the local community, maintaining contacts with the country of origin and family members and friends who continue to live there, solving family problems, etc. Migrants must make important decisions to solve those problems. But those decisions can provoke new conflicts.

For example, a professional who had a high economic and social status in his country of origin and who was forced to emigrate may have to accept lower-level professional and economic jobs in the host country. This generates a conflict between the need to obtain a means of subsistence and acceptation of the loss of status, which in turn can generate low self-esteem and depression. To resolve this conflict, the migrant should treat depression, but the difficulties in accessing health services and communicating with health workers prevent him from receiving treatment. The beliefs and taboos about mental problems can also play a negative role. In this situation that generates anxiety, the migrant must establish what the priority is: to obtain a low-skilled job or avoid a situation of loss of status and low self-esteem.

Ethnic Density and Migrants' Mental Health

Migrants often have the tendency to be organized in groups of people with similar origin and isolated themselves from nationals of the host countries. It is frequent that migrant women do not speak correctly the dominant local language after many years of living in the host country. They often maintain aspects of their culture, such as dress, food, rules of behaviour, etc.

This situation augments the problems for adapting to the characteristics of the host country and jeopardizes the relationships between the migrants and the local population.

The relationships between the densities of members of the same ethnic group in the neighbourhood with mental health are analysed in the hypothesis of "ethnic density" that propose that members of ethnic minority groups have better mental health when they live in areas with higher proportions of people of the same ethnicity. However the relationships between ethnic density and the frequency of mental problems are not definitive.

Ethnic density was considered a protective factor for mental disorders in older studies of admission rates in the USA. In recent multilevel studies for African-American people and Hispanic adults in the USA, ethnic density was considered protective against depression and anxiety. However, the situation is not similar for Hispanic and Asian American adolescents that had a higher level of depression in neighbourhoods with high ethnic density. In the UK studies show mixed results, with more evidence for ethnic density as a protective factor for psychoses. The most consistent results in different studies are those that show a protective effect of ethnic density for psychoses. However the different methods in the studies and the different results indicate that the effects of ethnic density on mental health are not sufficiently consistent [34].

Other studies show a more consistent relationship between ethnic density and mental health, suggesting that social environment plays an important role in patterning health problems in marginalized and excluded population groups [35].

Different studies indicate that cases of psychosis, including persecutory beliefs, are frequent among migrants and minority populations, especially when they live in neighbourhoods with low ethnic density. A proposed hypothesis is that there are various factors that influence the development of psychoactive states, such as victimization, discrimination or the feeling of not belonging to the community in which one lives. A safe ethnic identity can reduce low self-esteem and can also play a positive role in paranoia. A study conducted in female students in the United Arab Emirates analysed the relationship between language efficiency between Arabic and English, group identity and paranoia. The results indicated that those students who had a preference for Arabic and therefore had an identity closer to the local community had less frequency of paranoia than those students who preferred English, which indicated less preference for local iden-

tity had more frequent cases of paranoia. These results may explain the protective effect of ethnic density on psychoses [36].

Rupture of Social Status and Social Control and Other Factors Related to Mental Health

Migration is not only a change of place for living. It may mean an important change in social and economic status. The cultural and social framework that allows organize the life may be lost for the migrantsç. In the new conditions they may lose the psychological, emotional, cultural and social elements that are essential part of their development. Alcohol, drugs and risky sexual behaviour may be the wrong answer to the reduction of social control and the low self-esteem provoked by the migration. In some cases a group of migrants can maintain the social structure, but children and adolescents may became drug abusers and provoke a rupture in the social framework of the community.

An example of this process during internal migration is the transformation in the community of the Qom ethnic group in Argentina that migrate from northern provinces of Chaco to Rosario, Buenos Aires and other urban areas in Argentina. Information from health workers, teachers and social assistants that work with the Qom community indicates that adults maintain a traditional social structure and a strong religious organization. They have frequent contacts with the original populations in northern provinces. However some adolescents that live in urban areas lost the traditional values and consume drugs and alcohol. There is a rupture from traditional values in the community produced by drug abuse in adolescents.

Another example is the risky sexual behaviour in migrants working in mining and other industries in South Africa that can be related with the loss of social control when the migrant leaves their community. This situation can be considered one of the causes of the epidemic of AIDS in Africa.

Difficulties for Migrants to Access Health Services

Migrants, refugees and asylum seekers often have low-pay jobs where occupational risks are high. They frequently have economic problems and low social status and have difficulties for paying health services and the other costs of health, such as transport to health-care centres. They also have problem for paying diagnosis (radiographies, scanners, etc.) and medicines.

Others factors that influence the access of migrants and refugees to health-care resources are educational attainment, type of professional occupation and earning.

A solution can be a campaign to promote health literacy in migrants, including information about health and accident risks in the most frequent migrants and refugees' jobs and information about health insurances, rights for using health care and institutions that can help migrants and refugees. It is also important to inform about the cost of treatments and the cost of health insurances.

Acceptance of High Level of Pain

Recognizing low-level signals of health problems (as pain) and asking for care are an important issue for health. Pain is a signal for many health problems. The retard produces the development of the health problem and can reduce the possibilities for a solution. Some ethnic groups have big "resistance" to pain, and they ask for health care only when pain is difficult to support. Medical doctors in Argentina indicate that members of the Qom community (an ethnic group in Argentina) with rheumatic diseases withstood a very high level of pain before seeking attention.

Another example is dental care. When they have cavities or other dental problems, migrants or other vulnerable populations do not go to the dentist until the pain is almost unbearable. The delay increases the risk of having serious problems and losing teeth. The visible loss of teeth is a sign of poverty that can cause low self-esteem

and provoke difficulties in the search for employment and, in general, in social relationships.

Difficulties for Preventing Diseases, Asking for Care and Use of Health System

Any person makes decisions using different elements, such as analysis of the contexts, information, beliefs, emotion and memory. In very risky situations, decisions have big importance. Using correctly the information may be the difference between life and death.

Providing information may not be sufficient for promoting adapted behaviour. Emotions, beliefs and personal experience can interfere in making decisions and can provoke big problems.

There are a complex system of relationships between culture, psychology and the capacity to make adapted decisions. This system is difficult to analyse. The analysis of the epidemic of Ebola virus disease (EVD) in three countries of West Africa in 2014/2015 provides a good example about necessity of fast changes in beliefs and behaviours for preventing the epidemic, and the learning difficulties that jeopardize an adapted behaviour.

Difficulties of Communication Between Health Staff and Migrants

Health staff should adapt to a new kind of patients, who have different culture, beliefs and language. Improving the communication between health staffs and migrants and refugees is one of the important issues related with health literacy. Communication is a two-sided issue: health staff needs to develop cultural awareness, and migrants need to develop understanding of the new living conditions in the country and the health system.

Migrants and refugees frequently have different cultures than local people. They can also have difficulties in understanding local languages. These differences can be a barrier for the communication between health staff and migrants and refugees.

Low health literacy can be another important factor for difficult communication when the patients cannot explain correctly their health problems or when they cannot understand the information provided by the health staff. Promoting health literacy can facilitate the communication between health staff and migrants and refugees and promote correct adherence to treatment.

Psychological Problems

Low self-esteem, shame for not speaking well the local language and feeling to be discriminated can also interfere with health is migrants with these psychological problems are reluctant to go to health system for care. These problems can also be a barrier for the communication with health staff.

Explanatory Models

Migrants and refugees have magical and religious explanatory models such as fatalism, magical causes of diseases, beliefs that health problems are "punishment from god", etc. There are also "conspirator explanatory models", such as "AIDS is produced by countries that want to kill African populations" or "Ebola do not exist. It is only a way for governments for obtaining monetary assistance". Explanatory models can interfere with understanding health issues, looking for care in clinics and adhering to treatment.

Analysing the most frequent explanatory models of the target population is an important element for organizing culture-friendly health literacy campaigns.

Beliefs and Taboos Related to Health Issues

As any other population, migrant and refugees have non-scientific beliefs and taboos that can interfere with their communication with health-care staff and can prevent a correct adherence to treatment. Analysing the beliefs and taboos is an important issue for campaigns for promoting health literacy.

Some beliefs are based on correct empirical knowledge but they are expressed in magical terms. This kind of conceptions can be used in the campaigns for promoting health literacy for teaching basic scientific concepts.

It is important to take into account the beliefs about what is health and what is illness because they can interfere with the adherence to treatment, in particular when people "feel good" and interrupt the treatment or when they believe in magical solutions.

An example is the belief during the epidemic of Ebola virus disease in West Africa that drinking salt water is a way to prevent the disease or cure it; some people died of health problems due to drinking a big quantity of salt water. Others do not follow the preventive measures because they thought that drinking salt water was sufficient for preventing the contagion. Another example is the belief that Ebola is a punishment from God to sinners. Priests that have this belief promoted collective religious activities that concentrate hundreds of people in crowded churches, augmenting the risk of contagion of Ebola virus in the participants.

Different Patient Symptoms Related to Mental Health Problems

Some studies in the USA indicate that migrant populations and African-American communities have physical symptoms instead of psychological symptoms when they have mental health problems. This situation can be a source of errors in the diagnosis, for example, if the patient present pain in the chest when he has a mental problem and the medical doctor do not know that for some communities this is a signal for mental distress.

Language Problems

Migrants and refugees can have difficulties in understanding local languages. Producing teaching materials and organizing teaching activities in the languages spoken by migrant and refugee populations can be a solution. Using plain language, avoiding scientific and medical words and using examples from the culture of migrants and refugees are important issues for ensuring the success of a campaign in promoting health literacy. For ensuring the understanding of teaching materials, it is important to evaluate them to the members of the migrant and refugee populations or people who speak the same language and understand the culture.

Local population often do jokes about the pronunciation of migrants provoking low self-esteem in them.

Diffidence About the Health System

Migrants and refugees often have negative opinion about the science and the modern health approach. Traditional beliefs are one of the factors for the negative opinion. Other factors are negative experiences in the use of health systems. Other causes of diffidence are emotional problems in the relationships with health staff. In some ethnic groups, women are not allowed to be treated by men medical doctors. Migrants also dislike to talk about sexual behaviour. These problems can provoke problems in asking for help and in the communication with health staff.

Different studies in the USA show that African-American patients also have diffidence about the health systems.

Lack of Basic Knowledge Related with Health

Basic knowledge about health is an important element in order to understand health problems, prevent diseases, ask for care and adhere to treatment. Migrants and refugee lack this basic knowledge.

Understanding health problems, preventing them, asking for help, communicating with health staff and adhering to treatment are not easy for migrants that do not have basic scientific knowledge related with health. For this reason it is very important to focus a campaign for promoting health literacy by teaching basic scientific knowledge that can develop an understanding of the causes of the problems and the ways of solving them. New skills related with health should be understood as a logical response to the causes of the health problems and not as a simple list of tasks to be performed without explanation.

For example, the necessity of boiling water in order to reduce the risk of waterborne diseases, vaccinating children to prevent infectious diseases and washing hands before eating cannot be understood without basic knowledge about microorganisms. The lack of it transforms the information about these problems in a series of tasks to perform without knowing why it is necessary to undertake them.

The Needs of a Cultural Approach on Mental Health

Mental and physical illness patterns vary in differing cultures. The immigrant tends to respond to stress according to the cultures of his childhood environment. Mental health research relies primarily on members of dominant cultures, without taking into account the characteristics of minorities. Frequently the work of clinicians in mental health is based on a "Western" ethnocentric perspective that is different from the perspective of many communities in the world (including the Western communities themselves). The perspective that mental health workers have often does not take into account the culture of their patients, particularly migrants who have another culture [37].

The diagnosis and treatment of mental problems of migrants or members of marginalized communities can be erroneous if they do not take into account the characteristics of these populations, such as the ways of expressing in their organization the mental problems and the taboos and conceptions about those themes. An approach based solely on Western culture may leave aside positive characteristics of those communities, such as the possible support of family or friends. It is also important that those who work in social services and medical services consider that a migrant, especially a refugee or an asylum seeker, may have suffered from very traumatic experiences that are not expressed because of the communication difficulties mentioned.

A researcher proposes a change of paradigm to improve mental health services for migrants, marginalized or minorities. He noted the importance of taking into account the cultural factors that are transmitted to new generations such as traditions and customs, which largely determine beliefs, values, expectations and attitudes about mental health. That would allow greater sensitivity to the needs of migrants and minorities and improve the communication with them. Even the concept of "barrier to treatment" may not be applicable when the patient has a different understanding of mental health. The proposed approach, called "Culturally Infused Engagement", seeks to strengthen the alliance between the doctor, the family and the patient, allowing them to express themselves and their needs to be taken into account. This facilitates adherence to treatment and allows physicians to understand patient perspectives [15].

Frequency of Mental Problems in Non-refugee Migrants

Refugee and asylum seekers have differences in the frequency of mental problems than non-refugee migrants. For this reason we will provide information about mental issues for these two groups separately.

Studies developed in different countries indicate differences in the frequency of mental problems in migrants to the frequency in local population. A study realized in Canada indicates that the frequency of mental problems among migrants is lower than that of the local population at the beginning of the migrant life in the country. One possible explanation to this phenomenon is that immigrants in Canada must pass a series of filters before being accepted, including no problems on mental health. In this way there is a selection that reduces the frequency of mental problems in recent immigrants. However, the frequency of mental problems increases over time and becomes similar to the rest of the general population [38, 39]. Data from other countries indicated that the frequency of some mental problems is higher in migrants than in local population.

Mental health of migrants can be related to different elements, such as gender and marital status, state of residence, education, employment status and type of migration. In a research realized in Iran about mental health in migrants, there was a positive correlation between mental health and gender: males had better mental health status than females. Females also had more problems related with anxiety. Married people had better mental health than the single ones, and permanent migrants presented less mental health problems that seasonal migrants. One interesting finding was that people living with relatives had poorer mental health than people living alone [40].

Being in detention in prisons is also a factor that can increase the mental problems of migrants, refugees or asylum seekers. In a study in the USA with 70 asylum seekers detained in New York, New Jersey and Pennsylvania, it was found that 77% of participants have severe symptoms of anxiety, 86% of depression and 50% of PTSD. These symptoms were correlated with the duration of the detention. Participants who were released had a reduction of the symptoms, while those who remained in detention increased them [41].

Post-traumatic Stress Disorder in Migrants

There is growing evidence supporting the association between migration and PTSD. Some studies indicate that its prevalence is very high in migrants, reaching 47% of them for some groups of migrants. In some countries the frequency of PTSD is much higher (almost double) in refugees than in migrant workers [8].

Anxiety and Depression in Migrants

One of the most frequent mental problems that migrants suffer is depression. For example, the frequency of depression is high in some migrant populations such as Latinos in the USA, mainly provoked by language barriers and for the separation of their families or the feelings of loss. A study about mental problems of this migrant population indicates that 26% of the participants have symptoms related to depression. Low health literacy and other difficulties for accessing health-care services are factors that reduce the possibility of treatment. The study indicates the importance of measures such as training recent Latino immigrants aiming to improve their health literacy, provide them with information about symptoms of depression and promote their better access to health-care services. The study also indicates the importance of screening this population for depressive symptoms when they access health-care services [42].

Another study about depression of Asian women in the USA indicates that they tend to see depression as a personal weakness or a moral fall. For this reason, it is recommended that the paramedical health professionals explain to Asian patients that depression is a disease caused by neurochemical balance problems and is not

indicative of any weakness. This study indicates that women of Asian origin have a higher suicide rate than all the other groups of women in the USA [43].

Psychosis in Migrants

There is strong evidence in many countries that the frequency of psychosis in migrants and in minority ethnic population is higher than those of local population [44]. Studies conducted in the UK on migrants from Tristan da Cunha indicate that there is a high frequency of mental illnesses in migrants with paranoid symptoms and somatic symptomatology [45]. In Sweden, similar results were also obtained in a study of 839 people that indicated that black citizens, a group of recent immigration in Swedish society, were diagnosed with psychotic disorders more frequently than other ethnic groups [46]. African immigrants in Portugal are also diagnosed with schizophrenia more often than the local population [47].

A study conducted in Canada indicates that immigrants from Africa or the Caribbean Region with diagnoses of psychotic disorders had a higher percentage of maintaining that diagnosis than the local population. They also had a lower percentage change in the diagnosis of psychotic disorder to a diagnosis of non-psychosis [48].

A review of scientific articles of more than 30 years indicates that the history of migration and social status in the host country are factors that increase the risks of psychosis. These results are not explained by errors in the diagnosis or by psychopathological differences. That analysis suggests that migration is an important factor in explaining the frequency of psychosis in migrants [49].

In a study in the Netherlands, more than 21,000 pre-trial reports were analysed comparing the native Dutch population with the descendants of Africans and other minorities and whites from other Western countries, most of them from European countries. The results show that there were 19.8% of recommendations for admissions in psychiatric hospitals for Africans and other minorities; for white immigrants of European origin, the recommendations were 19.3% compared to Dutch natives (9.2%). According to the authors of this article, immigrants have a higher risk of being diagnosed with psychotic disorders and to be sent to psychiatric hospitals. This risk may be related to misunderstanding between psychiatrists and immigrants or with bias in symptomatology [50].

Problems in the Diagnosis for Psychotic Disorders in Migrants and Marginalized Minorities

Studies in different countries indicate that the patient's ethnic origin can determine the diagnosis on psychosis in multicultural societies [51].

African-Americans suffering from depression have a long history of being overdiagnosed with schizophrenia mental disorders and being underdiagnosed for mood disorders. Erroneous diagnoses affect these patients in various ways, increasing the attrition and drop-out of the treatments, reducing patient satisfaction, exacerbating chronicity and creating problems due to inappropriate interventions and the use of inappropriate psychotropic drugs [52].

A study synthesizing 24-year literature on the differences in the diagnosis of psychosis shows that ethnicity influences the diagnosis of schizophrenia. The study shows that in the USA during all those years, African-Americans have a schizophrenia diagnosis rate of three to four times higher than the Euro-Americans. Latinos also present a frequency of diagnosis of psychotic symptoms three times higher than the Euro-Americans and the Asians. An interesting result of the study is that people discharged after the first psychiatric hospitalization did not present differences in the diagnosis of schizophrenia, although African-Americans were discharged more often than the Euro-Americans with an unspecified diagnosis, such as psychosis [53].

A study of almost 3000 children and adolescents indicates that African-Americans and Latinos under the age of 18 had a rate of diagnoses of psychosis disorders twice as high as young Euro-Americans.

One study indicates that in forensic psychiatric treatment centres, African-Americans have a probability of being diagnosed with psychiatric disorder of 56%, while Euro-Americans have a probability of only 21% [54].

A psychiatrist in the USA reported that during 2 years she had to correct the diagnoses of 40 minority members, finding that 1 in 10 patients had been wrongly diagnosed with diseases such as schizophrenia.

In a study in medical centres for veterans in the USA, the diagnoses of more than 23000 elderly patients were analysed. The results show that Latin Americans and African-Americans have higher rates of psychotic disorders (24% and 23%, respectively) than Euro-Americans (18%) [55].

Different explanations have been given to this phenomenon. One of them is the influence of the prejudices of the psychiatrists in the diagnoses, although the data that have this explanation are not conclusive. Another explanation is that there are differences in the diagnostic criteria according to the race of the psychiatrist. Other authors take into account errors of interpretation of disruptive behaviours and socially deviants associated with African-Americans in the USA [13]. Some authors indicate that different ethnic groups have different symptoms when they suffer from mental problems [56]. An example is that African-American patients present organic symptoms, such as chest pain, when they suffer from depression [52].

Forcible Migration for Traumatic Situations in the Country of Origin

Millions of people escape from war, abuses in human rights, environmental disasters and extreme poverty. Frequently these factors are acting together.

The civil war in the former Yugoslavia, Syria, Iraq, Afghanistan, Sudan, South Sudan, Somalia, Colombia and Yemen; the successive wars in Gaza; the genocide in Cambodia of two million people, in Myanmar of the Rohingya ethnic population and in Rwanda of the near one million Tutsi and moderate Hutus; the dictators in Eritrea, Uruguay, Chile and Argentina; the action of terrorists in the Philippines, Nigeria and Cameroun; the massacres and the systematic rape of women in the east of the Democratic Republic of the Congo; the systematic kidnaping of children in Uganda; and the attacks on local population of gangs in Honduras, El Salvador or Mexico are examples of the causes that provoke the displacement of millions of people.

By the end of 2017, 68,5 million of people were forcibly displaced worldwide (refugees, asylum seekers and internally displaced persons), 19,6 million of them were refugees and 3,1 million were asylum seekers. The vast majority (86%) of refugees and asylum seekers are in developing countries. In some countries refugees provoke a great impact on the economy and on social and health systems. For example, Jordan and Lebanon have several million refugees who escaped the civil war in Syria. Uganda has millions of refugees from the civil war in South Sudan. In Lebanon more than 16% of the total population are refugees and asylum seekers [57].

Millions of people migrate also in order to escape extreme poverty. Environmental disaster also provoked migrations. Global warming can augment this issue in the future, especially in coast regions and in small island countries.

Very frequently the migrants lost everything when they escape from their country and should undertake long and harsh journeys before arriving to safe countries. The traumatic situations in the original country can provoke mental problems that can persist for a long time after the arrival to the host countries.

As for migrations between different countries, internal migrations can also provoke negative impacts on health, including mental health.

Mental Disorders in Refugees and Asylum Seekers

An immigrant sector that has frequent and serious mental problems is that of refugees and asylum seekers who often suffered from traumatic situations such as being tortured, participating in

armed conflicts, loosing family members or assisting to death. These traumatic situations produce serious and multiple physical and psychological sequels, which makes it difficult to diagnose and treat them [58]. In a study conducted in 1991 in the USA with Cambodian refugees who were not psychiatric patients, it was found that 86% had symptoms of PTSD, 96% had high dissociation rates and 80% suffered from clinical depression. The study demonstrated a relationship between the intensity of the trauma suffered and the severity of the symptoms [59].

The multiplicity of traumatic episodes suffered by refugees and the continuation of such situations in the host countries or the lack of family support determine that their mental health problems are different from those of other groups that also suffered from traumatic episodes, such as military veterans and victims of sexual violence [60].

Refugees and asylum seekers who have experienced violent situations before their arrival to Canada have a greater frequency of mental problems related to traumatic situations, including PTSD and somatic symptoms such as chronic pain. The risks of having mental problems are related to the stress in the country of origin, the stress and the situation of uncertainty during the migration and the experiences lived in the host country. To improve the effectiveness in the diagnosis and treatment of the mental problems of immigrants, the use of trained interpreters and culture brokers is suggested when cultural and linguistic differences hinder communication and understanding between patients and health personnel [61].

The analysis of the mental problems of 79 adult refugees from different Burmese ethnic groups newly arrived in Australia indicated a high frequency of mental disorders, 20% of these refugees had symptoms of anxiety, 9% of PTSD and 36% of depression. Also 37% of them had symptoms of somatization. The refugees indicated having problems in the host country, in particular concern about their family outside Australia and communication difficulties. No differences were found between men and women in the frequency of the mentioned mental disorders [62].

A study conducted with 854 war refugees from the former Yugoslavia in Germany, Italy and the UK showed that the prevalence of mental disorders varies substantially among the three countries. PTSD was associated with a greater number of traumatic experiences during the war, absence of combat experience, older age, a low level of education, great stress during migration and absence of temporary residence permit. The problems related to mood and anxiety disorders were related to more traumatic experiences during and after the major war related to immigration, the residence permit and the feeling of not being accepted in the country. The mood disorders were more associated with the older age, being a woman and not having a job, while anxiety disorders were related to the absence of combat experience. The use of substances was associated with being young, male and not having a partner. These results indicate that experiences during the war are more related to the frequency of PTSD, while experiences after migration were more associated with mood, anxiety and substance use disorder [63].

Depression and anxiety are also frequent in refugees. One example is the Karenni refugees on the border between Burma and Thailand, who have an average of 42% cases of depression and 42% cases of anxiety. These figures are related to violence, the harassment suffered and the difficulty in meeting basic needs [64].

In a Cambodian refugee camp on the Thai-Cambodian border, 82% of the refugees said they had depression, which was confirmed as severe depression in 55% of the cases. Symptoms of depression include problems with sleep, depressive mood, loss of interest in activities, weight changes, feelings of guilt and being useless almost daily. They have recurrent thoughts of death and suicide that includes attempts or plans to commit suicide. Those refugees suffered from torture, death of lovers and war; were prisoners; had serious accidents, such as explosions; and had serious health problems. They presented symptoms of generalized anxiety such as restlessness, fatigue, irritability, excessive worry and problems to relax and sleep and focusing on PTSD as intrusion, avoidance and hyperarousal.

Post-traumatic Stress Disorder (PTSD) in Refugees and Asylum Seekers

Migrants who suffered from traumatic situations in their country of origin, during their trip or in the host country can develop post-traumatic stress disorder (PTSD), in which characteristic symptoms develop after exposure to traumatic situations such as being victim of violence, living situations in which life is in danger, suffering from serious aggressions, being tortured, being raped, participating in armed conflicts or being involve in them, losing physical or moral integrity or observing this type of situations experienced by other people, particularly family members, friends or associates [8, 65]. There are also stressful situations in the host country that can provoke post-traumatic stress disorder, such as isolation, lack of capacity to communicate with local people, unemployment, difficulties for accessing health services, difficulties for obtaining refugee status, lack of opportunities for improving the quality of life, detention, bad housing, acculturation problems, etc. Health literacy can develop the capacity of patients and their families to recognize these symptoms and ask for care.

Each individual can respond in a different way to these emotional upset suffered before, during or after the migration. For this reason it is difficult to predict the trauma after-effects. Under certain circumstances traumatic situations provoke post-traumatic stress disorder. PTSD can have a lifelong negative impact on the affected individual. Recognizing the first signals of it and referring the person to health staff are a very important issue for reducing future mental problems. PTSD is also related to anxiety about the future situation in the host country. A study carried out in Canada with asylum seekers indicates that an important factor of stress is the idea of not obtaining political asylum. When the asylum is obtained, the symptoms of PTSD and generalized malaise are reduced [61].

A review of 161 articles that gave the results of 181 surveys in 40 countries, with more than 82,000 refugees and others who suffered from armed conflicts, indicates that there is a great variability between the rates of PTSD and those of depression. The adjusted average was 30,6% for PTSD and 30.8% for depression. Torture is the most important factor for PTSD followed by the cumulative exposure of potentially traumatic events (PTEs), time since conflict and levels of political terror. For depression, the most important factor was the number of PTEs, time since conflict, torture and residency status [66].

The results of 29 surveys with differences in sampling and assessment, with a total of 6743 refugees in 7 countries, showed great variations in the frequency of PTSD: in the most numerous studies, it was found that 9% of the refugees had symptoms of PTSD and 5% were severe cases of depression, with great evidence of psychiatric comorbidity. In other less numerous surveys, a frequency of 11% of PTSD was found in refugee children [67].

The impact of the war on mental health was verified in a study on students from Ahfad University for Women of Sudan, refugees in the Darfur camp, of which 80.9% had symptoms of PTSD. The main causes of these symptoms were having been exposed to combat situations, threats to life and the safety of relatives and the displacement from the village and home. The loss of family increased in those girls the feelings of isolation, humiliation, discrimination, guilt and shame for having survived, loss of identity, vulnerability, intimidation and isolation, which indicates the importance of the loss of the family in mental health in some communities [68].

An epidemiological survey was conducted in four countries between 1997 and 1999 to analyse the effect of traumatic situations of war and violence. In one of the countries, Algeria, the studied population had suffered from repeated terrorist attacks, with massacres; in another, Cambodia, the population suffered from the war against American army, the genocide carried out by the Khmer Rouge, the invasion by Vietnam troops and the civil war. In Ethiopia, the situation of refugees in refugee camps and, in the Gaza Strip, the situation of people in temporary shelters were analysed.

In all these countries, the frequency of PTSD was very high, 37.4% in Algeria, 28.4%

in Cambodia, 15.8% in Ethiopia and 17.8% in Gaza. The only risk factor for PTSD in all four cases was trauma related to armed conflict.

Torture was also an important risk factor for PTSD except in Cambodia where domestic stress, death of a family member, separation from family and alcohol abuse by parents were associated with PTSD. In Ethiopia and in Cambodia, psychiatric history and common diseases were also risk factors for PTSD, while in Algeria and Gaza an important factor was the poor quality of the refugee camps. Daily hassles were associated with PTSD in Algeria.

These results indicate that the causes of PTSD are different in the different populations that suffered from mass violence, armed conflicts and wars. From these data, the great importance for the diagnosis and treatment of PTSD that has the analysis of the specific situations suffered by the respective populations can be inferred [69].

In Syrian refugees in Turkey, the frequency of PTSD in these refugees was 33.5%, but the probability of having PTSD symptoms was 71% if it was a woman who had had a diagnosis of a psychiatric disorder in the past, had a family history of psychiatric disorder or having experienced two or more traumas [70].

PTSD is also a serious problem for health personnel who lived in conditions of war, for example, a study carried out in 2014 in the Gaza Strip, on more than 1100 nurses who suffered from prolonged stress in the attacks of the Israeli army, indicated that 69,4% of them had high levels of PTSD symptoms. The main symptoms were intrusion, avoidance and hyperarousal [71].

Refugees or migrants that present symptoms of PTSD can significantly increase those symptoms if they suffer from stressful life events in the refugee camps or in the host country. New stressful situations also produce a large increase in PTSD avoidance. For this reason it is important that the clinicians anticipate a possible reactivation of PTSD symptoms when patients are subjected to significant stressful stimuli [72, 73].

A study with Syrian refugees in Turkey indicates that eye movement desensitization and reprocessing (EMDR) can be effective in reducing PTSD and depression symptoms [74].

A programme for Palestinian adults who suffered from PTSD was evaluated in Gaza. The programme called Mind-Body Skills Groups consists of ten sessions that include meditation, guided imagery, breathing techniques, autogenic training, biofeedback, genograms and self-expression through words, drawings and movement. The results of the evaluation indicate that there were important improvements in the symptoms of depression, PTSD anxiety and the quality of life in general. After 10 months of the end of the programme, these improvements were maintained. Improvements in physical health and social relationships were also observed. The programme can be easily taught to health professionals [75].

The examples mentioned indicate that the intensity, type and frequency of PTSD symptoms are related to the traumatic situations experienced and the patient's interpretation of them, the meaning he gave to them and the emotions that produced to him, which it is related to his personal history, his psychological characteristics and the culture to which he ascribes. That is, there are three types of factors, the external (the violence suffered), the individual (the characteristics of each individual) and the social (the modes of interpretation that depend on each culture and each social group). We must also take into account the factors after migration, which, again, are external (living conditions), internal (the psychological characteristics of the patient) and social (the culture of the social group to which the patient ascribes).

The resilience of the traumas suffered depends on the situation that lives in the host country or in the refugee camps and the support that can be found in their family and community environment, which also depends on the culture of those groups.

According to the previous proposal, the relationship between culture, personal characteristics and situations experienced is a key element in the possibility of overcoming traumatic situations. We can think that in the cases in which the culture does not facilitate the expression of the traumas suffered (e.g. when mental illnesses are taboo), resilience is more difficult. Changing

the culture of a social group is very difficult. Perhaps a possibility is to inform that group of the importance of the support to those who suffered from traumatic situations and the negative effects that the shame of mental symptoms has on the persistence of these traumas. Education about mental disorders can be an important factor for resilience.

Effect of Torture in Refugees and Asylum Seekers

Many refugees from countries in civil war or dictatorships suffered from torture. Suffering from torture, war traumas and post-migration difficulties are factors that increase the likelihood that refugees will develop mental health disorders [76].

Different studies indicate that the aftermath of torture can be very serious, such as PTSD, generalized anxiety disorder, depression and somatic problems. Insomnia, isolation and loneliness may also occur [77]. A study conducted on Syrian Kurdish refugees that analysed the relationship between torture and PTSD found that 38% had serious symptoms of PTSD and 35% some symptoms of it [78].

A study in the Bhutanese refugee camps in eastern Nepal on 526 people tortured and a control group of 526 people of similar characteristics who had not been tortured showed that the tortured people had more symptoms of PTSD, anxiety and depression than those who were not tortured. The tortured also had problems in the musculoskeletal system and the respiratory system. The tortured Buddhists had less anxiety and depression than the non-Buddhists and the tortured men had less frequency of anxiety [79].

In a study with Tibetan refugees in India, it was found that 54.3% of those who were tortured had symptoms of anxiety, while only 28.6% of those who were not tortured had those symptoms. In both groups the symptoms of depression were presented in 14.3% of the interviewees. These results indicate that the effects of torture can have a long-term consequence on mental health [80].

The nature of the bad treatments and their duration has a direct influence on the severity of PTSD symptoms. A study of 550 former Palestinian prisoners in Gaza Strip indicated that the greater the exposure to physical, chemical or electrical torture, the more they suffered from intrusive re-experiencing, withdrawal, numbness and hyperarousal. Also the continuation of harassment by the Israeli military after the release of the prisoner augmented the importance of these symptoms [81].

Psychosis in Refugees

An important question to define refugee mental health problems is whether they have a higher risk of schizophrenia or other non-affective psychotic disorders than local populations or than non-refugee migrants. To answer this question, an extensive cohort study was carried out in Sweden, with the participation of a total of 1,347,790 people, of which 1,191,004 were Swedish with Swedish parents, 133,663 were non-refugee migrants and 24,123 were refugees, the majority coming from the Middle East and North Africa, sub-Saharan Africa, Asia and Eastern Europe and Russia.

The incidence was 38.5 per 100,000 people in the population born in Sweden, 80.4 per 100,000 people for non-refugee migrants and 126.4 for refugees. These differences were found in refugees from all countries, except those from sub-Saharan Africa who had the same frequency as the rest of the population born in Sweden. The results also indicated that the differences were greater for men than for women [82].

Comorbidity Between Psychosis and PTSD

Migrants who suffer from PTSD may have other mental problems simultaneously. In a study conducted in Denmark on 181 refugees suffering from PTSD, it was found that 74 (41%) of them had symptoms of psychosis, of which 66% were

auditory hallucinations and 50% persecutory delusions. Patients with psychotic symptoms associated with PTSD had personality changes after traumatic situations than other patients with PTSD but without associated psychotic symptoms. The study points out the difficulties in differentiating psychological symptoms from flashbacks related to PTSD [60].

Mental Health Problems in Refugee Children and Adolescents

Children and adolescents are particularly vulnerable to PTSD when they experience situations of violence or disasters, even when they see it on television or read it in newspapers. In some cases the frequency of PTSD in child and youth refugees is very high, as for Cambodian refugees in Canada that present a rate of 50% [5]. In the refugee camp of Moria, on the Greek island of Lesbos, the daily deadly violence, the very bad sanitary conditions and the overpopulation provoke such mental problems in the children that some of them of only 10 years of age tried to commit suicide [83].

A review of the literature on the mental problems of refugee children indicates that the prevalence of PTSD varies between 5% and 89%. These differences can be explained by differences in the degree of trauma suffered and the place where children reside: those who are refugees in countries with a high economic level, such as Canada, have lower PTSD rates than those in countries with a lower level of development or in countries close to the origin of the children. An explanation of this phenomenon is that more stable and safe countries give better protection to children and reduce the traumatic situations in where they live. Another factor that can explain the differences is the nature of the trauma suffered. For example, an accident or a tornado may denote PTSD symptoms, but phenomena provoked by systematic and violent human action, such as Nazi concentration camps, massacres in Rwanda or Cambodia or the recruitment of child soldiers, cause more distress and disorders. The proximity of the traumatic situation is also important, for example, the proximity to situations of war or massacres. Finally another factor is the type of trauma suffered, such as having seen a violent death or having suffered from rape or physical abuse [67].

A positive correlation has been found between the number of traumatic episodes suffered by the family, prior to migration or during the trip, and the cases of PTSD in children. Highest scores of PTSD in children are associated with the violent death of a family member.

Results were also obtained that indicate a positive correlation between the traumatic episodes experienced by the family in the host country and the depression scores of the children. High scores of depression in children are associated with their family difficulty in obtaining asylum and family economic problems [84].

Similar results were obtained in a study in Canada, where 394 primary caregivers with 639 children obtained the status of refugee. The results indicated that the traumas and post-migratory difficulties suffered by the caregivers were associated with subsequent PTSD, which in turn was associated with great harsh parenting which in turn determined high levels of behaviour problems in the children, such as hyperactivity, emotional symptoms, try them with other children. These results show that PTSD suffered by a primary caregiver influences directly in the children [85].

These studies show that children are sensitive to situations of stress lived by the family, be it before migration, during the trip or in the host country. For this reason it is important to analyse the situations that children and adolescents experience before or during their migration and those situations that live in the host country. The diagnostic of the PTSD symptoms in migrant children and adolescents is a very important element to be able to treat them [86].

Severe situations of stress, such as war, can cause serious problems in children with psychosomatic changes and behavioural changes that can lead to substance abuse, self-harm and even suicide. In a study conducted in Syria in children who suffered from war situations, interviewed children said that they frequently suffer from headaches and chest pain, have difficulty

breathing and in some cases temporarily lose the movement of the legs. Eighty nine per cent of the adults interviewed indicated that they saw children suffering from persistent fears, 71% said that the children wet the bed or urinated involuntarily in class or in public, while 48% saw children who lost the ability to speak or who suffer from speech impediments. The situation of violence suffered by the children can lead to violent behaviour, such as screaming, fighting with friends or bullying other children as was noted by 80.5% of the adults interviewed. Some children said they wanted revenge for the violence they and their families suffered [1].

One study analysed the differences between the frequency of depression and PTSD in children and adolescents in a Yazidi refugee population and found that children have less frequency of depression and PTSD symptoms than adolescents. The possible causes are related to that the adolescents have greater amount of siblings. Other possible factors related to depression are the older age of the parents, being a woman or having observed someone who suffers from violent situation or who died violently. Girls and adolescent women have a higher frequency of PTSD than boys and adolescent men [87]. However, these results are not consistent with those obtained in studies on Syrian Kurdish refugees, where no significant differences were found in the frequency of PTSD between men and women. It is possible that the cultural differences between the Yazidi population and the Kurdish population and differences in the trauma that they suffered explain these two results. However, the frequency of mental tests in general is more frequent in women in both populations [78].

Young refugees from the former Yugoslavia testify to have been tortured frequently. A study on 119 young Bosnian refugees presented 35–43% of PTSD diagnoses. Having no right to asylum in the host country may have influenced their situation. Significant predictors of PTSD were being a woman, problem-focused and avoidant coping strategies. No protective effects of social support were found. The conclusions of the study indicate the importance of investigating the effectiveness of social support and of the different coping strategies [88].

Forced migration is, as we pointed out earlier, the cause of many psychiatric problems in children. To differentiate the impacts of migration on mental health from those caused by life in the refugee camps, mental problems were analysed in the first days of resettlement in a refugee camps for Yazidi. Cases of PTSD and other mental problems were found. Children who had suffered from forced migration had more emotional and behavioural problems than children who had not suffered this traumatic situation. These problems were evidenced by great shyness when arriving in the refugee camps, avoiding contact with other children. They also said they feared being caught not feeling safe in the fields and having difficulty sleeping. In third of the children who suffered from forced migration, they were diagnosed with depressive disorders [89].

Changes in the behaviour of a child can be an indication of the consequences of the trauma suffered. For this reason parents and caregivers should observe any change in behaviour, and when they notice that the child is not talking as usually, or he presents a state of sadness, a violent behaviour or an increase in fears, they should consult a health agent [90].

The symptoms of PTSD in children can be persistent as demonstrated by a study about traumatized Cambodian children who maintained the symptoms of PTSD and depression during the 3 years that the study lasted. Frequently the symptoms of PTSD in children and adolescents are withdrawal, hyper alertness, emotional numbness and re-experiencing. Symptoms vary according to age and the conditions in which they occur. In children, temper tantrums and re-enactment behaviour are common, while adolescents tend to take risks. In addition to specialized services, diagnosis and treatment require an approach that encourages the participation of families and schools. Experience indicates that psychological therapies focused on trauma are effective and that single-session debriefing should be avoided [91].

Several strategies were used to give psychological and social support to children who suffered from severe stress or chronic stress. A

programme developed in Syria uses group artistic activities such as drawing, drama and music. The intention is to ensure that the children can process, express and communicate the feelings provoked by their traumatic experiences. The results indicate that the presence of an adult in which the children have confidence and can share their memories and feelings with them is important. Children feel less isolated, communicate more with other children and feel more protected by adults they can trust. This process allows the children to cope with the daily stress. The children are again children. Self-confidence and a positive belief in themselves and their identity can also help children to cope with stress [5].

The problems suffered by migrant and refugee children can impact their psychological and biological development that can provoke problems in education and jeopardize their future. Vulnerable children, for example, those living in extreme poverty, can have also this kind of problems. A research in Argentina in 2004 indicates that 20% of pupils in the first year in primary schools have retard in their psychological development and 30% of them have anaemia and other health problems. These problems where associated with poverty and low level of education of the mother [92].

Migrant children can be discriminated in schools. A research about racism realized in 11 countries suggests that a high proportion of teachers have negative conceptions related with children of minorities or for migrant children [93].

Reproduction of the Suffered Violence

The violence suffered by children can cause a desensitization and numbness of emotions, which increases the possibility of considering violence as a norm and developing violent behaviour. Loss of sensitivity to violence can be a way to adapt to it [94, 95].

The risk is that this situation will continue until they are adults who lack empathy and consider the violence as normal. These mechanisms can lead to a reproduction of violence in the future, either individually or at a collective level. Violence persists in the minds of those who suffered it, as psychological problems or as the development of a personality without empathy and violence. The millions of children suffering from serious situations of violence around the world make the search for solutions to this type of mechanism very important.

Reinforcing the social networks of traumatized children with aggressive behaviour and providing them with emotional outlets can be a solution to this kind of problem.

Open Questions

From the examples that we have presented, we can determine some serious problems. One of them is related to the communication difficulties between the clinician and the patient that can be a cause of misdiagnosis, in particular for depression and psychosis. The different methods we have shown are essentially based on developing the ability of clinicians to understand the culture of the patient when he or she is an immigrant or member of marginalized minorities and to establish a dialogue that stimulates the understanding and participation of the patient and their environment in the treatment of the disease. A complementary solution is to improve the patient's comprehension capacity, which in general is related to improving their health literacy and help them to overcome distrust, taboos and learning obstacles related with mental health. Therefore, the possible solutions to communication problems involve working in two domains, the training and awareness of clinicians and the education of patients and their communities.

The education of patients and their communities is possibly the most difficult subject to carry out, given the difficulties of communicating with communities that have another culture and in many cases another language that suffer from the problems of marginalization that we have mentioned and that may have distrust on health systems. Another element that hinders this solution is the lack of community education structures dedicated to health literacy.

Another serious problem, especially in the refugee camps and in areas of extreme poverty, is the lack of health structures that can diagnose and treat mental illness. Refugee camps with hundreds of thousands of people or shanty towns where thousands of people live crowded together present two very difficult problems: the stress of the situation (such as overcrowding; lack of resources; sexual, physical and psychological violence; nutrition problems; lack of future for young people; etc.) and the lack of health structures.

We believe that the possible solutions are related to a community approach that trains members of these communities in the detection of mental problems and in the information of existing health centres, when they exist or in some forms of treatment that allow to reduce the seriousness of those cases and avoid the risks they present to their families and other members of the community. We believe that the development of methods of education for a community development of mental health is a very big challenge for those who work in the field of mental health. However we also believe that the development of these methods and their concrete application will not only make it possible to improve the situation in the refugee camps and in the shanty towns, but it will make it possible to improve education for health in general.

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Part IV

Explaining Human Pathological Behaviors. From Brain Disorders to Psychopathology

The Transition to a Dimensional System for Personality Disorders: Main Advances and Limitations

37

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Introduction

The fifth edition of the Diagnostic and Statistical Manual of mental disorders preserved with minimal modifications the categorical classification of previous editions for personality disorders (PD), influenced by the traditions of Schneider and Kraepelin [1]. Although some researchers predicted an imminent advance towards the installation of a dimensional system, it was imposed a period of coexistence of models suggested by others [2]. Thus, Section III of the manual presented an alternative model, conceived as a palliative for possible deficiencies derived from the use of the categorical system.

There were proposed seven general criteria to diagnose and a double assessment that includes the level of personality deterioration and the existence of pathological features [3]. That is to say that despite the fact that certain authors have noticed the poor validation and the high overlap between the diagnostic criteria of each of the disorders included in the three clusters, the definitive leap towards a dimensional-factorial system has not been made. The explicit purpose of "preserving a continuity with current clinical practice" was explicitly stated, although the inadequacy of current nosography has been empirically recognized. This chapter does not propose an exhaustive analysis of the dimensional model of the DSM-5 but a review of its potential advantages and certain limitations attributable to this type of system, considering methodological aspects involved in the generation of evidence and underlying hypotheses and problems that remain unsolved.

Critics and Limitations of the Categorial Model for PDs in the DSM-5

Explicitly, the authors of the DSM-5 postulated that their categorical perspective implies the understanding of PDs as isolated and differentiable entities [4], which contrasts with the overlapping of diagnostic criteria between different categories and the number of possible combinations that could lead to confirmation in each case. In addition, it has been pointed out that the division into three clusters (A, "eccentric"; B, "dramatic, emotional, and erratic"; and C, "anxious, fearful") seems to be based on a simplicity criteria, despite the great amount of evidence that questions its empirical validation [1]. Even so, some of the arguments that justify their permanence cannot be dismissed either. It is due to the fact that the system expresses in a dichotomous way the decision linked to the "need or inadmissibility of administering treatment." Moreover, it facilitates the communication and training of clinicians and aims the discussion to large pathological groups supported by a long academic tradition [2]. As a counterpart, Esbec and Echeburúa [1] have indicated that it generates a discrepancy between the patient's symptomatology and the underlying theory, promotes inconsistencies between evaluators, and sustains superimpositions between the PDs –presumably sustained over time – and the clinical disorders traditionally ascribed to the axis I of multiaxial diagnosis.

In summary, the most relevant critics of the categorical model of the DSM seem to be based on four main axes:

1. There is no empirical evidence that validates the definition of the PDs in terms of 10 dichotomous variables.
2. It has not been precisely justified why a number of necessary symptoms are chosen – and not another – for a given diagnosis.
3. High levels of comorbidity and overlapping between categories hinder both the clinical and the planning of investigations [4].
4. The temporal endurance, the center of differentiation between PDs and clinical disorders, often is not observed in axis II diagnoses [2].

For this reason, a dimensional system would presumably help to solve certain difficulties by clarifying the diffuse limits between pathology and normality; solving the problem of comorbidity by exploring measurable traits in all people;

improving the reliability of the evaluation; and helping to capture the complexity of the pathologies [1].

The Alternative Evaluation of the DSM-5

Skodol et al. [5] presented the alternative model of personality evaluation that was later reflected in the final edition of the manual. Four elements composed it:

(a) A scale composed of **five levels of severity** in personality deterioration, including an assessment of nuclear and interpersonal functioning;
(b) **Five specific disorders**, defined by pathological personality traits (Antisocial/Psychopathic, Avoidant, Borderline, Obsessive compulsive and Schizotypal).
(c) **Six major domains** of pathological features (Negative Affection, Detachment, Antagonism, Disinhibition and Psychoticism / Schizotypy), with 4 to 10 more specific **facets** in each;
(d) New **General Criteria** for the diagnosis of PD based on extreme or severe deficits of the nuclear capacities and functioning of the personality.

The arguments and evidence provided by the authors to support the inclusion of each element are vast. Given the objectives of this chapter, we will present them in a summary way.

Regarding the *first*, it has been pointed out that *general severity* has been identified as the most important predictor of *current* and *prospective dysfunction* and that failures in the assignment of social categories or attributes appear significantly increased in those with personality disorders. Thus, it has been said that such individuals possess problematic states of the self, personal representations – or "inadequate narratives" – and poor self-regulatory strategies: phenomena also linked to the problematic *narcissism* that many psychological theories postulate.

Regarding *nosography*, they proposed a reduction in the number of disorders – including a narrative description of the types – and a dimensional evaluation of the degree to which the patient resembles each of them. They justified these decisions by the excessive comorbidity between the old categories, the limited validity of some, the arbitrary thresholds established, and the instability of the characteristics evaluated for the diagnosis. Regarding the *domains* and *facets*, they argued that its implementation would solve the problem of comorbidity and the overlapping of criteria, since it offers a complete characterization of the individual personality and explains the similarities or differences between people. The proposed assessment recognizes the existence of a continuity between normality and pathology, and its potential usefulness was postulated even when the existence of a PD is not verified.

Third, the *domains* – with some modifications – are explicitly inspired by the negative pole of each of the factors commonly known as the Big Five.

Finally, regarding the *new general criteria*, they indicated that a personality disorder cannot be defined only by extreme positions in certain domains but also implies a disorganization of the personality and a significant difficulty in developing the important aspects for an adaptive functioning, oriented both to itself and to others (see Table 37.1).

Limitations and Axioms Implied in Dimensional Perspectives

Although the dimensional approaches seem to overcome most of the difficulties attributable to the categorical approach, it is necessary to consider theoretical and methodological aspects that historically outlined some characteristics. In the first place, the term "dimensional" has been used to describe a large number of approaches and heterogeneous modalities aimed at quantifying personality pathologies. Thus, according to Trull and Durret [2], the simplest meaning refers to the practice of quantifying the levels of presence-absence of diagnostic criteria, independently of the nosography that is applied. Examples of this trend are the prototype models of Oldham and Skodol [6] and Westen and Shedler [7].

Table 37.1 Scheme of the alternative evaluation of PDs in DSM-5

Element	Aspect/type		Scale
1. Severity levels	*A. Self* Identity integration Integrity of self-concept Autodirectivity *B. Interpersonal* Empathy Privacy and cooperativity Complexity and integration of the representation of others		**0** = No deterioration **1** = Slight deterioration **2** = Moderate deterioration **3** = Severe deterioration **4** = Extreme deterioration
2. Specific disorders	Antisocial/psychopathic Avoidant Borderline Obsessive-compulsive Schizotypal		**1** = No match **2** = Slight match **3** = Moderate match **4** = Good match **5** = Very good match
2. Domains and facets	*A. Negative emotionality* Emotional lability Anxiety Separation insecurity Pessimism Low self-esteem Blame/shame Self-injury Depressivity Suspicion *B. Detachment* Social withdrawal Social detachment Privacy avoidance Restricted affectivity Anhedonia *C. Antagonism* Insensitivity Manipulability Narcissism Histrionics Hostility Aggression Oppositionalism Scam	*C. Disinhibition* Impulsiveness Distractibility Imprudence Irresponsibility *D. Compulsivity* Perfectionism Perseveration Rigidity Order Risk aversion *E. Schizotypy* Unusual perceptions Unusual beliefs Eccentricity Cognitive deregulation Dissociation propensity	**0 =** Very little or nothing descriptive **1 =** Slightly descriptive **2 =** Moderately descriptive **3 =** Extremely descriptive
General criteria	*Definition* Failure to develop a sense of self-identity and the capacity for interpersonal functioning *Criteria* The aspects of the first element must be verified. Adaptive failures must relate to extreme levels in one or more personality domains; they must be relatively stable at least since adolescence; they should not be better explained by the existence of another mental disorder or by the effects of substances or general health conditions		[*Without scale:* categorial]

A second definition consists of factorially identifying the traits underlying certain PD constructs: the 18-dimensional model of Livesley [8] and the 12-dimensional model of Clark [9] are examples of this perspective. Finally, there are approaches that transcend the strictly psychiatric-psychological constructs and integrate psychometric, neurobiological knowledge, etc:

Cloninger's Seven Factors model [10] and the Big Five Factors model are the main exponents of this perspective at present.

The Model of the Seven Factors

Cloninger describes temperament as the individual's ability to perceive and respond to sensory stimuli, citing a large burden of genetic inheritance [10]. The combination between temperamental variables, subsequent learning, and their interaction with environmental factors generates the expansion of their characteristics; it allows phenotypicity to distance itself from the inheritance and shapes the self as the combination of temperament and character. These two interact and mutually modulate through the propositional and procedural systems allowing learning that would render the hereditary neurobiological structure. It could be argued that Cloninger's model emphasizes the integrative character of all the components of behavior, admitting the genetic basis as the foundation of some personality traits, as well as the importance of environmental structures and the individual interpretations of them.

The Model of the Big Five Factors

Since the 1980s, innumerable evidence has been gathered showing that the aspects affected by the main classical dimensional theories can be precisely grouped into five main factors [11–14], one of whose numerous denominations can be detailed as follows: I, extraversion/introversion; II, pleasantness/hostility; III, responsibility; IV, emotional stability; and V, culture intelligence. Despite the favorable findings, limitations derived from at least two sources cannot be ignored: their foundation in the so-called lexical hypothesis and the characteristics inherent to factorial analyses.

On the "Lexical Hypothesis"

The model of the Big Five Factors is an heir of the lexical paradigm. It argues that the human sense or interest is codified or represented in language. The latter could thus be understood as a sedimentary deposit of people's observations, in which the necessary terms would be found to define the main characteristics of the human personality. In a critical review, Richaud de Minzi [15] noted that the current consensus on the existence of five superfactors can only be understood from a historical perspective. Thus, she indicated that Allport and Odbert [16], influenced by the interest of Germans Klages [17] and Baumgarten [18] on the analysis of language as a way of knowing the human personality, provided the first list of 4504 names ascribable to the determinant, stable, and consistent tendencies of the adjustment of the individual to his environment.

Later, Cattell [19–21] gradually revised the original list, finally arriving at a set of 12, using a factor analysis limited by the statistical resources of his time. Tupes and Cristal [22], for the first time, attributed reliability and recurrence to a set of five factors, although their work was not free of questionable statistical choices. For example, the synonymy between terms was not considered as a possible semantic explanation of their confluence. Although the "lexical hypothesis" – whose influence is often omitted – is attractive, it has not been proven. In the words of Mc. Rae and Costa:

> No one could suppose that an analysis of common terms for parts of the body would provide an adequate basis for the science of anatomy. Why should personality be different? [23]

About Factor Analysis

The use of factor analysis involves a number of decisions that may condition the fate of the results of a study [15]. The first one is the set of variables that goes under evaluation, whose structuring "prefixes" initially how many and which factors will be found. Thus, their grouping may be due to semantic and conceptual issues and not necessarily linked to the underlying structure of the personality. This condition is a reason for questioning the so-called universality [16] of the five-factor model, since it is not part of a previous psychological theory but rather derives remotely – as previously indicated – from Cattell's own list [14].

The Use of Self-Reports and the Conscious Assessment of Traits

Although the evaluation practices suggested by the authors of the different dimensional models are heterogeneous, most of them include the use of self-administered questionnaires, based on comparative, simple, general, and vague statements [24]. Among the most recognized are the MMPI-II of Hathaway and McKinley [25]; the NEO-PI-R of Costa and McRae [26]; the MCMI-III of Millon, Davis, and Grossman [27]; the 16-PF of Cattell of Eber and Tatsuoka [28]; the EPQ of Eysenck and Eysenck [29]; and the PANAS of Watson of Clark and Tellegen [30].

The Minnesota Multifaceted Personality Inventory [25] from its origins in the 1940s is one of the most widely used psychological tests worldwide due to its high standards of reliability and validity. The authors created the technique with an empirical-rational approach, comparing analysis of stories, attitudes, and ideas frequent in different patients with the characteristics exposed in the nosology of Kraepelin. Its adaptation to the Argentinian context in 1989 made it possible to exponentially increase its use, resulting in an indispensable technique in the clinical and legal spheres. Through 567 items of dichotomous response, the evaluated construct is pathological personality, understood as those lasting characteristics of a subject that are determinants of their behavior. The technique has three validity scales and nine clinical scales that allow obtaining information about different personality traits. The intrinsic difficulties associated to the instrument are related to its long extension and the outdated theoretical criteria and terms.

The NEO-PI-R test by Costa and McRae [26] is a self-conscious instrument, presented as a nonexclusive clinical test, suggesting its use in the educational and organizational context. It has the model of the Big Five as a theoretical basis and explicitly excludes standards from the rest of the literature concerned. One of the admitted weaknesses of the instrument is its low internal consistency. Some authors mention the natural impossibility that presents for retesting, for interrater observation, and even for longitudinal follow-up [31].

The MCMI-III instrument of Millon, Davis, and Grossman [27] is oriented with the diagnostic system ratified in the editions of DSM-4 and DSM-5 grouping the categories in the axes I towards the clinical disorders and II towards the disorders of the personality and mental retardation. Through a scoring system, it maintains a scale of values that indicate the severity of psychopathology.

Some criticisms about these instruments are focused on their limited contribution to the symptomatological description of depressive disorders and on methodological errors presented by the major depression and anxiety disorder scales: as described, they show a considerable number of false positives and false negatives. A professional review of each result is necessary [32]. The lack of literature regarding its methodological validity was also highlighted, with the lack of interevaluation cross-sectional studies or analysis of internal coherence regarding collateral data or forensic analysis. Some authors [33] consider the validation of anxiety scales to be poor, making it difficult to classify their severity and even to discriminate against depressive symptoms.

Cattell's 16PF test [28] consists of an evaluative questionnaire of 16 personality traits called first-order factors which are identified as affectivity, reasoning, stability, dominance, impulsivity, group conformity, daring, sensitivity, suspicion, imagination, cunning, guilt, rebellion, self-sufficiency, self-control, and tension. All individuals could be described based on these traits, and the difference in the extent of each of them would confirm the personality. Some of the questioning of the proposed instrument focuses on the critique of Cattell's model, which, among other things, subtracts from the semantic character of language and makes it prone to misunderstandings or coherence problems. On the other hand, authors like Nowakowska [34] question that Cattell has not been exhaustive enough in the verification of

his hypotheses about the personality before elaborating the instrument to analyze its features.

The personality questionnaire of Eysenck and Eysenck [29] called EPQ is used in a self-administered form, answering items in a dichotomous way. It results in a description of the personality that represents the combination of its intervening factors that can be classified in a double axis of four points: stability-neuroticism, extroversion-introversion, normality-psychoticism, and lability-veracity.

Basing his theory mainly on the model of the Big Five, many of the criticisms are made about the supposed anachronism of the model or the low methodological reliability, despite having obtained coefficients greater than 0.7 in internal coherence and retest. However, the same authors recognized certain psychometric weaknesses and reformulated the scales by adjusting the arithmetic mean. It has also been pointed out that the scales are not intercultural, so they are ineffective in certain social contexts and that, on the other hand, although the use of the first version of the test is discouraged, it is still the most used in the world. It can be inferred a lack of knowledge of their reviews or a considerable difference of values between different countries [35].

Finally, the PANAS affectivity scale elaborated by Watson, Clark, and Tellegen [30] evaluates positive and negative affectivity through 20 items with a Likert-like response. It supports its construct in the hypothesis that the characteristics of extroversion in personality are linked to positive affectivity and introversion or neuroticism to negative affectivity. Based on this postulate, depression and anxiety could be discerned, as well as the correlation of the values contributed by this scale with respect to other personality evaluations validating their reliability [36, 37].

Although this instrument has found some correlation with scales of other complexity, some authors argue that the dimensional analysis within the factorial construct tends to limit the expression of some characteristics of the subject, showing that negative affectivity can be quickly related to a determined set of symptoms. This tendency cannot be verified in terms of positive affectivity and anxiety symptoms or their consequences on personality traits [38].

All the aforementioned instruments can contribute to the evaluation of the level of prototypicity and the severity of a disorder, but this does not eliminate the need for a qualitative clinical assessment of the structure and functioning of the personality, as well as specific subjective and interpersonal difficulties. In this sense, the perspective of Westen et al. [39] makes an important contribution, since it emphasizes the approach by prototypes and proposes a protocol for recording the observations of clinicians [7] that is not limited only to the patient's conscious self-perceptions. In this way, intrapsychic and dynamic aspects of the personality [1] are included.

In the alternative system of the DSM-5, the clinician is in charge of collecting the information to perform a characterization by prototypes, domains, and facets. In any case, it would be important to consider which techniques were applied in the studies that have provided their theoretical foundations.

The Differentiation Between Health and Pathology

From a dimensional perspective, to define whether a personality has pathological characteristics, it is necessary to have "thresholds" or "cut-off points" [40]. Even when population statistical indicators can be established, any limit imposed is in some sense arbitrary – we remember that Skodol [5] admits continuity between normality and pathology – and may be useless for the actual treatment. The definitions that only consider the "universal" aspects bring with them the risk of a partial clinical judgment that obscures the particularities of the individual case.

As McAdams [24] warned, nuclear features indicate very little about the characteristics that differentiate people and motivate their actions: these aspects seem unattractive to the methodologies usually used in dimensional models.

Synthesis and Discussion

A Dimensional System Could Optimize Existing Nosography?

Given that the categorical constructs of the DSM have shown little empirical validity, it is reasonable to suppose that the dimensional perspective would make the definition of the PD more objective. However, the high use of self-administered questionnaires for the collection of information fosters a bias towards people's conscious self-assessments, while the factorial techniques underlying the theories generate reasonable doubts about their construction and validity. Likewise, such limitations do not invalidate the usefulness of the dimensional approach, although it is reasonable to note that without meticulous and systematized clinical observations, a vague, trivial, and atheoretical nosography could be reached, providing limited usefulness to understand the genesis and evolution of the pathologies. In this sense, we consider remarkable the proposals that involve registration protocols for professionals, as well as contrast methodologies with standard cases.

The construction of an optimal nosography should cover the currently existing taxonomy in the clinical and research areas and encourage the revision of its categories. Otherwise, the use of overlapped parameters that make it difficult to trace the evolution and treatment could be insisted upon.

A Dimensional System Would Enrich the Existing Theories?

Part of the resistance to the adoption of a dimensional methodology in the clinical context can be ascribed to the need to maintain continuity with theoretical constructs that have been shaped and perfected over long periods of time. All the theories concerning the structure of the personality are subject to criticism, but they also integrate valuable postulates and synthesize an effort of several centuries dedicated to the understanding of key aspects of human existence. In this sense, we consider that the revisions that are carried out from now on should contribute to a more complete understanding of the conditions that affect the development of the personality. Therefore, they should not be based on a mere statistical pragmatism that gives apparent objectivity to the systems we adopt for the study and the clinic.

The theoretical reformulations must not be exempt from scientific rigor and should incorporate the new contributions that neurobiology and cognitive psychology have made in this field. Every scientific model must have the precision to account for the observable and flexibility to explain the data that future advances may obtain. The arrival of determining classifications should not be – a priori – an obstacle to appreciate the singularities of each case.

A Dimensional System Would Simplify Clinical Decisions?

Among the aspects mentioned above, two are contradictory to each other: although theoretically the use of a dimensional system would clarify the limits between normality and pathology by focusing on dimensions attributable to all people, it would maintain the need to establish certain boundaries or cutoff points. In this sense, it may be pertinent that clinical decisions were based on the specific impact that the identified characteristics have on the patient's daily life: the difference between condition and health should be based on the presence of unwanted or disabling manifestations for the patient's personal and social development.

It is at this point that clinical expertise must be present and determine the process to be followed with each case. An important aspect to consider is the frequent egosintonicity of the symptoms: the self-perception of the patient would not in all cases be sufficient for an objective analysis, for which additional sources of information must be required. Therefore, caution should be exercised when making estimates regarding the intensity of a condition or the need for its treatment by the mere application of statistical procedures. At this point, we mention the need to review the tests of external validity

Table 37.2 Differences between the categorical and dimensional proposals

Categorial systems (DSM)		Dimensional systems	
Weaknesses	Strengths	Weaknesses	Strengths
Low empirical construct validity	Simplicity and communicability	Several theories adopt the lexical hypothesis based on nondemonstrable assumptions	They can be turned categorial for teaching purposes
High overlapping of diagnostic criteria	Adequate to large and traditional pathological groups	They start from factor analysis that can hold certain biases	They admit a continuity between normality and pathology
Assignment of comorbidities that make treatment design difficult	It makes it easier to decide whether to administer a treatment or not	They maintain the cutoff point problem	They do not propose comorbid pathologies but dimensions ascribable to all people
Possible disagreement between disorders and concrete symptoms		They are frequently based on self-administered questionnaires that only address conscientious aspects	They facilitate the understanding of the complexity of the disorders
Low validity of temporal criterion as a distinction between axes I and II			They can be enriched from comparison with clinical prototypes

and internal coherence of some instruments commonly used for diagnosis.

The following table outlines the route proposed by this review. Finally, possible future debates are opened, and some considerations are recommended to propitiate this path (Table 37.2).

Conclusions

The consideration of the dimensional perspective involves important contributions for the diagnosis and treatment of PDs. Although it maintains some difficulties – especially about therapeutic frontiers – it allows a more detailed description of the problems and could contribute to the definition of the relevant aspects of personal history and development, in which deepening is key to deciding therapeutic modalities. The comparison with prototypes is also a valuable resource for clinical training.

We consider that the conditions of this transition reflect the current effort to encompass the profound complexity of the human personality and to reconcile it as much as possible with the scientific refinement that is expected from any discipline. There are several personality theories currently recognized. It is unlikely that its synthesis will be reached: they start from different assumptions and have been built using unequal technical procedures. Perhaps this diversity contributes to maintaining high levels of effort that result, little by little, in a better approach to the ailments.

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Epilepsy and Psychiatric Comorbidities: New Approaches and Perspectives

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Introduction

Different studies indicate that patients with epilepsy report that their quality of life is significantly affected. In addition, it has been reported that patients with epilepsy (PWE) have higher rates of mental disorders than the general population, as will be discussed later on in this chapter. Some of the consequences of this disease have a well-recognized impact on the lives of patients, such as the severity of seizures, its frequency, and the perception of self-control and autonomy; but some others are not, such as social (discrimination, bullying), emotional, occupational, and cognitive performance.

Commonly, the main target of the usual treatments aims to diminish the frequency and severity of seizures; but the psychosocial problems are frequently left aside, making an important impairment of the quality of life of these patients and their families.

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Epilepsy and Depression

Mood disorders and depression within them are more frequent in people with epilepsy than in the general population [1]. This association between epilepsy and depression has been observed since the time of Hippocrates [2]. A new perspective emerged in the 1970s when research emphasized the study of depression in this population [3]. More recently, between 1990 and 2010, with the development of the new antiepileptic drugs, seizures have been greatly reduced, but the adverse effects are still very significant, and the risk of suicide has been reported with the use of some of them [4].

In recent years, some studies have shown interest in a different aspect to the reduction in the frequency of seizures: the impact of depression on the quality of life of those suffering from epilepsy. There is evidence of shared pathogenic mechanisms and a bidirectional relationship between depression and epilepsy [5, 6]. On the one hand, the psychosocial factors of suffering from a chronic disease such as epilepsy can predispose to depression. But on the other hand, depression and epilepsy share biological alterations, among them are the neurotransmitters involved in both pathologies (serotonin, dopamine, GABA, glutamate), the affected brain areas (amygdala, hippocampus, frontal lobe), and even the alterations of the hypothalamus-hypophysis-adrenal axis that generate a chronic state of cortisol increase [7].

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Selective serotonin reuptake inhibitors (SSRIs) used as antidepressants show anticonvulsant properties. Fluoxetine and citalopram decrease seizure frequency in experimental models of epilepsy. The anticonvulsant and antidepressant actions of these drugs may restore the HPA axis function and may be, in part, due to actions on GABA-A receptors. SSRIs have been shown to increase neurosteroid levels and normalize stress hormone levels in depressed patients. The evidence of HPA axis abnormalities in both epilepsy and depression implicate a common pathophysiology of these disorders and imply that HPA axis modulation may be a useful target for treating both disorders [7].

Besides, the iatrogenic effect generated by some antiepileptic drugs (AE) must be considered. As mentioned, they may present depression as an adverse effect (e.g., phenobarbital, phenytoin, topiramate, levetiracetam) [8]. Following the bidirectional relationship, a four- to sevenfold risk of developing epilepsy has been found in people who have depression with respect to those who do not have it, a risk that increases as the severity of the depression does [9].

An important point in the diagnosis of depression in patients with epilepsy lies in the fact that suicide has been indicated as one of the causes of preventable death of epilepsy. In addition to the iatrogenic effect of the AE already mentioned, the longer duration of epilepsy, the early age onset, and the high frequency of seizures have been associated with an increased risk of suicide [10].

When depressive symptoms are taken into account in temporal relation with the seizures, they can be premonitory of a seizure (preictal), present as aura (ictal), or subsequent to it (postictal) [11]. These forms of presentation of depressive symptoms are brief and stereotyped. Decreasing the frequency of seizures in cases of preictal, ictal, and postictal depression leads to a decrease in the appearance of depressive symptoms without needing any other therapeutic intervention in general. The term "interictal depression" is commonly used to describe the affective symptoms not temporally related to seizures, being the most common psychiatric disorder in people with epilepsy (PWE) [11, 12].

Temporal lobe epilepsy (TLE) especially when it becomes resistant (RE: resistant epilepsy) is the most frequent and has the highest comorbidity with depression. Depression prevalence has been reported between 24 and 72% if considered throughout life and around 30% if the current diagnosis is considered [1, 13, 14]. In addition, TLE has been described as a particular form of depression called "interictal dysphoric disorder" characterized by fluctuating course, irritability, anxiety, fear, somatic symptoms, psychotic symptoms occasionally, and, in severe forms, suicide attempts [15].

Finally, psychiatric disorders in general and depression in particular are considered factors of poor postsurgery prognosis of epilepsy. Those patients have shown a worse response to surgery in terms of seizures frequency [16, 17]. Nowadays, depression in patients with epilepsy continues to be underdiagnosed and/or untreated despite being recognized as one of the most important factors that deteriorate the quality of life, beyond the impact that the seizure frequency in RE has [10].

Epilepsy and Psychosis

The relationship between psychoses and epilepsy continues to be still controversial, despite the advances made in the matter. On the one hand, there would be an antagonism between both processes, where epileptic seizures would seem to protect the development of psychosis. On the other hand, there might be an agonism in which epilepsy would behave as a facilitator of the appearance of psychosis.

Psychotic disorders in patients with epilepsy (PWE) are often misdiagnosed and consequently lingering on needlessly. While early diagnosis is unanimously supported as a first step to avoid this problem, the necessity of switching from antiepileptic drugs with adverse psychotropic effects to others is more controversial [18]. Currently, epileptic psychoses have been categorized into four groups: chronic and acute interictal psychoses (IIPs), postictal psychosis (PIP), and the rare ictal psychotic events, such as nonconvulsive status

epilepticus. They comprehend 95% of psychoses in PWE [18]:

1. Acute interictal presentations: they are of short duration (days or weeks) and are usually observed in periods of low frequency of seizures. These forms of psychosis have been associated with the phenomenon called "forced normalization" and the appearance as a side effect of the administration of the new antiepileptic drugs, mainly topiramate and levetiracetam [19, 21–24].
2. Chronic postictal psychosis: they have a chronic course and are difficult to differentiate from schizophrenia. The clinical differences lie in a greater preservation of affection and the presence of visual hallucinations in the forms associated with epilepsy. They have been described mostly in patients with long-standing epilepsy (10 to 15 years) and poor response to antiepileptic treatment [19, 25, 26].
3. Postictal psychoses: they are the most frequent forms of presentation, constituting 25% of the total of the psychoses described in epileptic patients after episodes of repetitive epileptic seizures (seizures in cluster) or after a recent recrudescence of the seizures. They usually have a short duration (days or weeks) and resolve completely with antipsychotics at low doses, although in some patients with recurrent forms, they might become chronic. Clinically they constitute confusional psychoses, with disturbance of the clarity of the conscience, and the affective symptoms are usually prominent [19, 20].
4. Ictal psychosis: this form of psychosis corresponds to an epileptic status that clinically manifests as a behavioral disorder; obeys to epileptic discharges that extend over time; and is detectable in most cases with surface EEG [19].

From a physiopathological point of view, the theoretical explanatory models of the development of psychosis in an epileptic patient include, on the one hand, the experimental phenomenon of kindling and the physiopathological role of epileptic seizures as modifiers of cerebral plasticity. On the other hand, there is the phenomenon of "forced normalization," which is observed in some cases of epileptic psychoses, which appears simultaneously with the improvement of seizures.

The model of kindling, developed by Goddard and collaborators in 1969, is an experimental model that allows the reproduction of epilepsy in animals. Is based on repeated electrical stimulation of certain brain areas belonging to the limbic system, which results in discharges that progressively increase in amplitude, duration, and frequency, with propagation to other unstimulated areas. This phenomenon culminates with the appearance of spontaneous generalized seizures [27, 28]. The kindling constitutes a potential mechanism to explain the development of psychosis in an epileptic patient. According to this model, the recurrent ictal activity would favor the propagation of the abnormal activity from the epileptogenic focus to other areas of the CNS, generating secondary epileptogenesis. The ictal activity would provoke plastic changes that would involve the neurobiological mechanisms responsible for the development of the psychiatric symptomatology [29, 30].

What has been described up to now places epileptic seizures as a facilitating element in the development of psychosis. The controversial issue is that in some cases the opposite phenomenon is observed, where psychosis appears after the clinical and electroencephalographic improvement of the seizures.

The Hungarian doctor von Meduna (who studied the theoretical basis of electroconvulsive therapy (ECT), between 1935 and 1938) was the first to postulate an antagonistic relationship between psychosis and epileptic seizures. He maintained the theory that in epilepsy there would be a glial hyperfunction, while in schizophrenia the opposite would occur [31]. In 1953, Landolt published a study of serial electroencephalographic records of epileptic patients during a psychotic episode. The latter called it "forced normalization" [32, 33]. Towards the year 1965, Tellenbach uses the term "alternative psychosis" to refer to the clinical counterpart of the electrical phenomenon described by Landolt [34]. Slater and Beard pub-

lished a comprehensive report of chronic psychoses in PWEs [26]. Finally in 1988 the concept of PIP was clearly demarcated by Logsdail and Toone, which turned out to be a very important concept in our contemporary understanding of epileptic psychosis [18, 35]. This phenomenon has been observed by different authors in patients with severe psychiatric disorders during periods of improvement of epilepsy. With the introduction of the new antiepileptic drugs, reports of psychosis associated with seizure improvement increased. Postoperative psychoses have also been described, associated to the improvement of epilepsy [29, 31, 36].

An explanation suggested by some authors is that the compensatory brain inhibitory processes of the epileptic seizures would act on the epileptogenic focus, managing to prevent the propagation of the abnormal electrical activity. The epileptic activity would then be limited to subcortical structures, responsible for psychotic symptoms, not measurable by the surface EEG [36].

For the moment epilepsy seems to be a nonspecific precipitating factor leading to psychosis in patients predisposed to psychosis. This theory has accumulated strong supportive evidence, especially from large-scale family history studies. However, in some cases psychotic states closely appear during epileptic activity on EEG in continuous aura or spike-wave stupor. Sometimes PIP is closely associated with seizure activities and frequently can be stopped by eliminating seizures with either medication or surgical intervention [18].

The risk factors commonly considered for the development of epileptic psychosis are:

1. Severe and intractable epilepsy with an early onset of seizures (especially before adolescence)
2. The secondary generalization of partial seizures
3. The presence of a bitemporal or left focus [19]

The longer evolution time of epileptic seizures and the presence of bilateral hippocampal sclerosis could increase the risk of developing psychosis in patients with refractory partial epilepsy. A higher percentage of psychosis (19 to 27%) was observed among epileptic patients belonging to specialized centers in epilepsy, where a poor response to treatment predominates, than the frequency found in patients with epilepsy attended in nonspecialized centers, where a lower incidence of psychosis was found, closer to that reported in the general population (0.7 to 7%) [37].

In a study conducted on a total of 1285 epileptic patients, it was found that 24.3% of patients with TLE presented some type of psychiatric abnormality excluding mental weakness [19]. The frequency of psychotic states was 14.1% among patients with TLE and of only 3% among patients with idiopathic generalized epilepsy [38]. These findings had already been described by authors such as Slater and Beard, who found that 55 of the 69 patients studied with schizophrenic-like psychosis had a temporal epileptic focus [26].

In all subtypes of psychosis (PPI, PII, and PB), a significantly higher incidence of bilateral hippocampal sclerosis has been observed. Previously, other authors described that bilateral structural and electrical alterations constitute a risk factor for the development of psychosis. A possible explanation for these findings could be that in patients with unilateral structural compromise, there would be compensatory mechanisms that would take place in the healthy hemisphere and that could act as protectors of the development of psychosis, whereas this would not occur in those patients with bilateral structural compromise [37, 39].

Epilepsy and Anxiety and/or Stress

Stress is an adaptive mechanism that mobilizes the organism to respond fast and appropriately to threatening or challenging motivations [40]. It may be described as a state or a trait, either generalized or situational, and may be associated with psychosocial situations, psychiatric illness, or the effect of chronic medical illness. Regardless of its origin, it makes most medical illnesses worse [7].

In clinical studies of epilepsy patients, stress is the most frequently self-reported trigger of sei-

zures, more than other precipitants such as sleep deprivation, fatigue, diet, or even missed medication [41–44], with almost 50% of patients reporting feeling stressed prior to the seizure [7].

Mesial temporal lobe epilepsy (MTLE) will be specially considered as it is the most prevalent form of refractory epilepsy in adults, and its pathophysiology could be linked to stress [45]. Among a wide range of early life factors implicated in MTLE causation, stress might play an important role. There is a vast amount of neuroscientific data showing the enduring effect that it has on the anatomy and function in the hippocampus and amygdala, the key structures involved in MTLE. Early life stress might be implicated in the pathogenesis of both MTLE and the psychiatric comorbidity that often accompanies it.

The widest framework that englobes a multistage process of epileptogenesis is a "three-stage hypothesis" [46] that proposes:

- An initial neural insult perinatally or early in childhood
- A latent period, in which structural and functional changes take place at a cellular and molecular level
- A chronic stage, with spontaneous recurrent seizures

How these changes result in epilepsy is yet poorly understood, despite the accumulation of many insights. Nevertheless, the effect of stress might be highly relevant to all stages of epileptogenesis. Some evidence is direct, showing associations between experimental stressors and epilepsy; some other data are indirect showing effects of experimental stress on diverse biological processes implicated.

Neurobiology of Stress and Seizures

It is well-known that the physiological response to stress is mediated by the HPA axis. The parvocellular neurons in the paraventricular nucleus (PVN) of the hypothalamus control the activity of the axis. Corticotropin-releasing hormone (CRH) neurons release CRH into the hypophyseal portal system which acts in the pituitary gland to signal the release of ACTH. ACTH triggers the release of cortisol from the adrenal cortex in humans (corticosterone in rodents). The action of steroid hormones occurs through steroid hormone receptors, translocation of this ligand-bound receptor complex to the nucleus, and activation or repression of gene transcription by binding to a glucocorticoid response element in the promoter of glucocorticoid-regulated genes. Glucocorticoid signaling occurs via both a "fast" negative feedback mechanism, which is thought to involve non-genomic actions, and a "delayed" negative feedback mechanism that involves genomic actions. These effects are mediated by two different types of glucocorticoid receptors, mineralocorticoid receptors (MRs), and glucocorticoid receptors (GRs), respectively. Basal levels of stress hormones are elevated in PWE and are also increased after seizures.

The HPA axis is regulated by numerous different brain regions and neurotransmitters. Limbic regions, in particular the hippocampus, express an important quantity of mineralocorticoid and glucocorticoid receptors and are sensitive to regulation by glucocorticoids.

The hippocampus is considered to inhibit the HPA axis. It has a high density of mineralocorticoid and glucocorticoid receptors further implicating the hippocampus in the negative feedback regulation of the HPA axis. Finally, it has the capacity for neurogenesis in the adult animal.

Stress and glucocorticoids might inhibit adult neurogenesis. Hippocampal neurogenesis may also play a role in the regulation of the HPA axis. Interestingly, neurogenesis has also been implicated in changes in HPA axis regulation associated with depression. The effects of antidepressant drugs may involve enhanced hippocampal neurogenesis and consequent restoration of normal HPA axis function.

Stimulation of the amygdala increases glucocorticoid secretion, and lesions to it reduce glucocorticoid secretion. This structure expresses a high density of glucocorticoid receptors and a lower density of mineralocorticoid receptors, being implicated in the negative feedback regulation of the HPA axis.

The medial prefrontal cortex has a region-specific role in the regulation of the HPA. The infralimbic cortex projects to the bed nucleus

of the stria terminalis (BNST), the amygdala, and the nucleus of the solitary tract, all of them involved in activation of the HPA axis. The prelimbic cortex projects to the ventrolateral preoptic area, dorsomedial hypothalamus, and peri-PVN regions, which are involved in the suppression of the HPA axis. There is a high density of glucocorticoid receptors in the prefrontal cortex, implying that the prefrontal cortex may also be a site of negative feedback regulation of the HPA axis.

Finally, there are few direct connections between limbic structures and the PVN. The limbic system connects to the PVN via relay neurons, which are primarily interneurons in the BNST or peri-PVN region. For a more specific review, see Maguire et al. [7].

Early Life Stress and Neuroplasticity

Several forms of neuronal reorganization have been implicated in medial temporal lobe epileptogenesis. Stress biology has been implicated extensively in several of these aspects of limbic neuroplasticity [47], most notably the dendritic structure of CA3 pyramidal and amygdala neurons [48, 49] and dentate gyrus neurogenesis [47, 50, 51]. Both acute and chronic stress can suppress dentate gyrus neurogenesis and/or cell survival and can induce dendritic remodeling in CA3 [47]. Corticosterone elevation has a key role in these phenomena, but its effects are complex. Stress in early life can have short-, medium-, and long-term effects on the hippocampal structure [52–56], as can normal variations in maternal care [57].

Many of the genes identified in microarray analysis of epileptic limbic tissue participate in neuronal plasticity [58]. Seizures themselves, especially during childhood, are an important insult [59]. They possibly alter the development of brain circuits [60–64]. Hence, the neural reorganization seen in MTLE may be both cause and effect of seizures [65, 66].

Such effects of early life stress could be relevant to limbic epileptogenesis in different ways. First, they may play a role in seizures and epilepsies with onset in early life [67]. Second, they may contribute to the first stages of evolution of MTLE, long before clinical emergence of seizures. Third, they may create an enduring vulnerability to limbic epilepsy that emerges only with onset of neurodegenerative disorders or other cerebral insults or pathology in later life [68].

Several studies, derived from the experience of those who suffer from epilepsy, set the stage for a wealth of experimental data indicating that stress may impact and exacerbate epilepsy in at least four ways. (1) Life stress, particularly early life stress, may create a vulnerability for the incidence of epilepsy [41]. (2) Stress may play a role in the etiology of symptomatic epilepsy by exacerbating the causal event, such as traumatic brain injury, stroke, or status epilepticus [42]. (3) Stress may play a role in the process of epileptogenesis: the "silent period" that follows initial injury and is characterized by progressive cellular and network changes thought to underlie the ultimate onset of chronic seizures [43]. (4) Stress may increase the frequency or severity of seizures after epilepsy onset [69].

Early Life Stress and Endocrine Response in Later Life

Given the important role of glucocorticoids (corticosterone in rodents, cortisol in humans) in normal regulation of hippocampal and amygdala electrophysiology and neuroplasticity [70], there is ample reason to consider their role in limbic epileptogenesis, especially in the immature brain. Indeed, for mature animals there exists strong and consistent evidence that chronically elevated corticosteroids aggravate epileptogenesis [71–74].

Early life stressors can "program" HPA axis responsivity perhaps for the lifetime of the organism, as well as having a range of neurobiological and behavioral effects [75, 76]. The direction of HPA axis "programming" – whether hyper- or hypo-responsivity – varies considerably by timing and type of stressor and by species [77].

These alterations, which are affected by cognitive mechanisms (neural plasticity in reinforcing stress-responsive networks) and genetic transcriptional mechanisms (classical and epi-

genetic regulation of genes controlling the HPA axis), lead to adult animals that have an impaired stress response to aversive stimuli, including an increase in stress hormone release and impairment of HPA negative feedback [78–83].

Other hormones have been implicated in the modulation of seizures and epilepsy, notably neurosteroids [84–88] and sex hormones [7, 89, 90]. Both groups are implicated in psychiatric disorder and epilepsy and may have a role in the causation. These data from animal models demonstrate the influence of early life stress on several hormonal systems, influences which may be relevant to limbic epilepsy [68].

Early life stressors have been shown to have enduring effects on brain serotonergic function in rats [91] and nonhuman primates [92], with some preliminary evidence that this may also be the case in humans [93]. A further reason to consider serotonergic function associated to epilepsy is that SSRIs (serotonin specific reuptake inhibitors), widely used to treat depression, appear to have an anti-seizure action [94]. Fluoxetine, an SSRI, has been reported to reverse some of the adverse hippocampal effects of maternal separation stress [95]. Early life stress may also have an important impact on the development of white matter in the brain [96].

Effects of Prenatal and Postnatal Stress on the Development of epilepsy

Few studies have examined the contribution of early life stress to epilepsy in later life in humans. A population based cohort study tried to examine whether maternal exposure to stress increased the risk of epilepsy in the first decade of life. Hospitalization due to epilepsy was used as the outcome measure. However, the data shown did not suggest a strong association between prenatal stress and epilepsy [97].

There seems to be a "time window" for early life stress effects [68]. Neurobiological, behavioral, and endocrine effects of early life stressors and of exposure to stress intermediaries (such as glucocorticoids) vary by timing of exposure. There is some human [98] and animal evidence [99]. Rats exposed to early life stress exhibited significantly lower seizure thresholds and an accelerated rate of kindling, compared to early handled rats [73, 100].

The role of astrocytes in this process has come to be one of the most studied frontiers in epilepsy research, due to the effects of activated astrocytes and gliosis on regulating excitability via extracellular ions and neurotransmitters and to the association of glial scars with hippocampal sclerosis [101, 102].

While GCs are generally thought of as anti-inflammatory and indeed often used as therapeutic peripheral anti-inflammatory agents, they actually have pro-inflammatory roles within the brain [103].

Thus, stress would be expected to enhance pro-inflammatory pathways that are major aspects of epileptogenesis [44].

Aberrant neurogenesis in the hippocampus is also a hallmark of epileptogenesis [104, 105]. Generally, stress decreases neurogenesis at both proliferation and survival stages [106] but also decreases the percentage of precursor cells that adopt a neural cell fate [44].

Stress May Exacerbate Etiological Incidents and the Frequency or Severity of Seizures

One of the common occurrences across different types of precipitating incidents is immediate neurological injury and cell death. GCs exacerbate neural injury [107–110].

Breakdown of the blood-brain barrier (BBB) is also common across etiological incidents. Interestingly, stress also disrupts the BBB [111–113] and thus may directly contribute to postinjury BBB leakiness, likely through induction of pro-inflammatory pathways [114].

Corticotropin-releasing hormone (CRH) – which is released in the brain as the first step of the stress hormone response – causes an increase in neural discharge and modulates glutamatergic transmission [115, 116]. GCs themselves increase the release of excitatory glutamate [117], while

stress paradigms similarly induce an increase in extracellular glutamate and aspartate [118].

Epigenetic Approaches

Epigenetic mechanisms might underlie many of the gene changes associated to epilepsy. Chromatin structural alterations after seizures, including DNA methylation and histone modification, may have effects on genes relevant to the development of epilepsy, especially those coding for glutamate receptors and BDNF [119]. Early life stress, too, induces enduring epigenetic alterations of the promoter regions of relevant genes [99, 120, 121], and so a synergy between these chromatin modifications may result in alterations in gene transcription, leading to accelerated rates of epilepsy.

Serotonin-specific reuptake inhibitors (SSRIs) are known to affect processes involved in limbic epileptogenesis, such as dentate gyrus neurogenesis [122–124]; to mitigate effects of early life stress in experimental studies [95]; to moderate the HPA activation that is often a part of the depressed state [125]; and to reduce seizure activity in humans [94]. However, the actions of antidepressants on limbic epileptogenesis – both before and after the onset of overt seizures – are not yet known.

These diverse lines of evidence support the proposition that early life stress exposure may have a causal role in MTLE.

Epilepsy and Anxiety Disorders

In clinical practice and research alike, most of the attention has been focused on depressive disorders (DD), as they are the most frequent psychiatric comorbidity [126]. Although anxiety disorders (AD) are the second most frequent psychiatric comorbidity in patients PWE [126], they remain underrecognized and undertreated despite the fact that they have a negative impact on the life of these patients as DD [127].

Anxiety might be a symptom of the ictal phenomenon, either as a component of a partial seizure or as an aura, in anticipation of a seizure event, as a postictal phenomenon, as an adverse consequence of AED treatment, or as a psychiatric response to having a chronic illness. Seizures arising in limbic regions, such as in temporal lobe epilepsy, are most frequently associated with anxiety, and electrographic recordings support the role of limbic structures involved in anxiety associated with epilepsy. Furthermore, seizure severity is a predictor of anxiety levels [7].

Posttraumatic stress disorder (PTSD) is an anxiety disorder resulting from a consequence of experiencing a severe traumatic event, involving the threat of injury or death. Similar to other anxiety disorders, there is also a connection between PTSD and epilepsy [128]. Long-term changes in HPA axis activity have been associated with PTSD and may play a role in the comorbidity of PTSD and epilepsy [7]. A recent study has shown that individuals with PTSD had a higher incidence of developing epilepsy with an earlier onset of epilepsy than did the controls [129]. Besides, patients with epilepsy who report stress as a seizure precipitant are more likely to endorse a history of childhood traumatic experiences, particularly emotional abuse, compared with those who do not perceive stress as a precipitant [130].

As in the case of DD, AD have a negative effect on the life of PWE at several levels. For example, the presence of anxiety symptoms at the time of diagnosis of epilepsy was associated with a worse response to pharmacotherapy after a 12-month follow-up period [131]. In a South Korean study of 154 outpatient adults with epilepsy, the presence of anxiety symptoms was the most important factor in explaining a worse quality of life [132]. The effect of AD on the suicidality risk of PWE was illustrated in a Danish population-based study, in which AD increased the risk of completed suicides by 12-fold relative to people without epilepsy [133].

These data clearly demonstrate the need to identify and treat comorbid AD in PWE, in particular when they occur together with DD. Selective serotonin-reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRI) have become the first line of pharmacotherapy of primary AD. Although there are no controlled trials for the treatment of AD in PWE, there is a general consensus that these psycho-

tropic agents are equally effective and safe in PWE. Benzodiazepines are prescribed for the initial 4 to 8 weeks of therapy to achieve an early symptom remission, as the therapeutic effect of SSRIs and SNRIs may be delayed by 4 to 6 weeks. Cognitive behavior therapy (CBT) has been shown to be an effective nonpharmacologic treatment modality for primary AD. Furthermore, a combination of CBT and an SSRI or SNRI has been recommended in particular when AD and DD occur together [127]. Anxiety disorders are frequent in patients with refractory focal epilepsy, and clinicians should carefully examine their patients with this important comorbidity in mind [127].

Despite the enormous amount of information that links stress with epilepsy in different ways, there are still some doubts. According to a review done by Novakova and colleagues, the evidence from human studies for stress as a trigger of epileptic seizures is inconclusive. Prospective studies have yielded mixed results, and studies of life events suggest that stressful experiences only trigger seizures in certain individuals. There is limited evidence suggesting that autonomic arousal can precede seizures. For this author, stress is likely to exacerbate the susceptibility to epileptic seizures in a subgroup of individuals with epilepsy and may play a role in triggering "spontaneous" seizures [40]. Nevertheless, if all the data provided is taken into account, we can asseverate that stress plays an important role at least in the psychopathology of epilepsy.

Psychogenic Nonepileptic Seizures (PNES)

Psychogenic nonepileptic seizures (PNES) are diagnosed when disruptive changes in behavior, thinking, or emotion, resemble epileptic seizures (ES), are present without epileptic cause (paroxysmal neuronal discharges detectable by EEG), and are not originated from another medical illness. Usually, the PNES diagnosis emerges from an erroneous judgment of "epilepsy" which is frequently due to an inadequate interpretation by the physician of the episodes reported by the patient or their relatives. In the case of patients with epilepsy who develop PNES, it is the patient or the family who give the label of "epileptic seizures." Recognition of this entity increased significantly in the last years, following the setting up of Video-EEG.

Psychiatrically, PNES represents an underlying psychiatric disorder, categorized in Axis I of DSM-4, and many studies showed high rates of conversive, dissociative, and other coexisting disorders in these patients. Different psychiatric factors, like sexual abuse, trauma and posttraumatic stress disorder (PTSD), dissociative disorders, and personality disorders, have been associated with poor outcomes of PNES.

PNES accounts for approximately 20% of all intractable seizure disorders referred to epilepsy centers and have been found to occur also in epileptic patients with a frequency that ranges from 20 to 60%.

Psychiatric Diagnoses: DSM-IV and DSM-5

In a study ran by D'Alessio et al. [134], psychiatric disorders in patients with PNES, with and without comorbid epilepsy, were compared. Conversion and dissociative disorders were diagnosed frequently, considering PNES as the core syndrome.

More frequent disorders were somatoform disorders (pain, somatic complaints, autonomic dysfunction, etc.), affective disorders (major depression and dysthymia), and posttraumatic stress disorder (PTSD).

In Axis II, high rates of personality disorders were diagnosed in patients with PNES. The most common type of personality disorders found was Cluster B (borderline personality disorder and histrionic personality disorders) and Cluster C (dependent personality disorder).

Trauma and traumatic experiences have been considered a predisposal factor for developing dissociative experiences and dissociative forms of PNES. Historically since Freud and Janet, dissociation syndrome was related to traumatic experiences. Trauma, particularly sexual abuse, has been proposed by many authors as a major factor in the pathogenesis of PNES, and high

rates of PTSD have been usually reported among PNES patients.

Dissociation can be described as a structured separation of mental processes (perceptions, emotions, memories, and identity), ordinarily integrated in and accessible to conscious awareness. The quality of awareness and voluntary control of consciousness is disrupted in pure PNES and in dissociation, instead of complete loss of consciousness observed in PWE [134].

Nowadays, DSM-5 provides a new way of naming these disorders: "functional neurologic disorders" [135]. This terminology provides a causal neutrality, and it can also increase the knowledge and acceptance of the patient. In DSM-5, the term conversion disorder has been retained as an alternative expression that recognizes unconscious processes in the patient (but is less frequently used in neurology due to its implicit inference of causal psychological stressors, which are not always present, or they cannot be easily identified). For a more detailed review, see Espay et al. [136].

Psychiatric and Psychological Management of Epilepsy

Focusing on these psychosocial variables, different interventions have been developed, such as psychological treatments or psychoeducational and self-management programs, for different types of patients – adults, seniors, children, and adolescents, among others – and patient's family members.

In this section we will list some of the psychological treatments that have been developed for patients with epilepsy. For this we will refer to the type of intervention, the type of patients to whom these treatments are oriented, as well as the main objectives for outcomes.

Psychoeducational and Self-Management Approaches

In this section we will mention some treatments of "self-management" of epilepsy, understanding this as the health-related behaviors and adaptive activities that a person can perform to promote the control of seizures and improve their well-being.

(a) *Adults with Epilepsy*

For adults with epilepsy, different research groups developed short treatments (mainly 2-day long), with the aim of improving medical knowledge about the disease. Many of them obtained good results in terms of improving knowledge about epilepsy, less fear of seizures and improving treatment adherence [137], lower seizure frequency [138, 139], decreased depressive symptomatology, neurotic disorders, and a better understanding of the disease [140], compared with those groups that did not receive the intervention. Some of the interventions were designed to be delivered by nurses specialized in epilepsy [141–143].

Other interventions consist in the administration of a self-administered questionnaire that links the intention of taking antiepileptic drugs with a specific time and place or situation, in order to improve the behaviors related to the taking of medication in patients with epilepsy [144]. Participants in the intervention reported improvements in the number of doses they took, the days on which they correctly took the prescribed amount of medication, and the doses taken on schedule. Other interventions, of longer duration, were developed for the same purpose by different research groups. For example, Pramuka et al. [145] designed a 6-week intervention aimed at improving the self-efficacy and quality of life of adults with epilepsy. Although they did not find significant differences in relation to the general quality of life, they did find differences around specific dimensions, such as limitations in roles and emotions. Based on a rigorous evaluation to design a self-help program [146], Fraser et al. [147] developed the PACES (program for active consumer engagement in self-management) for patients with epilepsy. It is a group program to improve self-management skills and quality of life in these patients. In a randomized controlled trial, participants reported improvements in different dimensions of self-management measures and quality of life, both at the end of the intervention and at a follow-up at 6 months, compared with the control group

(a) *Children with Epilepsy and Their Relatives*

Based on the aforementioned MOSES (modular service package for epilepsy) study, Pfäfflin [148] evaluated an intervention for relatives of children with epilepsy (FAMOSES: modular service package for epilepsy for families). The group of parents who participated in the program reported improvements in knowledge about the disease, adaptation, anxiety about epilepsy, and seizure management. Also, the children of the participating parents reported a lower seizure frequency, compared with those in the control group.

In addition, Modi [149] evaluated the effectiveness of an intervention for parents of children with epilepsy aimed to improve children's treatment adherence. The intervention consisted in four face-to-face sessions and two telephone sessions during 2 weeks, oriented to problem-solving. Participants of the intervention group reported improvements in the adherence of their children's treatment, as well as greater knowledge about epilepsy.

Jantzen [150] designed a program for children and adolescents with epilepsy and their parents. It consists in a group program whose main objectives are to transmit knowledge about epilepsy in an age-appropriate manner, teach coping strategies, and strengthen the children's autonomy.

Empirically Supported Psychotherapies

Different treatment interventions have been designed for patients with epilepsy with cognitive and behavioral orientations, such as cognitive-behavioral therapies (CBT) and acceptance and commitment therapy (ACT). Among these, we find interventions for adult patients with epilepsy, patients with psychiatric comorbidities – especially depression – older adults and adolescents, and patients with drug-resistant epilepsy (DRE).

a. Adults with Epilepsy

Many of the cognitive-behavioral treatments for adult patients with epilepsy are aimed at improving the quality of life and the management of epileptic seizures. These have been designed both in group format [151, 152] and individually [153]. Also, given the characteristics of most cognitive-behavioral treatments, these include psychoeducational modules, training in self-awareness, compensatory cognitive strategies, and application of these strategies to everyday life [154].

In addition, many of the cognitive-behavioral treatments were designed to treat some of the most frequent psychiatric comorbidities in patients with epilepsy, such as mood disorders. For example, Davis et al. [155] used an intervention that included cognitive-behavioral techniques in patients with epilepsy and depression, with the aim of improving the management of depressive symptoms. They found improvements in anxiety and anger symptoms and an increase in social activity. Beck's depression inventory (BDI) scores also declined. Ciechanowski et al. [156] evaluated the effectiveness of a comprehensive cognitive-behavioral program, which includes training in problem-solving, behavioral activation, and psychiatric treatment, for patients with depression and epilepsy. They found improvements in indicators of depression, suicide risk, and psychological well-being, compared to the control group. In Mexico, Orjuela-Rojas et al. [157] compared SSRI and CBT in patients with TLE and major depressive disorder, finding improvements in depressive symptomatology and quality of life in both groups.

b. Specific Populations: Adolescents and Older Adults with Epilepsy

In adolescents, Martinovic et al. [158] evaluated the usefulness of a CBT group intervention to prevent depressive symptoms in teenage patients recently diagnosed with epilepsy.

For older adults, McLaughlin and McFarland [159] designed a 6-week cognitive-behavioral group intervention to improve depressive symptoms, frequency of seizures, and psychosocial functioning. Although they did not find significant differences between the CBT group and the control group in the indicators of depression and psychosocial functioning, they did find significant differences in the decrease of seizure frequency, in favor of the CBT group.

c. Drug-Resistant Epilepsy (DRE)

In the UK, Goldstein et al. [160] evaluated the results of a CBT intervention in patients with DRE with psychiatric comorbidities and/or psychosocial difficulties. They found improvements in the psychosocial problems associated with epilepsy reported by patients, although there was no effect in seizure frequency.

Lundgren et al. [161] developed an acceptance and commitment therapy (ACT) intervention for patients with DRE, with the aim of obtaining improvements in quality and satisfaction with life and seizure frequency. They compared ACT vs a support group (control group), finding that the ACT group obtained significant improvements in these measures. Analyzing the mediators of change, they found that many of the principles of ACT, such as acceptance, defusion, persistence, and value orientation, were involved in the improvement of these patients [162]. On another occasion, they compared ACT with yoga, concluding that both therapies used as complements are useful to obtain significant improvements in the quality of life of patients with epilepsy [163].

Also, in a prospective study with controlled patients with resistant epilepsy, Dewhurst et al. [164] evaluated the cost-benefit of an ACT intervention. They found significant improvements in quality of life, anxiety, social adjustment, self-esteem, depression, and seizure frequency. The authors stated that the results obtained justify the realization of RTC for this type of approach.

Conclusion

Epilepsy is a complex and multicausal disease. Some cases may be simple and respond easily to classic interventions, but others may be associated with a severe decline in quality of life. These patients should take us to a greater effort to understand their network of problems and to work as a team among professionals from different areas. Today it seems that only the multidisciplinary approach can generate an all-encompassing aid. Perhaps the greatest challenge lies in achieving adequate communication between the entire team, patients, and their families, since, at least in our experience, in health systems there is not a strong culture of teamwork and mutual collaboration.

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Integrative Objective Quantification of Individual Locomotor Behavior in Depressive Patients: Implications for Their Stratification and Personalized Treatment Monitoring

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Abbreviations

CCG	Cranio-corpo-graphy
LMB	Locomotor behavior
PMDs	Psychomotor disturbances
STEMPA	Spatial-temporal equilibriometric movement pattern analysis
STEPi	Spatial-temporal equilibriometric psychomotor index

Introduction

Psychomotor disturbances (PMDs) are cardinal features of endogenous (melancholic) depressions [1–12] with important diagnostic, pathophysiological, and therapeutic implications [9, 13–28]. They are the main objectively measurable dimension of depressive psychopathology [29–46] and could be informative on underlying neurobiological mechanisms [22, 47–57]. PMDs are frequently used to subdivide depressive disorders in order to stratify them into more homogenous subgroups for specifically targeted treatment approaches [1, 12, 36, 45, 46, 58–67]. According to the current diagnostic criteria of international classifications (ICD-10 and DSM-5), the endogenous (unipolar and bipolar) depressions involve PMDs with two opposite deviations from the norm (retardation *or* agitation). However, mostly the psychomotor retardation (slowness of movements) is explicitly discussed in the scientific literature as a defining feature of melancholic depressions [e.g., 13, 20, 23, 50, 61, 68]. Therefore, not surprisingly just this direction of psychomotor deviation from the norm has attracted the attention of almost all researchers in the field [e.g., 1, 5, 6, 13, 18, 20, 23, 33, 34, 48, 52, 57, 69–82]. Some of them have been specifically focused on the bidirectional associations between psychometrically rated locomotor slowness and depression severity [68–75]. On the other hand, psychomotor retardation in melancholic depressions has been frequently regarded as phenomenologically and neurobiologically similar to parkinsonian bradykinesia [76–82], thus being associated with underlying basal ganglia dysfunctions and striatal hypodopaminergia [15, 20, 23, 47, 83–97]. As parkinsonian bradykinesia involves inhibited locomotion, known as

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"parkinsonian gait," which is objectively measurable in neurological patients [98–100], analogous instrumental approaches for objective quantification of gait patterns have been proposed as a measure of depression-associated slowness of movements [17, 29–32, 35, 44, 76, 82, 96, 101–107]. Another objective and quantitative approach to depressive PMDs is the global assessment of patients' locomotor activity by means of actigraphy, cinematography, actometry, and accelerometry [4–6, 14, 18, 25, 26, 33, 34, 37–39, 41, 46, 48, 51, 52, 63, 66, 67, 108–118]. However, up to now these objective and quantitative instrumental methods have been restricted to research purposes, as they might discriminate patients from healthy controls only by statistical group comparisons, but not at the single-subject level. We must emphasize that available to date laboratory methods for comprehensive motion analysis are extremely sophisticated, expensive, and time-consuming, thus being inconvenient for regular clinical use. In their daily practice, clinicians continue to evaluate and measure depressive PMDs mostly subjectively – through observation and observer-rated scales [119–122]. However, such subjective approaches are difficult to generate precise and reliable measurements [28, 42, 44, 123–128]. Therefore, a novel, less sophisticated, inexpensive, and user-friendly instrumental method for a more precise and reliable objective quantification of PMDs at the individual-patient level is needed, so as to be applied in the clinical practice for improving diagnosis and treatment of patients with endogenous depressions.

Innovative Approach for Objective Quantification of Individual Locomotor Behavior

Consistent with this hitherto unmet need, we have developed and introduced in the daily clinical practice of psychiatry and neurology an original (internationally patented) method for integrative objective recording, representing, and measuring locomotor behavior (LMB) at the single-patient level [129]. It is based on the classical equilibriometric method "Cranio-corpography" (CCG), invented by Claus-Frenz Claussen, one of the founders of clinical equilibriometry as a branch of clinical neurosciences [123, 124, 130–136]. For more than 20 years, we are applying the computerized ultrasonic version of CCG (www.zebris.de), which allows a very precise spatial-temporal equilibriometric quantification of the head and body movements during execution of the classical locomotor "stepping test" of Unterberger [123, 124, 128–136]. The test consists of stepping in place with eyes closed for 1 minute, starting from the standard standing position of Romberg [123, 124]. The principle of the method is illustrated in Fig. 39.1.

The stepping test computerized ultrasonic CCG is easy to perform, user-friendly, reliable, reproducible, noninvasive, and not time-consuming (1 test = 1 min). Moreover, its results are understandable for clinicians, and the method could be routinely applied in the clinical practice by the treating psychiatrists themselves or by their technical assistants, e.g., nurses or students. Quantitative evaluation is achieved by the well-established polar reference net [123, 124, 130, 131]. Movements of the head and shoulder markers then appear as the radar-like images of four moving objects, progressing in an interrelated direction. The computer program automatically measures some standard CCG parameters, thus providing a very precise quantification of individual LMB, which could serve as an integrative objective measure of PMDs at the single-patient level. Our team has discovered that optimal equilibriometric quantification of PMDs in psychiatric patients might be achieved by three essential CCG parameters [123, 124, 128–130, 133, 136–142]: longitudinal displacement, lateral sway, and number of steps per minute (see below). Their numerical values are displayed on the computer screen, along with a visual comparison with the limits of normative data. Thus, immediately after the end of each test, it can be seen whether the value of a given CCG parameter is outside or inside the normal limits. Together with the numerical data, the graphical gestalt CCG images might also serve for discriminating between normal and abnormal LMB even at a first glance. As

Fig. 39.1 Stepping test computerized ultrasonic cranio-corpography

an illustration of such CCG visualization, we demonstrate a prototypical example of normal stepping test LMB in a healthy control subject (Fig. 39.2).

As could be seen, the gestalt CCG image of individual LMB visualizes the normal tendency for anterograde displacement of the body center of gravity that leads to unconscious and unintentional forward movement (anteropulsion) after every single step. Its total sum is measured (in centimeters) by the CCG parameter "longitudinal deviation." Our previous research has revealed [128, 133–135, 137–142] that lower values of this parameter might reflect psychomotor inhibition with underlying striatal hypodopaminergia, while higher values might reflect psychomotor activation with underlying striatal hyperdopaminergia. The CCG parameter "lateral sway" measures (also in centimeters) the maximal range of alternating (inverted pendulum-like) medial-lateral and lateral-medial head and body movements during the swing phase of locomotor cycle. This unstable one-leg position (see Fig. 39.1) leads initially to a medial-lateral displacement of the head and shoulders (contralateral to the lifted leg), followed by a reflexive (defensive) movement in the opposite direction that is aimed at preventing an eventual fall. Although such fluctuations of the upper part of the body are measured in space, they indirectly indicate the velocity (which in fact is a temporal measure) of unconscious and automatic psychomotor *reactivity* that helps to maintain equilibrium during stepping locomotion. So, large lateral sway (hypermetria) indicates slower psychomotor reactivity (brady-reactivity), while narrow lateral sway (hypometria) indicates hasty psychomotor reactivity (tachy-reactivity). The third essential CCG parameter that is relevant for PMDs is the "number of steps" (measured per minute). It reflects the self-paced rate of conscious and volitional, self-initiated, goal-directed psychomotor *activity*. A lower number of steps (bradykinesia) indicate psychomotor hypoactivity, while a higher number of steps (tachykinesia) indicate psychomotor hyperactivity. The normal numerical values of these three psychomotor CCG parameters could be conceived as indicating normal LMB in healthy individuals. Their lower and upper limits (provided by the computer program

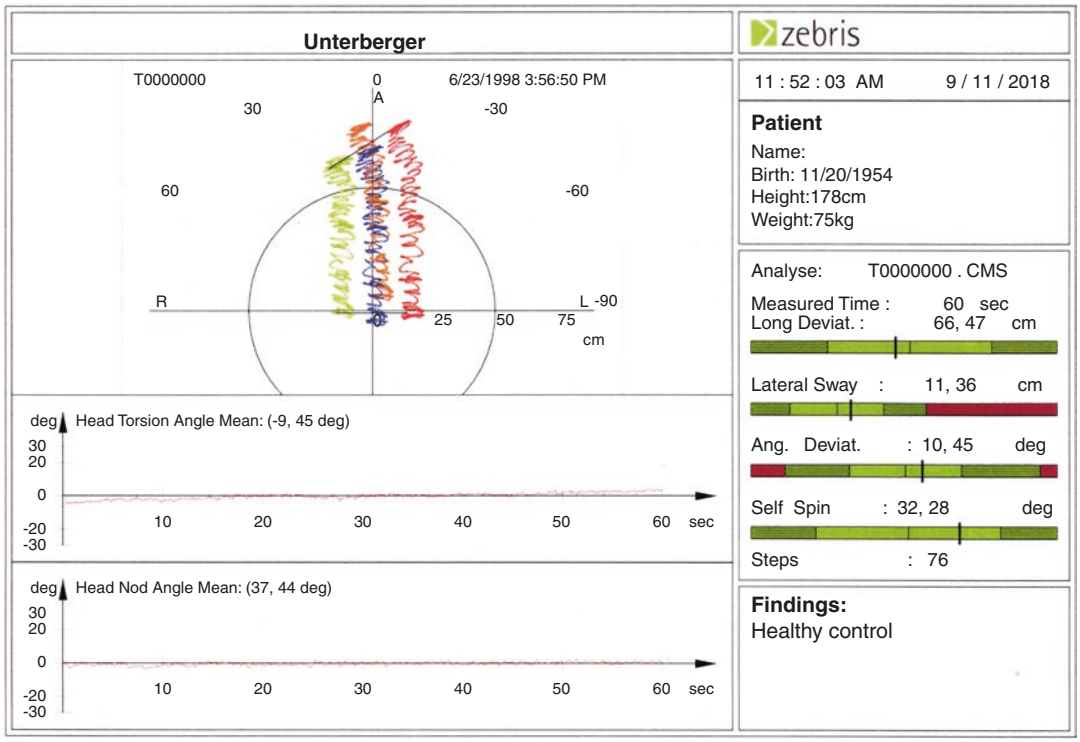

Fig. 39.2 Prototypical normal stepping test locomotor behavior in a healthy control subject

"Winbalance") have been determined after systematic investigations of hundreds of clinically healthy subjects.

Objective Quantification of Individual Locomotor Behavior in Depressive Patients

A representative sample of 200 depressive patients was selected from our computer database for further analysis. All patients were with clinical diagnosis "major depressive episode, recurrent" (F33), according to the ICD-10. They were included in our selection if they had stopped their previous medications so that their baseline CCG investigation has been performed before initiating a treatment for the current depressive episode. Graphical gestalt CCG images and quantitative values of the 3 psychomotor CCG parameters (quantifying their individual stepping test LMB) were compared case by case with those of 200 age-, gender-, height-, and weight-matched healthy controls. These objective graphical (qualitative) and numerical (quantitative) comparisons demonstrated that the selected sample of "unipolar" depressive patients in fact represents a heterogeneous group, which includes two contrasting poles of abnormal LMB, reflecting subclinical PMDs with *bipolar* deviations from the norm (with some intermediate variants between the two extremes). The one pole (–) combines markedly shortened longitudinal deviation, enlarged lateral sway, and lower number of steps per minute, thus suggesting subclinical but objectively measurable abnormal psychomotor inhibition (reduced unintentional anteropulsion, delayed automatic psychomotor reactivity, and decreased volitional self-paced psychomotor activity). As an illustration we demonstrate a prototypical example of such over-inhibited stepping test LMB (hypolocomotion) in a depressive patient (Fig. 39.3).

The opposite pole (+) of abnormal LMB combines markedly prolonged longitudinal deviation, narrower lateral sway, and higher number of

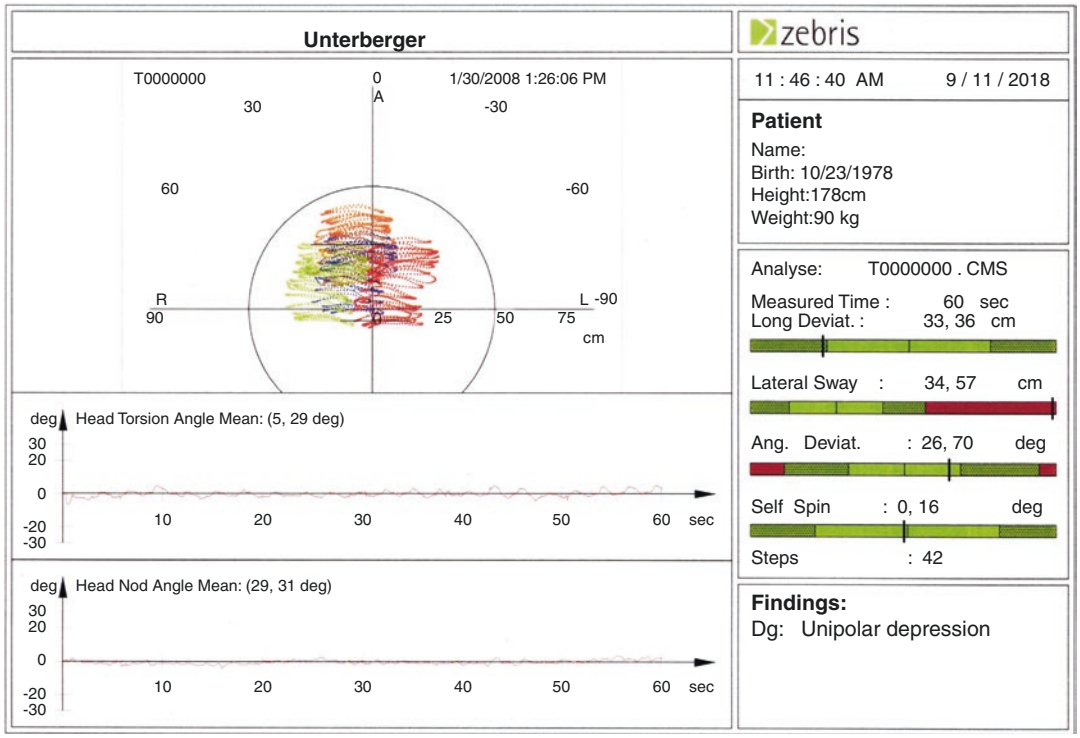

Fig. 39.3 Prototypical inhibited stepping test locomotor behavior in a depressive patient

steps per minute, thus suggesting subclinical but objectively measurable abnormal psychomotor activation (exaggerated unintentional anteropulsion, accelerated automatic psychomotor reactivity, and increased volitional self-paced psychomotor activity). As an illustration of the CCG characteristics of such over-activated stepping test LMB (hyperlocomotion) in a depressive patient, we demonstrate a prototypical example (Fig. 39.4).

Integrative Index of Individual Locomotor Behavior in Depressive Patients

From the prototypical examples presented in Figs. 39.2, 39.3, and 39.4, it is evident that the numerical values of the three essential psychomotor CCG parameters of stepping test LMB are divergent in their directions of deviation from the norm. For instance, higher values of lateral sway reflect decreased and retarded psychomotor reactivity, whereas higher values of longitudinal deviation and number of steps per minute reflect increased and accelerated conscious and unconscious psychomotor activity. Therefore, we have scored and summarized these three separate CCG parameters into a single concise and unidirectional measure of individual LMB, called "spatial-temporal equilibriometric psychomotor index" (STEPi), which is automatically calculated by our original computer program "STEMPA" (spatial-temporal equilibriometric movement pattern analysis). This index integrates the degree of subclinical psychomotor activation, on the basis of computer-measured values of the three psychomotor CCG parameters. The calculated values of STEPi for the prototypical examples, presented in Figs. 39.2, 39.3, and 39.4, compared to the respective computer-measured values of the three psychomotor CCG parameters, are demonstrated in Table 39.1.

It is evident that STEPi reflects in a more adequate and consistent manner the general direction of subclinical psychomotor deviation from the

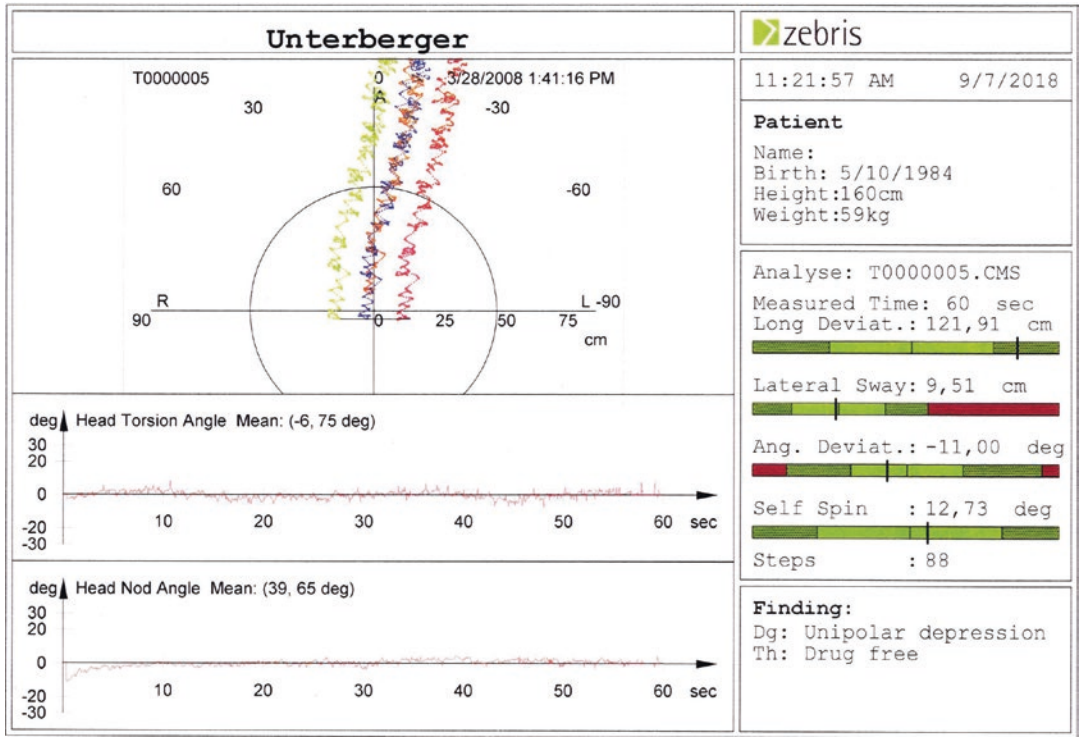

Fig. 39.4 Prototypical activated stepping test locomotor behavior in a depressive patient

Table 39.1 Quantitative measures of individual locomotor behavior in the prototypical examples

Psychomotor CCG parameters	Inhibited depression (*see* Fig. 39.3)	Healthy control (*see* Fig. 39.2)	Activated depression (*see* Fig. 39.4)
Longitudinal deviation (cm)	33.36	66.47	121.91
Lateral sway (cm)	34.57	11.36	9.51
Steps (per minute)	42	76	88
STEPi (degree of activation)	**4.05**	**44.47**	**112.81**

norm in a given depressive patient. At the same time, it is obviously a more sensitive indicator of abnormal (either over-inhibited or over-activated) LMB than each one of the three separate psychomotor CCG parameters. Our quantitative analysis of the individual values of STEPi in healthy control subjects has revealed that they vary between 20 and 80, with a mean value of about 50. So, the individual LMB of a given healthy control subject could be categorized as either relatively inhibited (STEPi between 20 and 50) or relatively activated (STEPi between 50 and 80). Accordingly, if the value of STEPi in a given depressive patient is inside the normal limits, then his/her individual LMB could be also categorized as either relatively inhibited or relatively activated. However, if its value is outside the normal limits, then the individual LMB could be categorized as either abnormally inhibited (STEPi <20) or abnormally activated (STEPi >80). The former could be viewed as a subclinical measure of psychomotor over-inhibition or hypolocomotion (Fig. 39.3), while the latter as a subclinical measure of psychomotor over-activation or hyperlocomotion (Fig. 39.4).

Objective and Quantitative Stratification of Depressive Patients

Our computer program "STEMPA" allows an automatic subclinical categorization of each concrete investigated patient, according to his/her individual value of STEPi. This type of categorization permits objective and quantitative stratification [143, 144] of the entire group of 200 depressive patients, as it is demonstrated in Table 39.2.

It is evident that the objective stratification of individual LMB in the investigated depressive patients distinguishes four psychomotor subgroups, involving two opposite poles: psychomotor inhibition (STEPi <50) or psychomotor activation (STEPi >50), each one of them with 2 degrees of deviation from the mean of the norm: relatively normal (STEPi inside the normal limits) or abnormal (STEPi outside the normal limits). Thus, the baseline LMB of every single depressive patient (measured by the quantitative value of STEPi) can be categorized as belonging to just one of the four stratified psychomotor subgroups. Table 39.2 demonstrates that 43.5% of depressive patients are with abnormal values of STEPi and their individual LMB is either abnormally inhibited (hypolocomotion) or abnormally activated (hyperlocomotion). Hypolocomotion substantially prevails (34.5%) over hyperlocomotion (9.0%). The remaining 56.5% of patients are with relatively normal values of STEPi, but again inhibited LMB substantially prevails (39.0%) over activated (17.5%). Analogous prevalence of inhibited (73.5%) over activated (26.5%) LMB is found in the whole sample of depressive patients.

The substantial prevalence of psychomotor inhibition could explain why almost all researchers in the field *statistically* reveal psychomotor retardation (slowness of movements) in patients with endogenous (melancholic) depressions *as a group*, in comparison with a group of healthy controls [1, 5, 6, 13, 15, 16, 18–23, 33, 48–52, 57, 61, 76, 77, 83, 86, 87, 92, 101, 108, 119, 145–153]. More specifically, objective signs of inhibited gait are commonly found in this group of patients [8, 17, 21, 29–31, 34–41, 44–46, 68–75, 78, 80–82, 96, 101, 102, 105, 106]. It is logically to predict that statistical comparisons at the group level between depressive patients and healthy controls would detect only the prevailing pole of psychomotor over-inhibition (hypolocomotion), but not the opposite underrepresented pole of psychomotor over-activation (hyperlocomotion). So, we suspect that without preliminary stratification a relatively small subgroup of depressive patients with hyperlocomotion would remain statistically undetected.

Dopamine Hypothesis of the Locomotor Bipolarity in "Unipolar" Depressive Patients

Our CCG investigations in psychiatric and neurological patients [8, 12, 22, 24, 28, 36, 44, 45, 60, 123, 124, 128–131, 133–142] attest that the subclinical locomotor bipolarity in "unipolar" depressive patients [60, 136, 138, 140, 141] is not nosologically specific. On the contrary, the two poles of abnormal LMB (hypolocomotion and hyperlocomotion) could be conceived as two transdiagnostic dimensional continua that cross the boundaries among the endogenous psychiatric categories (major depression, bipolar disorder, and schizophrenia) and even the border between psychiatric and neurological categories. On the one hand, we reveal hypolocomotion (Fig. 39.3) not only in a substantial proportion of depressive patients but also in some schizophrenic patients with prominent negative symptoms (syndrome of psychomotor poverty), as well as in most patients with idiopathic or neuroleptic-induced parkinsonian bradykinesia [123, 124, 128–131, 133–135, 137, 154]. On the other hand, we reveal hyperlocomotion (Fig. 39.4) not only in a relatively minor subgroup of depressive patients but also in some particular schizophrenic patients with prominent positive symptoms (paranoia with psychomotor excitation), as well as in most hypomanic or manic bipolar patients [123, 128–131, 133, 137, 139]. We might say that the negative pole of abnormal LMB is manifested by transdiagnostic hypolocomotion (Fig. 39.3), which could indicate

depressive motor slowness, schizophrenic psychomotor poverty, or parkinsonian bradykinesia, sometimes collectively considered as psychomotor indicators of "negative symptoms" [e.g., 80, 87, 154, 155]. The opposite (positive) pole of abnormal LMB is manifested by transdiagnostic hyperlocomotion (Fig. 39.4) that could indicate depressive, manic, or psychotic behavioral over-activation, which might be considered as a psychomotor indicator of "positive symptoms."

On the basis of our clinical, experimental, and theoretical research, we could admit that the phenomenological similarities in the objectively recorded and measured abnormal LMB are indicative for shared (transdiagnostic) underlying neurobiological mechanisms. Thus, the remarkable similarity between depressive hypolocomotion (Fig. 39.3) and analogous hypolocomotion in two well-known hypodopaminergic states, such as schizophrenic psychomotor poverty and parkinsonian bradykinesia, permits us to assume that the negative pole of abnormal LMB might be determined by underlying striatal hypodopaminergia [128, 133, 135, 137–142]. At the same time, the remarkable similarity between depressive hyperlocomotion (Fig. 39.4) and analogous hyperlocomotion in two well-known hyperdopaminergic states, such as schizophrenic and manic psychomotor excitation, permits us to assume that the positive pole of abnormal LMB might be determined by underlying striatal hyperdopaminergia [128, 133, 137–142].

These comparative locomotor dopaminergic analyses reasonably lead to the conclusion that the discovered locomotor bipolarity in depressive patients might be due to underlying dopaminergic bipolarity, with hypodopaminergia contributing to hypolocomotion and hyperdopaminergia contributing to hyperlocomotion [128, 137–142]. Such a dopamine hypothesis [138, 142] is in line with numerous findings in animal models of psychiatric disorders, which demonstrate that dopamine agonists (psychomotor stimulants and antiparkinsonian drugs) might produce hyperlocomotion, while dopamine antagonists (antipsychotic drugs) have the opposite effect and might produce hypolocomotion [94, 95, 156–165]. Besides, based on animal models, in vivo imaging of dopamine, functional magnetic resonance imaging studies, pharmacological evidence, and analysis of the treatment effects on the modulation of dopamine, the most recent version [94] of the old dopamine hypothesis of bipolar disorder provides convincing arguments that the depressive pole might be associated with underlying hypodopaminergia, behaviorally expressed as hypolocomotion, while the manic pole might be associated with underlying hyperdopaminergia, behaviorally expressed as hyperlocomotion. We could admit that the negative pole of abnormal LMB (hypolocomotion), supposedly attributable to underlying hypodopaminergia, should be viewed as prototypical for bipolar depression. In contrast, the positive pole of abnormal LMB (hyperlocomotion), supposedly attributable to underlying hyperdopaminergia, should be viewed as prototypical for bipolar mania.

According to our dopamine hypothesis of the psychomotor bipolarity in depressive patients, only those with subclinical hypolocomotion (Fig. 39.3) could be conceived as being linked with underlying *depressive* hypodopaminergia [47, 80–82, 84–97], while those with subclinical hyperlocomotion (Fig. 39.4) could be conceived as linked with underlying *manic* hyperdopaminergia and/or with antidepressant-induced dopamine dysregulation syndrome [88, 94, 95, 166]. Hence, we might conclude that the over-activated "unipolar" depressions are conceivably more adjacent to the hyperdopaminergic mechanisms of bipolar mania than to the hypodopaminergic mechanisms of bipolar depression. Such a conclusion is supported by the fact that in the last few years, behavioral over-activation is increasingly considered to be a defining feature of the manic pole of bipolar disorder [157, 159–173]. Moreover, since agitated recurrent depressions (known as "melancholia agitata") are classically conceived as mixtures of depressive mood and manic behavioral over-activation, several authors have proposed that such "unipolar" (pseudo-unipolar) depressions should be considered as mixed states (mixed depressions), which most likely belong to the bipolar spectrum [44, 60, 136, 138–142, 174–185]. In the same vein, psychomotor agitation in major depressive disorders

has been found to be a predictive factor of mood-switching (either spontaneous or antidepressant-induced), suggesting that it might be viewed as an indicator of bipolarity [136, 138–142, 168, 185]. In addition, a number of studies have shown that in the course of standard antidepressant treatment, agitated "unipolar" depressions are frequently treatment-resistant [186–189] and associated with elevated suicidal risk [178, 186, 189–193]. Therefore, antidepressants do not seem to be optimal for them, and instead mood stabilizers and/or atypical antipsychotics should be preferred [174, 178–180, 189–194]. From a similar point of view, we might assume that in "unipolar" depressive patients with hyperlocomotion (Fig. 39.4), depressive mood is paradoxically combined with manic behavioral over-activation. Such an ambiguous combination might be caused by underlying *manic* hyperdopaminergia with concomitant *depressive* downregulation in other (e.g., serotoninergic, noradrenergic, glutamatergic, or cholinergic) neurotransmitter systems [138–142]. Further studies are obviously needed, in order to clarify the multifaceted brain and mind mechanisms of these contradictory (mixed) depressive mood states with manic-like hyperlocomotion.

Clinical Implications of the Personalized Approach to "Unipolar" Depressions

We demonstrated that objective and quantitative stratification of the heterogeneous group of "unipolar" depressive patients has resulted in differentiating four relatively more homogeneous psychomotor subgroups (Table 39.2), each of them giving a chance for a more precise and differentiated treatment selection. As a minimum, we get the opportunity to choose divergent treatment strategies for patients with over-inhibited versus over-activated LMB. On the one hand, for baseline hypolocomotion (supposedly attributable to underlying depressive striatal hypodopaminergia), the therapeutical aim would be to achieve normalization through psychomotor activation (via eventual dopaminergic stimulation) at least above the lower limit of the norm. On the other hand, for baseline hyperlocomotion (supposedly attributable to underlying manic striatal hyperdopaminergia), the therapeutical aim would be just the opposite: to achieve normalization through psychomotor inhibition (via eventual dopaminergic suppression) at least below the upper limit of the norm.

Table 39.2 Subclinical stratification of the depressive patients, based on their individual locomotor behavior

Psychomotor subgroups of depressive patients	Relatively normal STEPi	Abnormal STEPi	Total
Inhibited LMB	**78 (39.0%)**	**69 (34.5%)**	147 (73.5%)
Activated LMB	**35 (17.5%)**	**18 (9.0%)**	53 (26.5%)
Total	113 (56.5%)	87 (43.5%)	200 (100%)

Based on the fact that agitated "unipolar" depressions are frequently resistant to antidepressant treatment but usually respond quite well to atypical antipsychotics and/or mood stabilizers [141, 174, 176–180, 184, 186–194], we could say that their underlying neurobiological mechanisms are in fact more manic than depressive. Therefore, such pseudo-unipolar depressions could be conceived as being attributable to underlying latent bipolarity that might predict forthcoming transition to manifest bipolarity with clinically identifiable manic or hypomanic episodes [138–142, 168, 185]. From an analogous point of view, depressive patients with manic-like hyperlocomotion (Fig. 39.4) could be conceived as being attributable to a latent phase of evolving bipolar disorder with initial (emergent) manic hyperdopaminergia, albeit so far with clinically expressed depressive mood. The same over-activated (supposedly hyperdopaminergic) mixed states could explain (at last partially) the often-reported suicidal behavior during antidepressant treatment in "unipolar" depressive patients [141, 142, 178, 186, 189–193]. We might conceive that such paradoxical suicidal behavior is attributable to antidepressant-induced striatal hyperdopaminergia [88, 94, 95, 138–142, 185, 193], which precedes the therapeutical antidepressant effect

on depressive mood and suicidal ideas. As psychomotor over-activation is a recognized risk factor for suicidal behavior in depressive patients [141, 142, 178, 186, 189–193], its early identification by means of objectively detectable hyperlocomotion (Fig. 39.4) could help clinicians to take appropriate prophylactic measures (e.g., by adding atypical antipsychotics and/or mood stabilizers to the current antidepressant treatment) in order to prevent a possible shift from passive suicidal ideas to active suicidal behavior. The same measures could be also effective in reducing treatment resistance to antidepressant drugs [187–189, 193]. Therefore, objectively revealing over-activated LMB in "unipolar" depressive patients could have important clinical implications for preventing the increased risk for suicidal behavior and/or antidepressant treatment resistance in them [138–142, 193].

Additional objective and quantitative stratification of "unipolar" depressions could be achieved, based on their longitudinal treatment response trajectories [144]. Our empirical data show that at least five such trajectories of the baseline *hyperlocomotion* could be differentiated: (1) improvement to relatively normal locomotor activation (normalization); (2) improvement to relatively normal locomotor activation but with a subsequent switch to the opposite direction of deviation from the mean of the norm (to relatively normal locomotor inhibition); (3) lack of significant dynamics toward improvement or worsening; (4) worsening despite the treatment; and (5) improvement to normalization but with a subsequent transition to the opposite pole of abnormal LMB (denormalization to hypolocomotion) during the treatment. At the single-patient level, the same five treatment response trajectories could be represented by changes in the quantitative values of STEPi: (1) from STEPi >80 to STEPi <80 but >50; (2) from STEPi >80 to STEPi <50 but >20; (3) STEPi is comparable; (4) STEPi is even higher than previously; and (5) from STEPi >80 to STEPi <20.

Analogous five alternative treatment response trajectories could be differentiated when the baseline LMB is *relatively activated* but remains below the upper limit of the norm (STEPi >50, but <80): (1) improvement toward the mean of the norm (further normalization); (2) improvement toward the mean of the norm but with a subsequent switch to the opposite direction of deviation from the mean of the norm (to relatively normal locomotor inhibition); (3) lack of significant dynamics toward improvement or worsening; (4) worsening above the upper limit of the norm (denormalization to *hyperlocomotion*) despite the treatment; and (5) improvement toward the mean of the norm but with a subsequent transition to the opposite direction of deviation, even below the lower limit of the norm (denormalization to *hypolocomotion*) during the treatment. At the single-patient level, these five treatment response trajectories could be represented by changes in the quantitative values of STEPi: (1) STEPi is reduced, although remaining >50; (2) from STEPi >50 to STEPi <50 but >20; (3) STEPi is comparable; (4) from STEPi <80 to STEPi >80; and (5) from STEPi >50 to STEPi <20.

Hence, we could state that the dynamics of *activated* LMB in every single depressive patient could be objectively followed up by longitudinal monitoring of the quantitative values of STEPi during pharmacological treatment. Depending on the level of baseline psychomotor activation, the possible treatment response trajectories for the individual patients are at least 10. Obviously, this is a step toward the goal of precision (personalized) psychiatry [195–198], which is part of the modern field of precision (personalized) medicine [195, 197, 199, 200]. More specifically, during antidepressant treatment, the escalation of baseline hyperlocomotion or the denormalization of relatively normal baseline psychomotor activation above the upper limit of the norm might significantly increase the risk for paradoxical antidepressant-induced suicidal behavior. So, in such cases the treatment strategy should be radically reconsidered, and antidepressants should be complemented or substituted by atypical antipsychotics and/or mood stabilizers. On the other hand, the denormalization of baseline psychomotor activation to the opposite direction of deviation from the mean of the norm to relatively normal or even to abnormal psychomotor

inhibition might be viewed as resulting from relative overdosing of the prescribed therapy, and accordingly its dosage should be gradually reduced.

Objective detection not only of baseline hyperlocomotion (Fig. 39.4) but also of baseline hypolocomotion (Fig. 39.3) in the group of "unipolar" depressive patients could likewise have important clinical implications. It is well-known that a significant proportion of retarded depressions could normalize their slowness of movement during antidepressant treatment [17, 19, 20, 23–25, 27, 28, 41, 147, 150–152]. Furthermore, our empirical data show that the psychomotor subgroup of depressive patients with *hypolocomotion* could be additionally stratified, depending on the treatment response trajectories [144]. At least five such trajectories might be differentiated: (1) improvement to relatively normal locomotor inhibition (normalization); (2) improvement to relatively normal locomotor inhibition but with a subsequent switch to the opposite direction of deviation from the mean of the norm (to relatively normal locomotor activation); (3) lack of significant dynamics toward improvement or worsening; (4) worsening despite the treatment; and (5) improvement to normalization but with a subsequent transition to the opposite pole of abnormal LMB (denormalization to hyperlocomotion) during the treatment. At the single-patient level, the same five treatment response trajectories could be represented by changes in the quantitative values of STEPi: (1) from STEPi <20 to STEPi >20 but <50; (2) from STEPi <20 to STEPi >50 but <80; (3) STEPi is comparable; (4) STEPi is even lower than previously; and (5) from STEPi <20 to STEPi >80.

Additional five alternative treatment response trajectories could be differentiated when the baseline LMB is *relatively inhibited* but remains above the upper limit of the norm (STEPi <50, but >20): (1) improvement toward the mean of the norm (further normalization); (2) improvement toward the mean of the norm but with a subsequent switch to the opposite direction of deviation from the mean of the norm (to relatively normal locomotor activation); (3) lack of significant dynamics toward improvement or worsening; (4) worsening with denormalization to *hypolocomotion* (below the lower limit of the norm) despite the treatment; and (5) relative improvement toward the mean of the norm but with a subsequent denormalization to *hyperlocomotion* (above the upper limit of the norm) during the treatment. At the single-patient level, these five treatment response trajectories could be represented by changes in the quantitative values of STEPi: (1) STEPi is reduced, although remaining <50; (2) from STEPi <50 to STEPi >50 but <80; (3) STEPi is comparable; (4) from STEPi <50 but >20 to STEPi <20; and (5) from STEPi <50 to STEPi >80.

We might say that the dynamics of *inhibited* LMB during pharmacological treatment in every single depressive patient could be objectively recorded and measured by longitudinal monitoring of the quantitative values of STEPi. Depending on the level of baseline psychomotor inhibition, the possible treatment response trajectories for the individual patients are at least 10. More specifically, the initial normalization of STEPi with a subsequent denormalization to the opposite pole of hyperlocomotion most probably represents a subclinical transition from "unipolar" depression to bipolar mania, as well as presumably from depressive hypodopaminergia to manic hyperdopaminergia. Such a dopaminergic-locomotor transition could be conceived as a side effect of the antidepressant drug treatment, most probably due to sensitization of the D2 receptors [88, 94, 95, 97]. From a clinical point of view, it could be even more important than the eventual subsequent transition to manic mood. Behaviorally inhibited depressive patients with suicidal ideas could realize a volitional suicidal act (and thus die) as a direct consequence of antidepressant-induced behavioral overactivation, while the manic mood itself is not so dangerous and threatening for the patient's life. Therefore, the over-activating effect of antidepressant drug treatment on the inhibited volitional LMB should be prevented by urgent reconsideration of the treatment strategy, which should lead to complementing or substituting antidepressants with drugs that block the

supposedly sensitized D2 receptors, e.g., atypical antipsychotics and/or mood stabilizers [138–142, 156, 160, 194]. Furthermore, our longitudinal data in depressive patients with baseline hypolocomotion demonstrate that the antidepressant-induced hyperlocomotion in them usually precedes the antidepressant-induced manic mood and could serve as a sign of impending transition to the manic pole of bipolar disorder [138–142, 185, 193]. So, it could be considered as an objectively measurable risk factor for such a transition. On the other hand, persistence or worsening of the baseline hypolocomotion despite the standard antidepressant treatment could be conceived as an objectively measurable risk factor for treatment resistance [187–189] and likewise should lead to a reconsideration of the treatment strategy and eventually to an augmentation with other classes of drugs in order to achieve an optimal treatment response.

We could generalize that at least 20 distinct treatment response trajectories in the heterogeneous group of "unipolar" depressive patients could be subclinically detected, thanks to the introduced integrative objective quantification of the patients' individual LMB. Through our computer database, now it is possible to compare the results from every single examination with the normative data and also with any previous results (whenever available) of the same patient. In such a way, it could be instantly determined whether the investigated individual LMB is inhibited or activated and whether it is inside or outside the normal limits, as well as whether the longitudinal trajectory of the baseline LMB indicates improvement, worsening, or stability. All these comparisons (based on the individual quantitative values of STEPi) are used by our computer program "STEMPA" to differentiate automatically not only between inhibited or activated and normal or abnormal individual LMB but also among distinct longitudinal treatment response trajectories.

Briefly, the routine clinical application of an original instrumental approach to "unipolar" depressive patients has enabled us to achieve an objective and quantitative prospective treatment monitoring at the individual-patient level, which is very close to the ideal of the so-called one-person trials [200] in the modern theory of personalized medicine in psychiatry [195–198]. At the same time, such psychomotor monitoring is in line with the modern tendency to use electronic devices to measure outcomes in clinical research [201]. Improved assessment methods will help PMDs become an important objective dimension of depressive psychopathology [14, 21, 26, 29–46, 63, 66, 76, 92, 96, 101–107, 109–119, 128, 136, 138–142, 146] that is informative on underlying neuropathology [48, 50–57, 75, 86, 91, 108] and antidepressant treatment response [22–28, 128, 147, 150–152, 202]. Particularly, the fast development of wearable inertial sensors as behavioral markers for routine use in clinical gait research [128, 129, 203–208] would permit further precision in the objective quantification of individual LMB as a new approach to PMDs and their dynamics in patients with endogenous depressions.

Conclusion

An integrative objective quantification of individual LMB is implemented for the first time in the clinical psychiatric practice. It allows a more precise stratification and further personalization of treatment in patients with "unipolar" depressions. Additionally, a composite STEPi is calculated in order to provide a user-friendly computerized distinction among treatment-relevant subclinical psychomotor subgroups despite their clinical-phenomenological resemblance. Thanks to the new approach, the neurobiological mechanisms underlying clinical symptoms become more accessible for objective evaluation and analysis. The integrative subclinical LMB is a nonverbal expression of the disease process in the brain, which is neither dependent on the subjective report of the patient nor on the subjective interpretation of the psychiatrist. It is under the level of consciousness and thus is immune against conscious simulation or dissimulation. The chance of detecting objectively measurable abnormalities at a single-patient level is

an incomparable precedent for the psychiatric practice, having in mind that even highly sensitive and expensive neuroimaging methods are not yet capable to detect such individual abnormalities. Application of quantitative locomotor analysis may yield further insights into neurobiological mechanisms of endogenous depressions and their optimal pharmacological treatment. Longitudinal monitoring of treatment response trajectories contributes to an objectively informed selection of treatment strategies and more effective suicidal prevention at the level of individual depressive patients.

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40 Epidemiological Perspectives in Psychosomatic and Liaison Psychiatry

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Introduction

In a background of controversy about the appropriate denomination of what has been called consultation-liaison psychiatry in the USA, the names psychosomatic psychiatry (PP) or psychosomatic and liaison psychiatry (PPL) are increasingly used in Spain [1, 2]. Both medical humanism and empirical or evidence-based science are fundamental philosophical bases in this psychiatric discipline, and its historical roots have been discussed in different publications [3]. Among the empirical bases of PP, there is now a generalized agreement about the high frequency of psychological morbidity in medical patients, and efforts have been done to improve its detection and eventual treatment [4]. The meaning of the psychological morbidity and its potential to influence the somatic, comorbid conditions has to be clarified. However, in this respect, some reputed nonpsychiatrists alerted about the real situation. Lord Platt, for example, in an influential paper, considered that one of the "greatest failures" of clinical science was the "almost complete neglect of psychological factors in disease…." [5]. While there is now evidence that psychological and psychiatric aspects of medical conditions have attracted considerable interest in some medical fields such as cardiology [6], there is wide space for the contribution of experts in psychiatry.

The evidence base of PP requires obviously being careful enough about some traditional psychogenic positions when trying to interpret in the previous century the role of psychological problems in the origin of somatic conditions. The critiques of psychiatrists such as M. Shepherd in this respect were particularly blunt [7]:

> So cavalier a disregard for the complexities of the mind-body relationship and the nature of causality could only have been taken seriously in the climate of opinion then prevailing.

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Shepherd's position was based on an epidemiological perspective, and this brings us to the subject of this chapter, since our nuclear contention is that epidemiological methods and studies may provide and have provided substantial empirical support to the field of psychosomatic psychiatry. The British professor summarized the potential of epidemiological methods in the following categories [8, 9]: (a) the completion of the spectrum of disease; (b) the establishment of outcome; (c) the actuarial assessment of morbid risk; (d) the evaluation of the efficacy of treatment; and (e) the conceptual construction of diagnosis and classification.

In this chapter, based on this outline, we review evidence to support our nuclear contention: epidemiological-type studies have provided important scientific information, often with direct clinical implications in the field of psychosomatic psychiatry. Moreover, the examples to review could suggest new directions in the research.

Studies on the Prevalence and Characteristics of Psychiatric Morbidity in Medical Patients

Epidemiological studies can certainly generate data related to the size and characteristics of the problem of psychiatric morbidity, which are fundamental to document service needs. Classical studies very early documented a high prevalence of psychiatric morbidity in medical wards [10], a finding we confirmed in a series of studies in different medical wards at the Johns Hopkins Hospital in Baltimore [11–13]. Relevant reviews have further supported the early findings [14]. However, wide differences were observed in documenting prevalence rates, and methodological problems were detected, such as the limited representativity of samples, so that it was difficult to reach solid conclusions. Still, during the classical study of the European Consultation-Liaison Workgroup (ECLW), it was estimated that the prevalence of psychiatric morbidity was approximately 30% [15].

More recent, relevant studies in specific clinics or diseases have reported results consistent with investigations in general wards. For example, Singer et al. [16] reported that one-third of the cancer patients in acute care hospitals suffers from mental health disorders and need appropriate treatment. Mehnert et al. [17] have observed, in a proportional, stratified random sample based on the nationwide incidence of all cancer diagnoses in Germany ($n = 4020$), that the 4-week total prevalence for any mental disorder was 31.8% (95% CI, 29.8% to 33.8%). In another important study in a national cohort, Søyseth et al. [18] documented that the current prevalence for mental disorders and psychological distress in patients undergoing evaluation for lung transplantation was 41.5% and the lifetime prevalence was 61.0%. Similarly, Ferreira et al. [19] have reviewed the literature on the prevalence of psychiatric conditions in psoriasis, which may range from 24% to 90%.

Important studies have also been conducted in primary care patients. Classical, early investigations reported that psychiatric disorders in this setting were similarly frequent [20] and were commonly associated with substantial disability [21]. In the same direction, the classical, WHO Collaborative Study carried out in 15 centers worldwide demonstrated the burden of psychological problems in this setting [22]. We also documented in the Zaragoza study, in a representative sample of primary care centers, that 27% of attenders with a new illness episode had psychiatric morbidity [23]. In relation to these reports and close to the primary care investigations are the studies showing the association of physical and psychological morbidity in the general population, as shown in the early reports of Eastwood [24] or Simon and Von Korff [25]. While the findings of these studies were still largely descriptive and correlational, they suggested a causal hypothesis. We have also confirmed in the general population of the older the "clustering phenomenon" and the bidirectional association between psychological and somatic morbidity: the probability of psychiatric morbidity increased significantly in individuals with somatic diseases and vice versa [26].

In relation to the characteristics of the psychiatric morbidity found in medical settings, mood and anxiety disorders have been the main diagnosis reported, although other specific disorders, such as sleep disorders, sexual dysfunction, or even substance-related disorders, have also been found in some studies [19]. Depression has specifically been investigated in medical settings. Walker et al. [27] reported in a systematic review of adults with cancer that the estimated prevalence of depression was as follows: 5–16% in outpatients, 4–14% in inpatients, 4–11% in mixed outpatient and inpatient samples, and 7–49% in palliative care. However, they concluded that only a few studies met adequate inclusion criteria, so that the prevalence estimates were imprecise, and suggested that future studies should use more stringent quality criteria. In relation to this, the same authors have published more recently an important, systematic review and meta-analysis of interview-based studies [28]. They have reviewed 158 relevant articles in general hospital inpatients and found that the average of the reported prevalence of depression was 12% (95% CI 10–15). They concluded that this likely prevalence is high enough to make it worthwhile screening hospital inpatients for depression and initiating treatment where appropriate, but again, higher-quality research is needed to further explore the reasons for the observed heterogeneity in estimates.

Important studies have also been carried out in relation to anxiety in medical settings. For example, Broen et al. [29], in a systematic review of Parkinson's disease patients, found that the average point prevalence was 31%, although, again, a wide discrepancy in the rates reported was observed, ranging 6–55%. The prevalence of health anxiety problems in medical clinics has been reported in another relevant UK study of more than 40,000 patients: 19.8% of them had significant health anxiety, although the prevalence levels varied by clinic, with neurology (24.7%) having the highest prevalence [30]. Moreover, the so-called "organic" conditions, certainly delirium, are often observed in medical patients referred for psychiatric consultation [31], suggesting that these disturbances are also frequent in the medical wards [32].

In relation to the characteristics of psychiatric disorders, the issue of severity is obviously of paramount importance, since it may determine the need for specialized interventions. For example, in the Zaragoza study in primary care, we concluded that most patients had mild/moderate severity levels of psychiatric morbidity and only 2% had severe morbidity [23]. The estimation of the European Consultation-Liaison Workgroup (ECLW) study was that the frequency of psychopathological problems reaches almost one-third of medical inpatients, but it is also important to not overestimate the specialized care needs, since only approximately 10% of them would require a consultation with the specialist. Nevertheless, the prevalence rates are in contrast with the average consult rate, which was only 1.4% in the European Union hospitals [33]. Therefore, the suggestion is that most cases in medical wards may go undiagnosed and untreated.

In view of the prevalence of disorder, the issue of the systematic screening of psychiatric morbidity in medical settings has been raised. Nevertheless, Wilson and Jungner's principles [34] should be considered in this respect, since no systematic screening should be implemented unless there is an available specialized workforce to treat all the patients detected. Only a few solid studies ventured to recommend such screening, in the specific example of cases of depression in medical wards [35]. However, in relation to this, the ECLW considered what the present workforce of PP units is unable to treat all morbidity cases in medical wards and, therefore, should prioritize patients with higher care needs. It was in this way that the concept of "complex cases" emerged and appropriate screening instruments were developed, including the INTERMED interview [36]. "Complex cases" are considered those with commonly severe medical morbidity, accompanied by comorbid psychiatric problems as well as social problems and difficulties with the healthcare system. We have shown in a multicenter study that the prevalence of "complex cases" is 27% in internal medicine inpatients and 5% of cases have severe complexity [37]. Highly

reduced health-related quality of life (HRQOL) and increased healthcare costs have been reported in such cases [38], as well as increased emergency room visits and hospital admissions [39]. A self-assessment version of INTERMED has recently been developed to screen for these cases of biopsychosocial complexity [40]. On the other hand, the challenge of treating "complex" cases with comorbidity is apparent, since the outcome of fragmentation of medical care has been shown to result in the persistence of medical and behavioral problems, increased complications, greater disability, and increased costs [41].

The documentation in epidemiological-type studies showing a high proportion of psychiatric morbidity among medical patients going undetected and therefore untreated [11–13] was the base of the so-called "liaison" strategy, intended to improve the ability of nonpsychiatrists to detect and eventually treat the morbidity [42]. This problem has persisted, although there was increasing evidence about the negative consequences of the psychiatric morbidity [43, 44]. Furthermore, the problem of underdiagnosis has more recently been documented in reviews such as the one by Ferreira et al. [19] in dermatology patients. This takes us to another, related epidemiological subject: the outcome of psychiatric morbidity in medical patients.

Studies on the Outcome of Psychiatric Morbidity in Medical Patients

Epidemiological-type studies include the follow-up of conditions detected at baseline. In relation to psychiatric morbidity in medical patients, this type of longitudinal studies is more ambitious and should provide data about the natural history of conditions and about the outcome, which is relevant to know the implications of the morbidity initially detected; moreover, in case of negative outcomes, these studies could generate a causal hypothesis.

The negative implications of the psychiatric morbidity were very early reported in the literature. Amplification of symptoms [45, 46], poor adherence to medical treatments [47], functional disability [48], and increased medical utilization [49, 50] were all reported in influential studies. We also documented a higher mortality among oncological patients with depression and particularly in those with cognitive impairment [32] and a negative outcome in the 1-year follow-up of somatizers detected in the Zaragoza study of primary care [51].

More recently, important studies have similarly documented the negative outcome of psychiatric morbidity in medical patients. Scott et al. [52] have shown the association of different mental disorders with subsequent chronic medical conditions in a world survey in 17 different countries. Similarly, Berg et al. [53], in a cross-sectional study of 14,239 patients using patient-reported outcomes at discharge and national register data, have documented negative consequences in patients with heart disease, which include increased mortality. Remarkable, recent longitudinal European studies have similarly documented a negative outcome of psychiatric morbidity among cardiovascular patients in a meta-analysis of 10 different studies [54], in irritable bowel syndrome patients in a study with 1964 eligible long-distance travelers [55], and in an 18-year follow-up study of 9514 patients with diabetes [56] or following orthopedic surgeries [57].

The epidemiological data on both the prevalence and the negative outcome of psychiatric morbidity in medical patients certainly have implications for treatment. They may also suggest etiological hypotheses, but different epidemiological designs are required for the study of causality and risk factors.

Studies on Psychopathological Risk Factors of Somatic Morbidity

As shown in the previous paragraphs, there is abundant evidence about the association of somatic and psychiatric morbidity, but, obviously, association does not necessarily mean causation. Some classical, early reviews con-

cluded that not much was known about the nature of this association [58]. Later on, a number of studies suggested that somatic morbidity could lead to psychiatric disturbances [59]. On the contrary, the evidence about the possibility that emotional and psychopathological disturbance could lead to somatic diseases was much weaker, and the last-century, psychogenic theories of somatic conditions were seriously criticized on the bases of insufficient empirical support. The position of M. Shepherd in this respect was very clear [7]:

Since then, there is a body of literature suggesting the negative outcome of the psychopathological morbidity in medical patients, as documented by the studies reviewed in the previous paragraphs. In this context, a number of epidemiological-type studies have faced the challenge of testing the hypothesis related to potential, psychopathological risk factors of somatic conditions.

During the ZARADEMP study in a representative cohort of nearly 5000 individuals aged 55 or more years living in the community, we tested in Zaragoza, Spain, a rather provocative hypothesis: depression might increase the risk of incident diabetes. Standardized instruments and methods were used throughout, and, evidently, we controlled for other risk factors of diabetes, including gender and age, family history of diabetes, body mass index, hypertension, statin use, functional disability, smoking, alcohol consumption, etc. We found that clinically significant depression increases 65% the risk of type II diabetes, and the attributable risk of depression would be 7%, which is a very considerable proportion of the population [60]. C. Lyketsos [61] wrote an editorial related to this article, arguing in favor of the treatment of depression in the community to prevent the onset of diabetes. Moreover, during the ZARADEMP study, we have also reported that clinically significant depression increases the risk of Alzheimer's disease [62] and of mortality [63]; and more recently we have similarly documented that clinically significant anxiety increases the risk of Alzheimer's disease [64].

All these studies have clear implications for the treatment of psychiatric morbidity and the prevention of somatic diseases. In addition, they should stimulate studies trying to explain the mechanisms underlying the increased risk documented. A number of other relevant, recent studies point in a similar direction. Probably the best examples relate to cardiovascular morbidity. Depressive symptoms have been considered to be important predictors of cardiovascular morbidity and mortality [65]. Although some researchers considered that the link between depressive symptoms and cardio-metabolic conditions has yielded inconsistent results [66], the inconsistencies might be related to different depressive symptom profiles studied [67]. On the other hand, the association between depression and cardiovascular morbidity could be mediated by type D personality [54, 68].

Somatic conditions different from cardiovascular diseases have similarly been associated with psychopathological risk factors in relevant, recent studies. Some examples follow: illness anxiety and somatic symptom burden have been shown to predict the development of irritable bowel syndrome [55]; psychological and culturally influenced factors have been considered to have an important role in the development and persistence of low back pain [69]; and postoperative depressive symptoms have been reported to predict pain at discharge in orthopedic surgery patients [57] and perceived stress in midlife to predict disability in old age [70].

Studies on the Efficacy of the Treatment of Psychiatric Morbidity

The contribution to the studies related to treatment, including the interventions in the psychological morbidity in medical patients, is one of the less familiar aspects of epidemiological methods. In fact, in writing about drug trials, M. Shepherd [71] very early regarded them as of crucial importance in the evaluation of treatment

and considered them to be essentially based on epidemiological methods. The randomized trials:

> …reflect the look of the statistician, who inevitably tends to think less as a physician and more as a metaphysician, specializing therefore in the description of the types of proof which are appropriate to various types of assessment.

Difficulties in this area were very soon identified, such as the problems posed by the wide assortment of natural histories and wide range of conditions of psychiatric disorders in medical patients or the influence of natural variations in severity, including spontaneous relapse or modifications parallel to the course of the somatic disorder. Still, relevant studies in this area were conducted very early, such as the drug treatments for depression in the medically ill reviewed by Stoudemire and Fogel [72] or the controlled trial indicating that depressive illness in general medical settings responds to drug treatment [73]. In specific disorders, such as poststroke depression, Gonzalez-Torrecillas et al. [74] reported the efficacy of tricyclic antidepressants to improve the affective syndromes but also the functional disability and even the cognitive impairment. Early controlled trials using psychotherapy were based on the same principles and include Goldberg et al.'s [75] studies on the attribution techniques in somatizing patients or the reports on the reduction of medical costs in chronic somatizers [76].

More recent studies have also shown the efficacy of "integrated care" in medical patients with psychological morbidity, which may be crucial to prevent fragmentation of care [41]. The review by Huffman et al. [77] was very relevant in this respect. In different controlled trials, it was shown the efficacy of collaborative care management of multiple anxiety disorders and late-life depression in the primary care setting [78] or in the treatment of depression in patients with a serious somatic illness such as cancer [79]. A stepwise psychotherapy intervention for reducing risk in coronary artery disease was conducted in a multicenter, randomized trial in depressed patients with coronary artery disease (CAD), which was beneficial for depressed CAD patients with type D personality, although the more global results were negative [80].

In addition, some rather recent European studies may be good examples of trials inspired by epidemiological-type methodology, such as those included in a systematic review and meta-analysis of interventions in cancer patients with emotional distress and poor quality of life [81]; the study on the self-management skills in depression in chronic disorders [82]; or in the rehabilitation of cardiac patients [83]. Among the examples, other studies with similar methods but negative results may be included, such as those with escitalopram in medical patients with depression [84].

Other recent and novel studies incorporating the methodology of randomized controlled trials in this area include the subject of advanced illness and palliative care. Agar et al. [85] studied the efficacy of neuroleptic treatment for symptoms of delirium among patients in palliative care; and other researchers have studied the efficacy of meaning-centered group psychotherapy (MCGP) for improving psychological well-being in patients with advanced cancer (Breitbart et al., [86] or for improvements in personal growth and environmental mastery in cancer survivors [87]. It is relevant to observe that the psychological-type, randomized controlled studies have been particularly abundant in the area of psycho-oncology. Other examples include Epstein et al. [88] study of the effect of a patient-centered communication intervention on oncologist-patient communication. A series of randomized controlled trials, reviewed by Wang et al. [89], have approached the effects of life review interventions on spiritual well-being and psychological distress in patients with terminal or advanced oncological diseases.

In addition, innovative electronic health techniques have been studied with epidemiological-based designs. Andersson et al. [90] conducted a systematic review of studies comparing Internet-based approaches with face-to-face psychologi-

cal treatments for psychiatric and somatic disorders, with promising results. Similarly, Strain et al. [91] have reviewed the use of electronic health records (EHR) for the management of depression as a systemic medical illness.

It is also remarkable that the effectiveness of consultation-liaison psychiatry in the general hospital, assessing parameters such as cost-effectiveness and reduction of length of stay, may be approached in adequately controlled studies, with a methodology similar to the randomized controlled trials [92–94].

Studies on the Conceptual Construction of Diagnosis and Classification

Epidemiological data on the characteristics, course, outcome, or morbid risk of psychiatric disturbances in medical patients may be of help in the refining of the existing or developing new classificatory systems. The efforts launched by WHO to develop ICD-10 or the APA to develop the DSM-5 in the field of general psychiatric disturbances were preceded by relevant epidemiological studies. This previous work helped to improve the problems leading to disagreement in communication between psychiatrists, related to variations at the levels of observation, the inferences drawn from such observations, and the nosological schemata employed by clinicians. However, new efforts are persistently required to improve the diagnosis and classifications, and, for example, we did new, additional work to reliably use ICD-10 in the psychiatric disturbances in medical patients [95].

Moreover, the general impression is that the final consensus of experts in the committees in charge of official classifications may often be taken in the absence of sufficient empirical evidence. We have recently observed the weakness of the category "mild neurocognitive impairment" in DSM-5, a nosological category with implications for liaison psychiatry, particularly in primary care [96]. The conversion rate to dementia in the general population was much lower than expected in view of reports in the general literature. An editorial related to this article was critical with this DSM-5 category, underlying the importance of conducting adequate epidemiological-type research to support the categories in the system [97].

It is obvious that experts on both ICD and DSM systems have done important efforts to get face and content validity for the categories eventually included, and the documented work supporting the reliability of the categories has been impressive and probably unique in any medical field. Reliability is a crucial, preliminary stage, but documentation to support the general validity is unavoidable before a category may be implemented on solid bases. One of the main difficulties for the development of a valid classificatory system in this setting derives from the complexities of psychiatry in medical patients. We have argued in favor of the application in this setting of the Johns Hopkins' perspectives [98]. These perspectives get away from narrow views, abhorred in our field, of what constitutes psychiatric disorder; yet, they are unambiguous and useful from the heuristic point of view: the disease perspective, which is useful in disorders such as the psychopathological syndromes directly attributable to and "explained" in Jasperian terms by diagnosable brain diseases, such as poststroke depression [99]; the dimensional perspective, quite useful to conceptualize, for example, the minor psychiatric morbidity found in primary care in this setting; the behavior perspective, relevant in somatizing patients; and the life story perspective, which can also be the primary way to "understand" in Jasperian terms the depression in some patients following situations such as being informed of a diagnosis of cancer. While all these perspectives may be needed in a particular case, we have also argued that conjectures to be tested should differ depending on the type of disturbance to be studied.

The weaknesses of some official categories in both ICD-10 and DSM-IV are exemplified by the category previously called somatoform disorders, which has suffered radical changes in DSM-5 (and probably in ICD-11). It has long been known the strong social and cultural influences in somatic

syndromes and the consequent implications for the nosology of somatoform disorders [100]. The possibility that this old category might include different conditions has been discussed but also the possibility that it is one disorder with many names [101]. The potential contribution of epidemiology in this type of disorder has to do with abundant documentation showing its high prevalence and negative outcome [102]; moreover, clinical experience but also epidemiological-type research advised against emphasizing the centrality of medically unexplained symptoms [103]. Somatoform disorders are now called "somatic symptom disorder and related disorders" (SSRD), but it is not only the denomination which has changed; since the concept, the construct has been modified [104].

The nuclear aspect of this chapter is that epidemiological methods may help in refining or eventually reconstructing a category for which the disease model works poorly and may result in slow progress. Among the efforts to validate the category, some studies emphasize the use of questionnaires and other assessment instruments such as the SSD-12 [105]. The challenge, though, is the identification of the appropriate "gold" or "reference standard." In case the new category is not mature enough, it is difficult to validate the appropriate instrument against such category and vice versa: in such circumstances, the risk of "circular reasoning" is obvious.

Epidemiological-type studies might be designed to support the discriminant validity of the category, which could be achieved in the hands of experts, standardized clinicians. However, the difficulties are also apparent, since the overlapping with categories such as "illness anxiety disorder" in the new DSM5 category or with more traditional categories such as "somatized anxiety and/or depression" in categories described by Goldberg [51] is difficult to disentangle. The predictive validity of the category could also be tested in longitudinal, epidemiological studies. Variables such as duration and intensity of the symptoms, as well as their impact on the patient's functioning, and outcomes in domains such as quality of life, healthcare utilization, and treatment satisfaction might help in clarifying the overlapping categories. External validating criteria could similarly be tested in this respect. Some studies have reported the potential use of quantitative electroencephalography in differentiating patients versus healthy controls and also in patients with somatic symptom disorder versus patients with depression [106]. In any case, it is obvious the interest in the challenging somatization-type disorders, as shown by important, multicenter research projects [107, 108].

Summary

It is possible to provide, from the epidemiological perspective, an evidence-based contribution to the field of psychosomatic psychiatry. There are some possible ways: (1) epidemiological studies documenting the size, the characteristics, and the outcome of psychological morbidity in medical settings, including new paradigms such as the one on "complex patients"; (2) similarly documenting the psychopathological risk factors of somatic conditions, with obvious implications for the etiological knowledge; and (3) describing studies using an epidemiologically inspired methodology for randomized trials and examples of research providing data relevant to the classification in this field. All these ways may serve as contributions to psychosomatic psychiatry.

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Emotions and Cognitions in Bipolar Disorder

41

Michel Bourin

Introduction

The regulation of cognitive activities and the control of the emotional experience are determining parameters of the adaptation of an individual to its environment. Organic or functional disturbances of these processes are likely to induce pathologies clinically characterized by behavioral disorders, especially in the field of social interactions, and an alteration of the level of functioning. Thus, psychiatric diseases are frequently studied as models of pathology of the interactions between cognitions and emotions. There is cognitive impairment in people with bipolar disorder both during and between episodes. In addition, scientific studies describe a worsening of disorders during evolution.

Cognitive functions are the mental activities by which we develop a representation of our world and ourselves. They allow us to adapt to the situations we face. There are several cognitive functions recognized:

- *Language*, oral and written, whose expression and understanding can be dissociated in their attacks.
- *Attention* and speed of information processing.
- *Memory*: In the short term, and working, when the short-term stored information is worked to be restored: limited storage capacity. The long-term memory is subdivided into declarative memory, itself subdivided into episodic memory (memories) and semantics (knowledge and concepts), and a perceptual representation system (shape/structure of objects): unlimited storage capacities. The verbal memory and visual memory represent the two elements of a subdivision also commonly used.
- *Executive functions* allow the adaptation of our response to the information received: planning, mental flexibility, and inhibition.
- *Social cognitions* condition our interactions with others through the recognition of facial emotions, theory of mind, and attributive style, which group together the tendency of the subject to think for others/to determine the causes of an event.
- *Metacognition* is the knowledge/awareness of our own cognitive abilities.

Given the limitations of the diagnostic criteria defined by the nosography classifications, new readings from the clinic are needed to understand the complexity and heterogeneity of mental illnesses, particularly mood disorders. The dimensional approach of symptomatology consists of a description of clinical or psychopathological dimensions, such as cognitive and emotional disorders.

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The aim of this chapter is to take stock of the neuropsychological profile of the bipolar patient during the euthymic period, in particular concerning working memory. We will also discuss functional disability and therapeutic perspectives to improve cognitive performance and therefore functioning.

Bipolar Disorder

As a serious global public health problem, bipolar disorder affects between 1% and 6.5% of the population according to the diagnostic criteria retained [1]. This pathology is in the sixth position in terms of years of life lost and/or lived with a handicap [2]. The excess mortality of multifactorial bipolar patients can be explained by a risk of suicide 12.3 times higher than that of the general population [3]. The functional impact is major, since two thirds of the subjects suffering from bipolar disorder are concerned by a professional disinheritance, while four-fifths of them describe a significant alteration of the family and social functioning. The consequences of bipolar disorder are all the more worrying since this pathology could frequently start during adolescence or even in childhood [4].

Conventionally, bipolar disorder is a cyclical mood disorder in which acute phases (depression, mania, mixed mania, hypomania) alternate with periods of normothymia considered as asymptomatic [5]. However, certain clinical data, such as the demonstration of hypomanic symptoms during certain depressive syndromes (mixed depression) [6] or the demonstration of persistent cognitive and emotional disorders during the euthymic phases, have allowed to identify subsyndromic or atypical forms of bipolar disorder that only a dimensional approach to symptomatology would allow to recognize and manage. The exploration of cognitive functions and emotional processes in bipolar patients is currently unavoidable, both for the purpose of diagnosis and prognosis [7].

According to the World Health Organization, bipolar disorder is one of the world's ten most costly and disabling diseases. The usual management of this pathology includes a psychiatric follow-up whose frequency is adapted to the thymic state and the setting up of a mood stabilizer treatment. However, the future of patients with bipolar disorder remains unsatisfactory. The psychosocial repercussions, aggravated by the frequent presence of somatic and psychiatric comorbidities, are important: socio-occupational disinheritance, low socio-economic level, and lower quality of life. Functional impairment is thus found during the phases of acute decompensation but also in the euthymic period via various factors, including cognitive factors. In fact, 30% of euthymic bipolar patients have cognitive deficits, which can be objectified by neuropsychological tests. Nearly two thirds have subjective cognitive complaints [8]. Managing cognitive disorders appears to be an important issue to improve the functioning, the quality of life of the patient, and the stability of the disorder [9].

Cognitive Deficits of Bipolar Patients

Attention disorders, working memory and executive functions are reported in patients with a diagnosis of bipolar disorder [10]. More specifically, we find in bipolar depression disturbances of attention, an alteration of immediate and delayed memory recall capabilities as well as an alteration of the executive functions involved in particular in decision-making and problem solving. These disturbances, which do not differ qualitatively from those found in unipolar depression, could partly be attributed to a decrease in motivation, to concentration problems, or to psychomotor retardation [11].

The few studies that have evaluated cognitive functions during manic or hypomanic episodes find, in these patients, deficits in attention and executive functions related to errors due to impulsive choices [12]. Cognitive functions seem to frequently normalize when returning to euthymia. However, inconsistently, cognitive disorders are reported in normothymic patients. However, it is likely that the importance of these

disorders is overstated by the inclusion of patients with subs sub-syndromic thymic symptomatology. Moreover, the frequency of addictive behaviors, especially alcohol, and the importance of suicide attempts in these patients may be a bias factor in the evaluation of cognitive disturbances directly induced by bipolar disorder [13]. Cognitive deficits in the euthymic period affect the memory and attention domains, the speed of treatment, and the executive functions. The overall intellectual level, however, does not appear to be altered. No significant difference in intelligence quotient is found between patients with bipolar disorder and healthy controls [14]. Finally, patients diagnosed with bipolar disorder most often benefit from complex treatments including one or more mood stabilizers. Data on the impact of these treatments on cognitive functions are contradictory, with longitudinal studies comparing patients treated with untreated patients being impossible to perform because of their unethical nature [15]. For example, some lithium studies report a slight disruption of attention and working memory and a negative effect on the speed of information processing. These data are moderated by a [6–]year longitudinal study, which showed no decline in cognitive performance in patients treated with lithium and data showing a neuroprotective effect of this treatment [16]. Attentional difficulties are sometimes reported with valproate and carbamazepine, two other mood-stabilizing treatments. Finally, other treatments commonly used in the treatment of people with bipolar disorder may impair cognitive function [17]. For example, benzodiazepines may impair memory function, and neuroleptics may be associated with attention maintenance difficulties. Given the links between thymic episodes and cognitive difficulties, the absence of structural brain damage, and the lack of evidence for a degenerative process, cognitive disturbances are more likely to be related to neurological functional alterations involving mood and cognitions. In addition to thymic episodes, the persistence of impaired cognitive functions should lead to sub-syndromic thymic symptomatology, treatment side effects, or disturbances associated with concomitant disorders that may contribute to cognitive deficits [18].

During normothymic periods, the overall intellectual functioning is comparable to that of healthy subjects. In contrast, the performance of bipolar patients is significantly disrupted in many neuropsychological tests. According to a meta-analysis [19], the executive functions are mainly concerned, as evidenced by the results obtained in the Wisconsin card sorting test which implies planning, categorization, and inhibition capabilities [20]. Category fluency and working memory are affected in most studies. The performance of euthymic bipolar patients is lower than that of control subjects on the color Stroop test, which explores cognitive inhibition and selective attention capabilities by measuring the interference between the two dimensions of the same stimulus [21]. The purpose of this test is to name the color of the stimuli, which can be nonwords (control condition) or color names written in a different color. In this last condition, called interference, the two dimensions (the word and the color) are in competition. The denomination time of the color is then greater than in the control condition. This "Stroop effect" translates the interference of the automatic reading of the word on the name of the color. In addition to selective attention, sustained attention is also disrupted [22]. Memory and learning abilities are lower than those of healthy subjects, especially in a verbal modality. On the other hand, tests evaluating visuomotor skills, on the other hand, showed contradictory results.

Cognitive deficits during the euthymic period seem to be the result of multiple factors, both developmental and neurotoxic, and remain influenced by some markers of bipolar disorder. Authors have proposed a multifactorial model that can account for cognitive disturbances observed during the course of this disorder [23]. These would be the result of:

- The initial etiopathogenetic process and neurodevelopmental abnormalities
- The course of the disease, in particular the number and duration of thymic episodes, the

age of onset, and the duration of development of the disorder
- Psychotropic treatments both by their iatrogenic or neuroprotective effect
- Comorbidities, both psychiatric and somatic
- The aging of the subject

These cognitive disorders are more marked during the acute phases of bipolar disorder. Many clinical arguments (slowdown or psychomotor hyperactivity, distractibility, impulse control disorders, behavioral disinhibition, limited awareness of disorders) testify to the existence of underlying cognitive deficits. In the depressive phase as in the manic phase, the different aspects of executive functions, verbal memory, selective and sustained attention, categorial and verbal fluency, visuomotor and spatial skills, and, according to the studies, global intellectual functioning, are deficient compared to healthy subjects [24]. The importance of cognitive deficits during acute episodes made it possible to suggest that these deficits were secondary to functional brain abnormalities characterizing these episodes. Several studies in functional imaging during depressive states have described hypoactivation of the prefrontal cortex in its dorsolateral, subgenual, anterior cingulate, and medial portions. It was shown that activation of the dorsolateral and anterior cingulate cortex decreased, while that of the ventral prefrontal cortex increased [25]; the opposite phenomenon could be observed in mania [26].

These changes in brain metabolism may reflect changes in local synaptic activity and neurotransmission involved in cognitive deficits present during acute episodes. Supporting this hypothesis, numerous studies have shown an inverse correlation between the intensity of depressive symptomatology and the level of activity of the prefrontal cortex; for example, it was shown [27] that hemodynamic activity in the left dorsolateral prefrontal cortex correlated with the intensity of depressive symptomatology.

In addition, cognitive impairment is thought to correlate with structural brain abnormalities found in patients with bipolar disease. It has been shown that deficits in tasks requiring rapid information processing or involvement of attentional processes are correlated with hyperintensities of the white matter in the frontal cortex. These structural abnormalities may favor alteration of cognitive function over time in bipolar patients, explaining the association between the number of episodes and neuropsychological performance. Thus, patients whose clinical history is punctuated by multiple thymic relapses present significantly greater ventricular enlargement than patients assessed during their first episodes [28]. According to all these findings, cognitive impairment is a status marker of bipolar disorder. However, the persistence of cognitive impairment in euthymia may challenge this model. In addition, a functional MRI study performed during a color Stroop test showed that activation of the left ventral prefrontal cortex was aborted in bipolar patients compared to healthy subjects, regardless of their affective status [29]. Thus, the functional changes associated with episodes of thymic decompensation are not enough to explain cognitive disorders.

Some arguments suggest that the disturbance of cognitions is a premorbid anomaly underpinned by genetic vulnerability factors, constituting a trait marker of the disease. Thus, bipolar patients with a family history of bipolarity show visuomotor and attentional performance inferior to those observed in sporadic cases of the pathology [30]. Studies have shown that neuropsychological outcomes in healthy relatives of bipolar patients are lower than in control subjects; in particular, an inhibition deficit in the color Stroop test, marked in euthymic bipolar patients, would also be observable in their relatives [31]. These subclinical cognitive abnormalities, called cognitive endophenotypes, would be privileged tools. Deficits of cognitive function are largely implicated in the functional disability of patients with bipolar disorder [32]. The presence of residual cognitive deficits is associated with a lower level of functioning [19]. These cognitive, long-lasting, and sometimes severe deficits can also contribute to the direct costs of the disease: patients with poor cognitive functioning may have limited insight, leading to poor compliance, increased risk of relapse, and increased hospital-

ization. In addition, the management skills of daily living are more marked in these patients, constituting a factor of institutionalization. Indirect costs (professional disinheritance, family burden) are also favored by the cognitive disorders of bipolar patients.

Emotional Processes in Bipolar Disorder

Mania and depression have long been regarded as the two poles of a one-dimensional axis, which reflects the use of the term "bipolar." The thymic exaltation of the maniacal patient (playful joviality, morbid euphoria, extravagant presentation, hyper syntonic contact) is the counterpart of the sadness of the depressed mood (lived generally pessimistic with many feelings of dissatisfaction, devaluation, and moral pain characterized by the unpleasant tone of the affect). However, the increasingly frequent description of mixed forms (mixed mania, dysphoric mania, agitated depression, mixed depression) suggests understanding the pathology of emotions on a non-qualitative but quantitative side. Thus, emotional hyperreactivity is at the very heart of bipolar illness; its persistence during euthymia would reflect persistent emotional instability, likely to favor thymic relapses when a stressful life event occurs [33]. In addition to disorders of the emotional experience, whose quality and intensity are inappropriate to the context, abnormalities in the recognition of emotional stimuli have been reported in bipolar patients. Compared to healthy subjects, depressed patients have an overall deficit of recognition of fundamental emotions [34, 35] especially joy and sadness [36]. Studies suggest that in manic patients, there is a lack of identification of facial emotions compared to healthy subjects [37]. Explorations of emotion identification in euthymic patients yield conflicting results [38]. It is therefore not yet established whether the disorders of the recognition of the emotions constitute a marker of trait or state. Emotional processes in bipolar disorder engagement in potentially harmful activities for the identification of the alleles involved in the development of the disease.

The disorders of the recognition of the emotions could concern specifically certain categories of emotion. Thus, many studies find a preferential treatment of faces expressing fear [39]. It was found better performance in bipolar patients at recognizing disgust [40] and to the recognition of fear [41]. In a study exploring emotional expressivity, depressed subjects had fewer electromyographic variations than healthy subjects during emotional presentation [42].

Bipolar disorder appears to be associated with an increased size of limbic structures, particularly the amygdala and caudate nucleus [43]. A decrease in the volume of the prefrontal and subgenual cortex is, moreover, one of the most consistent results [44]. In the depressive phase, the presentation of an aversive stimulus after induction of sadness is accompanied by a reduction of metabolism in the lateral prefrontal cortex but an increase in the anterior cingulate cortex and insula [45]. The functional exploration of emotional tasks in manic patients is still poorly documented. Nevertheless, interesting results emerge from studies conducted during tests of executive functions; hypoactivity of the prefrontal cortex is observed, but hyperactivity of the anterior cingulate cortex and ventral striatum correlates with the intensity of mania [46]. These results may reflect a tendency for these patients performing tasks correctly to have to make special efforts to regulate their affective behaviors [47]. In a similar way, hypoactivation of the prefrontal cortex contrasts with hyperactivation of subcortical limbic structures in patients with euthymia in response to facial expressions of fear [48]. All of these data suggest, on the one hand, a hypersensitivity of the ventral system involved in the identification of emotions and the production of affective states, on the other hand, a deficit of the dorsal system of regulation of emotional behavior. These neurofunctional abnormalities could be at the origin of certain specific symptoms of the depressive and manic phases, in particular the thymic and emotional lability and the distractibility.

Emotion Disorders and Cognition Interactions

The deficiency of identification of the facial emotions testifies to the difficulties encountered by the bipolar patients for the realization of complex emotional tasks which call for the bringing into play of high-level cognitive processes. Thus, the emotional nature of the stimulus could influence cognitive functioning.

The use of neuropsychological tests based on the use of emotionally charged material reveals that the processing of information is biased according to the nature of the emotional content. Such biases could be involved in maintaining or even developing symptomatology [49]. In depressed patients, one of the first studies to highlight this phenomenon evaluated the recall of past experiences compared to a population of healthy subjects. The results indicated that, compared to control subjects, depressed subjects, invited to recall pleasant and unpleasant memories, evoked unpleasant memories more quickly than pleasant ones, especially since the depressive syndrome was severe [50]. Subsequently, such biases have been described in the processes of evaluation, social judgment, decision-making, attention, and memory [51]. The demonstration of an emotional bias is more recent in mania. The performance of depressed and manic patients was compared to those of the healthy subjects during a task requiring the activation of the processes of inhibition and emotional treatment, the emotional go-no-go. Patients must respond as quickly as possible to negative or positive emotional valence words and inhibit their response to stimuli in the emotional category in competition. Both groups of patients show mood-congruent moods: negative stimuli in the depressed, positive stimuli in the maniacs [52].

The emotional Stroop test is a version of the color Stroop test designed to explore the influence of the emotional valence of stimuli on the processes of inhibition and selective attention [53]. The stimuli words used in this test are neutral or strong emotional valence. In the majority of studies, the Stroop effect is more pronounced when the emotional valence of words is relative to participants' concerns. This is particularly true in subjects with emotional disorders: the Stroop effect is all the most important as the emotional valence of the words specifically refers to the psychopathology of the disorder.

Such attentional bias towards emotionally salient information for the subject results in a reduction in the amount of attentional resources available to name the color of the stimuli; this emotional interference results in an increase in the execution time of the task [54].

Bipolar patients in the depressive phase exhibit an attentional bias towards items whose valence, depressive, is congruent with mood [55]. However, the authors also observed a depressive bias in manic patients. It was previously found, in hypomanic patients, a propensity for interference with depressive items, but not with cheerful items [56].

These results suggest that manic symptoms may be defenses against depressive emotionality. Consistent with this hypothesis, we have shown that the interference effect was pronounced in manic bipolar patients when the emotional valence of the words referred to depressive mood; however, our results also showed an interference effect on manic theme words; thus, bipolar patients in the acute phase have an inhibition deficit regardless of the valence, negative or positive, of the stimuli; such an interference effect has not been observed for neutral stimuli. Emotional interference could thus represent a cognitive marker of emotional hyperreactivity [57]. In patients with bipolar disorder in the normothymic period, the naming time of the neutral and affective stimuli during an emotional Stroop test is, as in the acute phase, greater than that of the control subjects, which confirms that the inhibition deficit would be a trait marker [58]. In addition, the relative hypoactivation of the medial ventral prefrontal cortex, involved in inhibition processes, and the amygdala, a fundamental limbic structure in the treatment of emotions, shows that emotional interference in euthymic bipolar patients is associated with functional abnormalities of the cortico-subcortical networks underlying cognitive and emotional functioning.

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Cannabis and Psychosis: A Causal Association

42

Eduardo A. Leiderman

Introduction

The Addiction Office of the United Nations has reported that 2.5% of the human population consumes cannabis [1]. In Argentina, a recent study found that 17% of the population consumes or has consumed cannabis [2]. Moreover, 18.4% of this group is dependent on this drug. Almost 3% of the teenagers between 12 and 17 years old has consumed marijuana in the last month, and 20% believes that to use occasionally cannabis is not risky. These numbers show us that cannabis use is highly expanded. Therefore, it is quite necessary to study and investigate the consequences of its use and to spread the information between the users.

Cannabis components act on the endocannabinoid system of our body. Basically, there exist two well-known receptors in this system: CB_1 and CB_2. CB_1 is presynaptically expressed in glutamatergic and GABAergic neurons. It modulates the release of these two neurotransmitters. This receptor exists principally in the cerebellum, hippocampus, and frontal neocortex. Also, it can be found in the basal ganglia, thalamus, spinal cord, liver, lung, and adipocytes. CB_2 receptor is mainly in the periphery: lymph nodes, spleen, endothelial, and immune cells. It acts as an immunomodulator.

The neurotransmitters of the endocannabinoid system are the following:

1. Anandamide (AEA): It was discovered in 1992. It is synthesized in the cell membrane and is transported from the synapsis down to the interior of the cell. It is a partial agonist of CB_1 and CB_2 receptor.
2. 2-Arachidonoylglycerol (2-AG): It is the most abundant in the brain and is total agonist of CB_1 and CB_2 receptor.

When the intracellular calcium augments, the postsynaptic neuron releases the endocannabinoids. They are retrograde neurotransmitters and act on the presynaptic neuron mainly inhibiting the release of GABA and glutamate. These endocannabinoids also decrease the release of dopamine, serotonin, acetylcholine, and norepinephrine. They intervene in neurodevelopment, neuronal proliferation, migration, synaptogenesis, metabolism, emotions, pain, memory, and learning [3].

Cannabis plant has more than 100 components. Cannabinol was the first one isolated in 1899, cannabidiol (CBD) in 1963, and delta-9-tetrahydrocannabinol (THC) in 1964. THC is the main component of herbal cannabis and constitutes 1–20% of its total. It is a partial agonist of

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CB_1 and CB_2 and produces the principal cognitive and psychotomimetic effects. Cannabidiol, on the other hand, is less than 4% of the plant. It has antagonistic effects in receptors CB_1 and CB_2 and inverse agonism in CB_2, which produces hypotension, anxiolytic, anticonvulsant, anti-inflammatory, and antipsychotic effects. It reduces the psychomimetic and amnestic effects of THC [3].

Schubart et al. [4] described an inverse relationship between cannabidiol content in marijuana use and autoreported positive symptoms in a sample of 1877 subjects in the Netherlands in 2011. These facts led researchers to test this substance as an antipsychotic. In 2012, Lewecke and others [5] published a study that compares the use of cannabidiol with amisulpride in 39 patients with schizophrenia that were between 18 and 50 years old. After 4 weeks, both drugs reduced positive, negative, and general symptoms, measured by the PANSS scale, without difference in efficacy between them. However, cannabidiol was better tolerated than amisulpride, with less extrapyramidal symptoms, weight gain, or hyperprolactinemia. The antipsychotic effect of cannabidiol was interpreted as a result of its partial agonism at dopamine D_2 receptors and the upregulation of the concentrations of the endocannabinoid anandamide due to the inhibition of its reuptake and degradation [6].

In spite of the deleterious effect of THC, in the USA, studies of confiscated cannabis have revealed that concentration of THC has increased in the last years as well as the THC/cannabidiol rate [7].

Cannabis-Psychosis Association

The association of cannabis and psychosis is well known since a long time ago. Jacques Moreau (1804–1884), a psychiatrist, was a member of the Parisian group, "The Club of the Hashish-Eaters" (Club des Hashishins), dedicated to the exploration of drug-induced experiences. Members of this group included Victor Hugo, Alexandre Dumas, and Honoré de Balzac, among others. In 1845, Moreau wrote the book *Du haschisch et de l'alienation mentale* (Haschisch and the mental alienation) where he first described the association between cannabis and psychosis: "…psychotic reactions…lasting a few hours, but occasionally as long as a week…the reaction seemed dose related and…included paranoid ideation, illusions, hallucinations, delusions, depersonalization, confusion, restlessness and excitement…" [8].

Nowadays, we can describe three types of psychotic outcomes with cannabis:

1. The acute transient psychosis that starts with cannabis use and may last hours or days
2. The acute persistent psychosis that also starts with cannabis exposure but outlast the period of intoxication and therefore can last weeks
3. The chronic recurrent psychosis that may start with exposure or after weeks and months but lasts forever and is clinically indistinguishable of schizophrenia (although some particularities have been observed)

As we see, the association between cannabis and psychosis was well known for the past two centuries. However the kind of relationship was controversial: while some researchers described a causal relationship, others supposed that cannabis was a self- medication form for psychotic people. How can the type of relationship between cannabis and psychosis be disentangled?

Robert Koch, the Nobel Prize winner of 1905 created postulates that can be used for the determination of the cause of infectious diseases:

1. The organism must always be present, in every case of the disease.
2. The organism must be isolated from a host containing the disease and grown in pure culture.
3. Samples of the organism taken from pure culture must cause the same disease when inoculated into a healthy, susceptible animal in the laboratory.
4. The organism must be isolated from the inoculated animal and must be identified as the same original organism first isolated from the originally diseased host.

These postulates work very well with the infectious diseases but not too well for chronic diseases with several causal environmental contributors. With that in mind, Austin Bradford Hill (1897–1991) an economist, epidemiologist, and statistician proposed nine principles to establish a causal relationship between a presumed cause and an observed effect. Indeed, he definitively demonstrated the casual relationship between smoking and lung cancer in his case-control study with Richard Doll, "Smoking and carcinoma of the lung: preliminary report" published in London in 1950 [9] .

The nine principles of Hill's criteria for causation are:

1. Strength of association: the larger the association, the more likely that the association is causal. This can be observed in observational studies with odds ratio (the possibility that the condition appears in a sample compared with the possibility that it appears in other sample) or similar statistical measures. In cohort studies, relative risk (the ratio of the probability of an outcome in an exposed group to the probability of an outcome in an unexposed group) is used.
2. Consistency or reproducibility or replicability: The likelihood of an effect is increased if the association is observed by different persons in different places with different samples and different study designs.
3. Specificity: The more specific an association between a factor and an effect is, the bigger the probability of a causal relationship. However, it is not conclusive due to the fact that a factor can cause other illnesses or diseases than the one studied.
4. Temporality: The cause always precedes the effect (as the Latin expression says: "post hoc ergo propter hoc").
5. Biological gradient: Greater exposure should generally lead to greater incidence of the effect. It refers to the dose-response relationship.
6. Plausibility: A plausible biological mechanism between cause and effect is needed to explain the causal association. But we must keep in mind that the interpretation of biological plausibility depends on the current state of knowledge.
7. Coherence: The cause-and-effect story should make sense with all knowledge available to the researcher. The cause must be reasonable and not contradict science principles.
8. Experiment: Evidence drawn from experimental manipulation may lead to the strongest support for causal inference. But sometimes there are ethical restrictions for such experimentations.
9. Analogy: The existence of other cause-effect relationships analogous to the one under study supports a causal interpretation.

Strength of Association

Nowadays there are a lot of epidemiological studies that reveal an association between cannabis and psychosis:

The first study that observed this association was published by Andreasson et al. in 1987 [10]. These authors asked a cohort of 45,570 Swedish conscripts about their consumption of cannabis. The cohort was followed 15 years. The relative risk for schizophrenia was 2.4 in the group that reported use of cannabis at least once compared with nonusers. The relative risk increased with increasing consumption level and was 6 among those who had used it more than 50 times. Relative risk remained high after being controlled by many other factors such as psychiatric diagnosis at conscription, alcohol consumption, school adjustment, relative medicated for nervous problems, etc.

This cohort was further analyzed by Zammit et al. [11] 12 years later and also found that cannabis was associated with an increased risk of developing schizophrenia. This risk has a dose-dependent fashion both for subjects who had ever used cannabis and for subjects who had used only cannabis and no other drugs (adjusted odds ratio [OR] 1.2 and 1.3, respectively). The adjusted odds ratio for using cannabis more than 50 times was 6.7. Similar results were obtained when analysis was restricted to subjects developing schizo-

phrenia 5 years after conscription to exclude possible prodromal cases.

The Dunedin birth cohort study consist of 1037 individuals born in 1972–1973, followed until age 26 [12]. It obtained information of psychotic symptoms at age 11 and drug use at ages 15 and 18 from self-reports and assessed psychiatric symptoms at age 26. In this study, the use of cannabis by age 15 was associated with an increase in schizophreniform disorder at age 26 (OR 4.5). However, this risk was reduced and no longer significant when psychotic symptoms at age 11 were controlled for.

Another research in North Finland studied a sample of 6534 people from a cohort of persons born in 1986 [13]. Information on prodromal symptoms of psychosis and cannabis use was asked at age 15–16, and individuals were followed until age 30. The risk of psychosis in those who had used marijuana was elevated (hazard ratio of 2.85) being higher with more use (hazard ratio 6.5 in individuals that had tried cannabis 5 or more times). The association remained statistically significant even when adjusted for prodromal symptoms, parental psychosis, and other substance use.

In 2011, we surveyed 862 individual residents at Buenos Aires City [14]. We asked about psychotic like experiences and cannabis use among other questions. We found in this sample that cannabis use increased the risk of having psychotic-like experiences (OR 2.5). Almost 30% of those that use cannabis had at least one psychotic-like experience, while only 14% of the nonusers had at least one.

Replicability

Nowadays, we had a great amount of data that confirmed the association described in the previous studies [15–20]. Cannabis use increases the risk of having psychosis, and the presence of previous psychotic symptoms moderates the effect but do not suppress it.

Arsenault presented in 2004 a revision of studies [21] and concluded that cannabis use confers a twofold increase in the relative risk for later schizophrenia. Henquet carried out a meta-analysis of prospective studies [22]. The pooled odds ratio observed was 2.1 and could not be explained by confounding or reverse causality.

Recently, Marconi et al. [23] published a systematic revision that investigates the association between the degree of cannabis consumption and psychosis and a meta-analysis to quantify the magnitude of the effect. Ten studies were inserted in the meta-analysis enrolling a total of 66,816 individuals. Higher levels of cannabis use were associated with increased risk for psychosis in all the included studies. A logistic regression model gave an OR of 3.90 for the risk of schizophrenia and other psychosis-related outcomes among the heaviest cannabis users compared to the nonusers.

Although there is a lot of evidence about the association between cannabis and psychosis, we must be aware of its limitations: the presence of high heterogeneity between the measures used across the studies, lack of controls for previous psychotic symptoms or other drug consumption, low statistical power, use of self-reports with their corresponding bias, etc.

Specificity

Associations between cannabis and other disorders were studied, showing some contradictory data: a representative sample survey of 10,561 Australian adults revealed that cannabis use was associated with higher rates of depressive or anxiety disorders. However, these associations disappeared after the effects of demographics, other drug use, and neuroticism were considered. At the same time, the relationship between cannabis dependence and psychosis remained statistically significant after these analyses [24].

One study conducted by the Prevention Research Center at the John Hopkins University enrolled 2311 first grade children from 43 classrooms in 19 urban elementary schools in a Mid-Atlantic metropolitan area of the United States. The assessments were done on 1494 individuals from 1985 (at age 6 years) through 2002 (at age 21 years). The use of other drugs was controlled.

The estimated risk of young adult depression on adolescent with cannabis problems was not significantly different (between females and males) from that for comparison adolescents for either females or males [25].

Recently Manrique-Garcia et al. published a study based in the same cohort of Swedish men that initially revealed the epidemiological association between cannabis and psychosis. Only those subjects with a high level of cannabis use had an increased crude hazard ratio for depression (HR: 1.5), but the association disappeared after adjustment for confounders. There was also no association between cannabis use and bipolar disorder [26].

On the other hand, some studies showed some kind of association between cannabis and depression or anxiety. One international study of the World Health Organization collected data from 85,088 subjects from 17 countries. The association between early-onset cannabis use (before age 17) and depression spells was studied. Modest results were obtained (risk ratio: 1.2), and when adjusted for childhood conduct problems, they decreased to nonsignificant [27].

A recent meta-analysis of 31 studies showed a small positive association between anxiety and cannabis use (OR: 1.24) or cannabis use disorders (OR: 1.68) or between comorbid anxiety and depression related to cannabis use (OR: 1.68). The temporal association between cannabis use and anxiety disorders was studied in a small subset of five studies. This analysis showed that those using cannabis at baseline were more likely to have symptoms of anxiety at follow-up (OR: 1.28). However, acute cannabis use was not ruled out, and some of those anxiety symptoms may be the result of heavy cannabis acute use [28].

As we can see, up to date we cannot rule out the absence of associations between cannabis and other psychiatric disorders. However, these associations, if they truly exist, do not seem to be as consistent and strong as the relationship between cannabis and psychosis.

Temporality

The evidence of this condition is quite important because is essential to prove the causation. It has been a controversial topic because many authors had considered the self-medication hypothesis. According to this hypothesis, the fact that cannabis is used as a palliative for the suffering psychotic symptoms may be a consequence and not a cause of these symptoms [29, 30].

This topic was approached in different studies. We chose three studies that show that the use of cannabis precedes the presence of psychotic symptoms supporting the causation hypothesis. The first was the Christchurch health and development study. They followed a cohort of 1265 children born in the Christchurch urban area in mid-1977. This cohort was followed from birth to age 21. Data were gathered on cannabis dependence and psychotic symptoms at age 18 and 21 in a sample of 1053 subjects. Psychotic symptoms at the time of the preceding assessment (2 or 3 years before) were included as confounding factors. Even adjusting for preceding psychotic symptoms as well as other important confounders such as other drugs dependence, major depression and anxiety disorders, exposure to adverse life events, sociodemographic variables, neuroticism, parental conflict, and psychiatric disorder prior to 16 years old, the rate ratio was 1.8 (95% CI: 1.2–2.6). This means that cannabis use contributes to the development of psychotic symptoms independently of preexisting psychotic symptoms and social or other psychiatric factors [15].

The second was the Kuepper et al. longitudinal and prospective study. It was the most similar to an experimental study. The authors analyzed the data of 1923 individuals of Germany (aged 14–24 years old at the time of the baseline interview in 1995) gathered in a baseline survey and follow-ups (mean of 3.5 years [T2] and 8.4 years from baseline [T3]). First, they excluded all individuals with cannabis use at baseline and also individuals that suffered any psychotic symptom at T2. Incident cannabis use over the period from

baseline to T2 increased the risk of psychotic symptoms between T2 and T3 (odds ratio: 1.9). There was no evidence for self-medication effects, because when individuals that had psychotic experiences at T2 were followed, no evidence of increase of cannabis use was found [20].

The third and last study considered was published in 2016. The participants were 1009 students of first and seventh course of Pittsburgh public schools. Data about self-reported frequency of cannabis use, subclinical psychotic symptoms, and confounding variables was gathered annually from age 13 to 18. The study showed that for each year adolescent boys that consumed marijuana, their expected level of subsequent subclinical psychotic symptoms rose by 21%. This effect persisted even after a year of cannabis abstinence. For each year that adolescents had weekly cannabis use, the predicted odds of experiencing paranoia and hallucinations rose by 133% and 92%, respectively. After controlling for confounders, changes in prior and current psychotic symptoms did not predict increases in weekly cannabis use as the self-medication hypothesis would forecast [31].

These studies are only some examples of a great amount of evidence that had been collected during the last years. They certainly showed that the temporal direction of the association points to a cannabis causation of psychosis.

Biological Gradient

There are plenty of studies that can illustrate this postulate. The first epidemiological study that observed the association between psychosis and cannabis also noted the difference in the strength of the association depending on the frequency of cannabis use. While those Swedish conscripts who had ever used cannabis 2–4 times had an OR of almost 2 of having schizophrenia, the ones that had ever used more than 50 times had an OR of 6.7 [11].

Another study based in the same cohort studied in the Kuepper et al. study described above also observed a biological gradient. Henquet et al. studied 2437 young people aged 14 to 24 years. After adjustment for age, sex, socioeconomic status, urbanicity, childhood trauma, predisposition for psychosis at baseline, and use of other drugs, tobacco, and alcohol, the OR for psychotic symptoms at follow-up 4 years later was 1.67. There was a dose-response relation with increasing frequency of cannabis use. Using cannabis at baseline less than one time per month, the individuals had an OR of almost 1 of having any psychotic symptom at follow-up, but when the frequency of use increased up to almost daily, the OR increased up to 2.23 [22].

Moore et al. conducted in 2006 a systematic review of the evidence of association between cannabis use and occurrence of psychotic or affective mental health outcomes. They included seven studies that looked over the association of cannabis and psychosis. Six of them that examined a linear trend across cannabis use frequencies or compared higher- with lower-frequency users reported a dose-response effect. While the OR of having a psychotic outcome was 1.4 for the individuals that had ever use cannabis, the OR for heavy users was almost 2 [32].

Marconi et al. performed 10 years later a similar systematic review and a meta-analysis to quantify the magnitude of the effect between the degree of cannabis use and psychosis. Eighteen studies for the systematic review and 10 for the meta-analysis fulfilled the inclusion criteria (reporting cannabis consumption using a dose criterion). Higher levels of cannabis use were associated with increased risk of psychosis in all the included studies. The pooled analysis reported approximately a fourfold increase in risk for the heaviest users and a twofold increase for the average cannabis user in comparison to nonusers [23].

These data show the relevance for public policy to apply drug reduction programs to decrease the frequency of cannabis consumption if stopping its use is not possible now.

Plausibility: Coherence

In recent years, neuroimaging, postmortem analysis, and animal experimentation have shaped the building of the endocannabinoid hypothesis of schizophrenia. This hypothesis assumes that the

hyperactivity of the endocannabinoid system can increase the risk of developing schizophrenia or psychosis in general, mainly by dopamine neurotransmission augmentation.

Some evidence of endocannabinoid system disturbances has been accumulated lately. Studies on animal models of schizophrenia have repeatedly shown a reduction of CB_1 receptor levels in different brain regions such as the prefrontal cortex (PFC), hippocampus, substantia nigra, and cerebellum. However, neuroimaging studies have demonstrated an increase binding for CB_1 receptors in the dorsolateral PFC, the posterior anterior cortex, the nucleus accumbens, and the pons of patients with schizophrenia. Also, a decrease in peripheral level of CB_2 receptors was found in treated and non-treated schizophrenic patients. Polymorphisms of CNR2, the gene encoding CB_2 receptors, are observed in schizophrenic patients. At the same time, neurotransmitter levels of the endocannabinoid system are disturbed. Anandamide levels are increased in plasma and CSF of schizophrenic patients, and 2-AG has been found higher in animal models [33].

THC could exert its psychogenic effect by disrupting the regulatory role of the endocannabinoid system in GABA and glutamate release. This alteration in glutamate release can disturb the neurodevelopment of the individual due to anomalies in synaptic connections, neuronal differentiation, pruning and migration, and disturbances shown in schizophrenic patients [34]. Also, cannabinoids have been shown to induce firing of dopaminergic mesolimbic neurons (as seen in psychotic patients) and induce dopamine release in the stratium in animals. THC inhibits, via CB_1 activation, the release of glutamate onto GABA neurons that project from the nucleus accumbens to the ventral tegmental area. These neurons normally produce an inhibitory effect on the firing of dopamine neurons back to the nucleus accumbens. Their inhibition causes increased dopamine release in the striatum that is implicated in the pathogenesis of psychotic symptoms. However, the CB_1-GABAergic disinhibition of dopamine neuronal activation could not be demonstrated in humans yet [35].

The evidence that cannabidiol can act as an antipsychotic allows the researchers to implicate the endocannabinoid system in the pathophysiology of schizophrenia albeit in an indirect way. This provides a plausible mechanism by which cannabis consumption may cause psychosis.

Caspi et al. showed that a functional polymorphism in the catechol-O-methyltransferase (COMT) gene moderates the influence of adolescent cannabis use on developing adult psychosis [36]. He collected blood samples for genotype analysis from 803 individuals of 26 years old belonging to the Dunedin cohort of persons born between April 1972 and March 1973. He observed that adolescent cannabis use was associated with increased risk of schizophreniform disorder in adulthood among Val/Val individuals (OR: 10.9) and to a lesser extent among Val/Met individuals (OR: 2.5), but not among Met/Met individuals (OR: 1.1) [36]. Individuals carrying the Met/Met allele genotype have the lowest COMT activity, while the Val/Val genotype have the highest, causing a decrease of dopamine levels that are associated with poor cognitive performance and prefrontal functioning. However, the association between Val/Val COMT genotype and risk of psychosis in cannabis users was not confirmed in later studies [37, 38].

Other studies showed an interaction between genetic polymorphism and risk of psychosis in cannabis users. Van Winkel et al. studied 801 patients with nonaffective psychosis and 740 unaffected siblings in the Netherlands and Belgium. They observed that subjects who carried two copies of the C allele of the rs2494732 polymorphism of the AKT1 gene were especially at risk of schizophrenia and schizotypy, respectively [39]. The AKT1 gene codes a protein kinase that forms an integral part of the dopamine receptor signaling cascade in the striatum. It also inactivates the glycogen synthase kinase (GSK-3) by phosphorylation. The interaction between AKT1 and GSK-3 has been implicated in several cellular processes such as apoptosis, cell proliferation, cell survival, and metabolism. THC activation of the AKT1 by phosphorylation can influence in D2 receptor signaling increasing the vulnerability to psychosis.

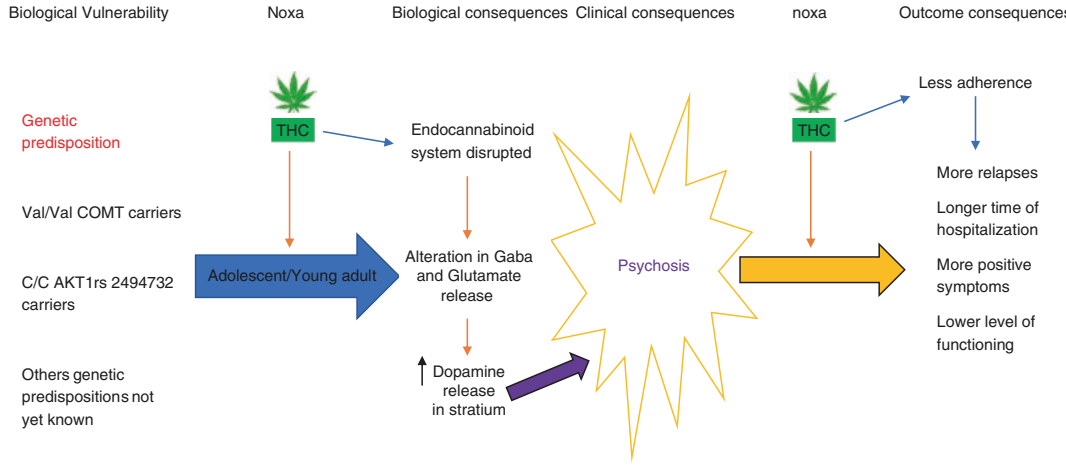

Fig. 42.1 Cannabis and psychosis: predisposition, biological, clinical consequences, and outcome

The moderation of the genetic variation in the AKT1 gene on the effect of cannabis use in increasing the risk of a psychotic disorder was confirmed in a later study [40]. In a case-control study of 489 first-episode psychotic patients and 278 control subjects, Forti et al. showed that carriers of the C/C genotype of the AKT1 rs2494732 with a history of cannabis use have more than twofold risk of having a psychotic disorder when compared with T/T carriers (OR: 2.18). Among daily cannabis users, the C/C carriers showed a sevenfold increase in the risk of psychosis compared with T/T carriers (OR: 7.23) [40] (Fig. 42.1).

Experiments

Many experimental studies have been conducted in humans to test the psychomimetic effects of cannabis. We will only present three of them.

In one study, 22 healthy individuals with a mean of 29 years old who had never been diagnosed with a cannabis abuse disorder were given 2.5 and 5 mg of delta 9 THC by intravenous route in a double-blind condition [41]. Delta 9 THC increased scores of the PANSS positive symptoms subscale with a symptoms' peak 10 minutes after drug administration. Subjects had suspiciousness, conceptual disorganization, depersonalization, derealization, thought disorder, altered perceptions, loosening of associations, grandiosity, and loss of insight. THC also produced an increase in the PANSS negative subscale reporting blunted affect, reduced rapport, psychomotor retardation, and emotional withdrawal. It also increased scores of the PANSS general symptoms such as somatic concern, guilt feelings, tension, and unusual thought content [41].

In a later study, 22 healthy adult males were given 2.5 mg of THC intravenously under double-blind conditions [42] . Psychotic symptoms were assessed by investigators using the positive subscale of the PANSS and by subjects using the CAPE. The scores on the PANSS positive subscale increased with a peak at 30 minutes post-THC administration returning to baseline levels by 120 minutes. CAPE scores also increased peaking at 30 minutes and returning to baseline levels at 80 minutes post THC administration. Both scores were highly correlated. The most reported item in the CAPE scale was ideas of reference (42).

A recent study also showed the psychotomimetic effects of THC in cannabis users. A randomized, double-blind, crossover design was used to compare the effects of THC, CBD, and their combination in 48 cannabis users [43]. The drug was administered by inhalation. THC and its combination with CBD increased the scores

on the Psychotomimetic States Inventory (PSI) (especially perceptual distortions and cognitive disorganization) and on negative items of the BPRS. CBD reduced PSI scores in light users but not in frequent users [43]. This should be taken into account when the antipsychotic effect of CBD is considered.

These experimental studies demonstrate the psychotomimetic effect of THC in humans and thus, increase the proof of the cannabis causation of psychosis.

Analogy

One of the most common analogies to the causal relationship between cannabis and psychosis that has been described is between smoking and lung cancer [9]. Although not every person that smokes cigarettes gets lung cancer and the time elapsed between the noxa and the illness can be years or decades, the relationship is certainly proved. Tobacco smoking is responsible of approximately 85% of all lung cancers with an additional fraction caused by secondhand smoke exposure in nonsmokers [44]. Cannabis has a much lower risk of causing psychosis (an estimated 14%) but still important [32].

Another analogy may be the risk of psychosis produced by head injuries. It has been proven that head injury in the childhood increases the risk of having psychosis years after, although the reason is not yet clear [45, 46]. The risk is higher in those individuals with genetic predisposition and there is no evidence that those persons with risk of schizophrenia would be more prone to have head injuries than others [45, 46]. Unlike cannabis causation, there is no evidence of biological gradient [46].

These examples demonstrate that there are other models in medicine and specifically in psychiatry with certain similarity to the causal relationship between cannabis and psychosis. This analogy has in effect some flaws that arise principally because of the heterogeneous character of psychosis and schizophrenia in particular.

Impact of Precedent and Current Cannabis Use in Schizophrenic Patients

Regarding the characteristics of schizophrenia disorder in patients that had previously use cannabis, we have contradictory findings. Sarrazin et al. studied 171 schizophrenic patients consecutively admitted in a university-affiliated hospital in France. Forty-one of them (18.2% of the sample) had a cannabis use disorder before or at the time of the onset of schizophrenia. The mean duration of the illness was 11.5 years. They compared the demographic and clinical variables of the schizophrenia subjects with and without preonset cannabis use disorder. The group with preonset cannabis use disorder (CUD) was younger and had more males. But the mean age at onset of schizophrenia, the presence of a family history of schizophrenia, mood disorder, or suicide attempts have no statistical difference between both groups. The researchers also observed no significant difference between groups in terms of symptom dimensions or number of hospital admissions concluding that their findings argue against "cannabis associated schizophrenia being a distinct disorder" [47].

Gupta et al. conducted a meta-analysis comparing the symptoms of patients with psychosis with or without a history of substance use (not only cannabis). Twenty studies met the inclusion criteria. No significant differences were observed in terms of positive, negative, depression, or global function between former substance users or non-substance users. A subanalysis found no difference between former cannabis users and non-substance users [48].

Other authors found instead some demographic and clinical differences in schizophrenic patients between the former cannabis users and the nonusers. Ringen et al. studied a naturalistic sample of patients with DSM IV schizophrenia spectrum disorder. Six hundred eighty-one patients were recruited from 2004 until 2013 from major hospitals in Eastern and Central Norway. The sample was dichotomized based on the presence or not of premorbid cannabis use.

Individuals with former cannabis use were more often male, more often single and had more severe psychotic symptoms and impaired functioning (although fewer lifetime psychotic episodes). Higher levels of premorbid cannabis use were associated with higher levels of current psychotic symptoms, and the earlier the use of cannabis, the lower the age of onset [49].

Arendt et al. also observed lower age of onset of schizophrenia in patients with cannabis-induced psychosis. The researcher's extracted data of 535 patients with cannabis-induced psychosis from the Danish Psychiatric Central Register and followed them for 3 years. Schizophrenia-spectrum disorders were diagnosed in almost half the sample. The mean age at time of first treatment for cannabis-induced symptoms was 27 years, while the mean age at onset of the comparison group (2721 patients with no history of cannabis-induced psychosis) was 33.8 years. Age at onset of males and females with cannabis-induced psychosis was on average 6 and 4 years before, respectively, than the comparison group [50]. However, a Swiss study only found lower age of onset of psychosis in those patients with a cannabis use disorder, starting age 14 or younger [51].

There exist also an increasing number of investigations performed studying the impact of cannabis use in the psychotic illness. A study in the Netherlands also found higher severity of positive symptoms in patients with a psychotic disorder and cannabis use disorder, although there is no difference in pre-psychotic symptomatology among ultrahigh-risk (UHR) subjects. However, the researchers found a negative correlation between the amount of cannabis used recently and negative symptoms in the UHR patients [52].

A meta-analysis including 30 studies of UHR individuals found that those subjects had high rates of cannabis use and that UHR cannabis users have higher rates of unusual thought content and suspiciousness than nonusers [53].

A prospective study conducted in London followed 245 first-episode psychotic persons for at least 2 years. Continued cannabis use predicted poor outcome including risk of relapse, number of relapses, length of relapse, and care intensity at follow-up. Between 20% and 36% of these adverse effects of continued cannabis use on outcome was mediated through the effects of cannabis use on medication adherence [54].

A meta-analysis of 24 studies compared clinical variables of psychotic patients with or without cannabis use, as well as with continued or discontinued use, that were followed at least 6 months. They found that continued cannabis users had a greater increase in relapse of psychosis than nonusers and discontinued users, as well as longer hospital admissions. Continued cannabis use after onset of psychosis predicted an adverse outcome, more severe positive symptoms, and a lower level of functioning than for individuals who discontinued cannabis use or never used cannabis [55].

All these studies taken together clearly argue against the use of cannabis in those patients with high risk or already diagnosed with psychosis. Considerable efforts should be made to avoid the initiation of cannabis use or at least to discontinue it in those already using it (Fig. 42.1).

Health Implications

Although all these evidences support a causal relationship between cannabis and psychosis, we cannot discard any confounding factor that may take part in the equation. But if it exists, it has still remained elusive to the researchers.

If cannabis doubles the risk of psychosis, we can suppose a 2% risk of schizophrenia for individuals taking cannabis without taking into account the presence of genetic predisposition or other risk factors. But considering first-order relatives, the risk may increase to 20%, and this would be a probability less feasible to be accepted.

Hickman et al. have calculated that it may be needed to stop cannabis use in around 10,500 of light cannabis users in men and up to 29,000 of light cannabis users in women to prevent one case of schizophrenia or psychosis. But the number needed to prevent one case of schizophrenia

is reduced to 2800 in heavy cannabis users in men aged 20–24 years old and to 5470 in women aged 25–29 years old [56].

We can conclude that all these data warn against the indiscriminate use of cannabis and warrant the recommendation to first-order relatives of patients suffering schizophrenia to prevent using cannabis or to stop using it. We can also infer that cannabis users should avoid taking heavy doses or increasing THC content of the cannabis used. Teenagers, especially genetically predisposed individuals, should be advised of these detrimental consequences, and main efforts should be done to discourage the use of cannabis or to treat the addiction in those already dependent in this population.

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Gut Microbiota Biomarkers in Autism Spectrum Disorders

43

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Introduction

Autism spectrum disorders (ASDs) are a heterogeneous group of neurodevelopmental disorders characterized by difficulties in social interaction, communication, and cognition with repetitive behaviors and restricted interests [1]. The etiology of ASD has not been determined, suggesting both genetic and environmental contributions. No specific gene causing ASD has been identified, although genetic alterations have been found in 10–20% of ASD cases [2]. Genetic mutations could appear spontaneously or be triggered by epigenetic modifications induced by environmental agents [3–5]. Epigenetic mechanisms are currently known to be involved in neurogenesis, neuronal plasticity, learning and memory, and in several neurological disorders [6, 7]. This information points to interactions between gene and environmental factors that may have an important role in understanding the etiology of ASD [8, 9].

Indeed, ASDs are complex metabolic disorders involving the immunological, gastrointestinal (GI), and neurological systems [10, 11]. Immune dysfunction and gastrointestinal (GI) inflammation are also common in individuals with ASD and contribute to the severity of behaviors seen in the disorder [12, 13]. Recent evidences suggest that multiple factors, like nutrients, infections, inflammatory conditions, and toxins, could induce gut microbiota changes, altered intestinal permeability, and abnormal immune responses. The development of ASDs is associated with the combination of these factors and genetic predisposition [14, 15].

Microbiota-Gut-Brain Axis

The gut microbiota is a collection of trillions of microorganisms inhabiting the gastrointestinal tract (GI) [16–19]. It has been suggested that the microbiota is an important mediator of gene-environment interactions, modulating host gene expression through dynamic and reversible epigenetic mechanisms [20, 21]. Furthermore,

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dietary factors can result in epigenetic alterations that lead to disease susceptibility [22, 23].

Bacterial community composition varies along the digestive tract and shows changes throughout life from birth to old age [24, 25]. Although the gut microbiota of each individual is unique at the genus and species levels, immediately upon birth, the healthy gut is gradually colonized by maternal bacteria, of which the dominant phyla are *Lactobacilli* and *Streptococci*. These are followed later by *Enterococci* and *Enterobacteriaceae*, with the subsequent emergence of *Bifidobacteria, Bacteroides* spp., and *Clostridium* spp. [26]. However, the healthy adult GI tract is colonized predominantly by *Firmicutes* and *Bacteroidetes*, with a smaller proportion of *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* [16, 25, 27].

Under normal physiological conditions, these microbes not only are commensal and mediate food digestion but also enhance our immune system thus preventing pathogens from invading our tissues and organs [28]. Indeed, there is a growing body of evidences showing that the gut microbial community and its metabolic products impact in the host health, participating in digestive functions, influencing the immune system and metabolic functions, as well as in the synthesis and metabolism of nutrients, short-chain fatty acids (SCFAs), secondary bile acids, hormones, amino acids, vitamins, and tryptophan metabolites [25, 27, 29–33]. In addition, gut microbiota participate in host immunity stimulation, in preventing overgrowth or infections with pathogenic bacteria and contributing to the maintenance of intestinal barrier [34, 35].

Host genetics, dietary changes, immune imbalances, stress, and environmental factors like antibiotic treatment may disrupt the balance between beneficial and harmful microorganisms in the gut [26, 36]. Microbiota alterations (dysbiosis) may affect the physiology, immunity, and susceptibility to infectious diseases of the host, thus playing a role in certain disease processes [27, 37]. In this line of investigation, an increasing number of studies have provided evidences that gut microbiota is involved on the bidirectional signaling between the gut and the central nervous system (CNS), termed the "microbiota-gut-brain axis" [38]. The brain regulates gut motility, intestinal transit, and integrity via the hypothalamic-pituitary-adrenal (HPA) axis, thus affecting the gut microbiota composition and function. Indeed, the CNS influences the gut permeability, secretion, and nutrients availability for gut microorganisms, and it also modulates microbial gene expression through hormone secretion [39–41]. In addition, the gut microbiota plays an important role on the development of the intestinal structure, the mucosa-associated lymphoid tissue, as well as in the gut immune regulation [42, 43]. Microbiota alterations can disrupt the integrity of the gut and the blood-brain barriers with the consequent transport of bacterial metabolites and immune mediators from the gut through the bloodstream and thus exert systemic effects. Moreover, the access of these metabolites throws the cerebrospinal fluid into the CNS that can trigger the activation of resident immune cells, especially microglia [37, 44]. It is important to highlight that gut microbes play a critical role regulating CNS functions; their metabolites activate endogenous CNS signaling mechanisms, through nervous, endocrine, immune, and metabolic pathways [39, 40]. Indeed, neurotransmitters are produced by different gut microbial species: gamma-aminobutyric acid (GABA) is produced by *Lactobacillus* and *Bifidobacteria* [45, 46], dopamine by *Bacillus* and *Serratia*, norepinephrine by *Escherichia* and *Saccharomyces* [47], and acetylcholine by *Lactobacillus* [47, 48].

Thus, the gut microbiota plays a role in brain activity modulation and has an impact on neurodevelopment and behavior [37, 41]. Therefore, abnormalities in gut microbiota composition can dysregulate neuroactive molecule synthesis leading to more susceptibility to neurodevelopment disorders [49–51].

Gut microbiota contribute to the availability of the essential amino acid tryptophan, the precursor of serotonin synthesis. Serotonin is a neurotransmitter in CNS and plays a role regulating GI motility and secretion and is affected by the microbiota composition [52].

A growing list of observations associates alterations in brain-gut-microbiome communication

with the pathogenesis and pathophysiology of several psychiatric and neurologic disorders, including Parkinson's disease [53], depression [54], multiple sclerosis [55], and autism spectrum disorders [38, 56].

Microbiota in ASD

Several studies suggest that abnormal gut microbial composition may be concurrent in patients with ASD and GI disorders. The comorbidity of GI symptoms (constipation and/or diarrhea) and neurodevelopmental alterations support the role of disturbances within the microbiota-gut-brain axis in ASD [11, 57]. In this respect, researchers have recently provided evidences of diminished beneficial microbes or increased harmful toxin-producing species in the gut of patients with ASD [58]. Indeed, children with ASD and GI dysfunction show increased populations of *Firmicutes* and decreased abundance of important commensals: *Bifidobacteria, Bacteroidetes,* the mucin-degrading *Akkermansia muciniphila,* as well as *Alistipes*, *Bilophila*, *Dialister*, *Parabacteroides*, *Veillonellaceae*, *Prevotella*, and *Coprococcus* [59–62]. Moreover, intestinal dysbiosis and increase of *Desulfovibrio* and *Bacteroides vulgatus* are correlated with ASD severity in patients with ASD and GI dysfunction [63, 64]. *Sutterella*, a mucose-associated microbe, and *Ruminococcus torques* have been found overrepresented in significant numbers in ASD children with GI dysfunction [61, 62]. Recent research suggests major changes in the microbiota composition in ASD patients, with overgrowth of *Collinsella, Corynebacterium, Dorea,* and *Lactobacillus* [60].

Moreover, different studies have identified intestinal overgrowth of spore-forming *Clostridium*, a group of bacteria species recognized to produce powerful pro-inflammatory neurotoxins. In fact, their presence is highly correlated with GI symptoms in ASD children [63, 65]. In addition, differences in the *Clostridium* groups, *C. bolteae* and *C. cluster I* and *XI* [66], and a higher incidence of *C. histolyticum* have also been reported [65]. There are also evidences that constipation is correlated with increases in *Escherichia/Shigella* and *Clostridium cluster XVIII*, in ASD patients [60].

The microbiota is also constituted by fungal microorganisms, termed mycobiota, and their overgrowth can contribute to dysbiosis [67]. In this respect, *Candida* species are the most abundant pathogenic fungus found in children with ASD, the majority of which are *Candida albicans* [60], whose overgrowth may have immune consequences [68, 69]. Other studies in ASD patients have shown dysmorphic yeast with hyphae formation, reveling invasive and adhesive form of *Candida*, as well as fluconazole-resistant species such as *C. krusei* and *C. glabrata* [68, 70].

Metabolomics and Microbial Metabolites in ASD

Metabolomics is a research field that studies the complete set of chemical compounds involved in an organism metabolism. It is widely used for detection, identification, and quantitative measurement of different metabolite profiles in biological fluids. Thus, metabolomics studies are useful tools for the characterization of disease biomarkers and environmental interactions [71–73]. For this, metabolite profiles are usually analyzed for the screening of biochemical pathways involved in carbohydrates, tricarboxylic acid cycle (TCA), amino acid, and lipid metabolism. Metabolomics patterns allow exploring cellular energy and mitochondrial metabolism, neurotransmitter pathways, vitamin or mineral cofactors relationship, and also biochemical mechanisms influenced by gut microbiota [25, 40, 71, 73, 74].

Metabolomics alterations were observed in ASD patients and may be linked to genetic and environmental factors [74]. These metabolic abnormalities include alterations of TCA energy production, ammonia detoxification, reduced synthesis of omega-3 DHA, and abnormal cholesterol metabolism [75]. The urinary organic acid test allows detecting and quantifying biomarkers, so it is a useful tool to find metabolic

alterations in ASD children. Abnormal levels of organic acids can indicate metabolic dysfunction related to inherited or acquired enzyme deficiencies as well as nutrient insufficiencies, toxicants and drug effects, or even microbial overgrowth [76, 77].

Mitochondria are critical for many basic cellular activities throughout the body, and their dysfunction is known as an important risk factor for key cellular abnormalities that could induce disturbances in different organs and physiological systems [78]. Mitochondrial dysfunction has been described in about 5% of the patients with ASD. Distinct clinical features have been depicted between ASD patients and the group of ASD patients with mitochondrial dysfunction [79]. Moreover, patients with ASD and mitochondrial dysfunction have generally not shown specific genetic mutations. That is to say, the absence of specific genetic mutations observed in the majority of ASD patients implies that mitochondrial dysfunction in these individuals could be acquired rather than genetic [79]. Abnormal levels of different organic acids associated with mitochondrial dysfunction have been found in urine of ASD patients [73, 80–82]. A marked increase in several Krebs cycle metabolite analogs (citramalic, tartaric, and 3-oxoglutaric acids) has been shown in the urine of ASD patients [81, 83]. For instance, higher levels of tartaric acid in urine of ASD patients are associated with bacterial overgrowth in the gut [81, 83]. Also, elevated excretions of lactate, pyruvate, alanine, and ammonia have been reported as additional markers of ASD associated with mitochondrial dysfunction [79, 84].

Moreover, several studies have found high levels of succinic acid in urine of ASD patients. These findings were associated to Krebs cycle enzymes deficiencies or to inadequate levels of cofactors like CoQ10, vitamin B2, vitamin B6, or magnesium. Succinic acid is a dicarboxylic acid also related to aerobic and anaerobic bacterial overgrowth [81, 83, 85–90]. In addition, greater urinary excretion of Cis-acotinic acid has been found in ASD patients [74]. Elevated levels of this metabolite are related with aconitase deficiency, the enzyme that catalyzes the isomerization of citrate to isocitrate in Krebs cycle [73, 74]. Furthermore, citric acid, another analog of Krebs cycle, has been found elevated in urine of ASD patients [83, 87–89]. This finding points to possible alterations in protein metabolism or an amino acid deficiency in ASD patients [83]. In addition, the ketone body 3-hydroxybutyric acid was found elevated in the urine of ASD patients. The increment of this metabolite suggests mitochondrial electron transport system dysfunction due to cytochrome oxidase enzymes deficiency [73].

Adipic, suberic, and azelaic acids are dicarboxylic compounds resulting from fatty acids' incomplete omega-oxidation by cytochrome P450. Increased urinary levels of these metabolites in ASD patients may be due to deficiencies in beta-oxidation mitochondrial pathways and deficits of carnitine or vitamin B2 (riboflavin) [77, 91–95].

There is a growing body of evidence indicating that abnormalities in different metabolites are related to bacterial overgrowth in ASD. The function of some metabolites derived from the gut microbiota is associated with clinical characteristics of ASD: they regulate metabolic pathways and immune system of the host, influencing the susceptibility to disease and the phenotype [74, 83, 86, 96].

Metabolomics studies also have shown evidence of alterations in tryptophan, kynurenine, and serotonin pathways in children with ASD [74, 97, 98]. These compounds are related with the kynurenine pathway, involved to N-methyl-D-aspartate (NMDA) receptor agonist [99]. Indole compounds like indole-3-acetic acid and indolyl lactate are produced by bacterial breakdown of tryptophan and were also found elevated in ASD patients. These metabolites have been related to diminished levels of serotonin synthesis [99]. These findings suggest that alteration of the metabolism of tryptophan, a precursor of the serotoninergic system, would have implications on brain function and might give insightful information about ASD pathogenesis and progression [82].

Glycolic acid was also seen elevated in urine of a cohort of children with ASD, suggesting a

strong connection with yeast overgrowth in the GI tract [74]. Increased levels of urinary glycolic acid in combination with oxalic acid are observed in primary oxaluria type I, an inborn error of metabolism [100]. Elevated glycolic acid without oxalic acid elevation has been associated to ASD, and this fact may be the result of gastrointestinal yeast overgrowth or due to glycerol dietary sources [87].

Higher levels of oxalates have been reported in urine samples from children with ASD. In fact, oxalates and oxalic acid may derive from diet and human metabolism and also from fungus overgrowth (such as *Aspergillus, Penicillium,* and *Candida* spp.) [101, 102]. Indeed, intestinal bacteria metabolism generates aromatic compounds such as hippurate and phenylacetylglutamine from diet resources, as benzoic acid and phenylacetic acid, respectively [73]. Also, P-hydroxyphenyllactate is produced by *bifidobacteria* and *lactobacilli* from tyrosine metabolism and plays a role as an antioxidant [103]. Altered urinary levels of these aromatic compounds were observed in ASD patients and have been linked to alterations in gut microbiota [82, 86–90]. Therefore, 4-hydroxyhippuric acid, a common microbial end-product derived from polyphenol metabolism by the gut microbiota, was found to be increased in the urine of ASD patients [74, 104, 105].

There are findings that point out that tyrosine gut metabolism is secondary to bacterial overgrowth. Hydroxybenzoic acid, a microorganism product from catechin diet, was found increased in the urine of ASD patients, as well as p-hydroxyphenylacetic acid, which is formed by gut metabolism using tyrosine as precursor [82, 83]. Furthermore, phenylalanine, tyrosine, 4-hydroxyphenylacetic acid, and homovanillic acid metabolites are involved in the synthesis of neurotransmitter catecholamine through the tyrosine pathway and have been found increased in urine of ASD patients [74, 80].

Altered patterns of bacterial metabolites have been seen in a large number of children with ASD compared to healthy control children. These metabolites included increased levels of 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), a phenylalanine catabolic compound produced by the anaerobic bacteria *Clostridia sp.* in the gastrointestinal tract [74, 106, 107]. This metabolite is associated with behavioral, gastrointestinal, and neuropsychiatric effects [74]. Elevated urinary levels of HPHPA have been shown also in depression, schizophrenia, seizures, chronic fatigue syndrome, colitis, tic disorders, muscle weakness, or paralysis [74, 106].

P-cresol, another microbial metabolite produced by *Clostridium* species, was significantly elevated in children with ASD and was associated with repetitive behaviors constipation, and with the severity of ASD [102, 108–111]. Furthermore, increased glutamine concentrations were positively correlated with *Clostridium* sp. overgrowth in ASD patients [112]. Additionally, in the urine samples from children with ASD, other metabolites such as 2-(4-hydroxyphenyl) propionate and taurocholenate sulfate were found significantly increased, whereas 5-amino-valerate levels were lower [97]. In addition, metabolomics studies have shown higher urinary excretion of 3,4-dihydroxybutyric acid in ASD patients [74]. Higher concentrations of this metabolite were found in succinic semialdehyde dehydrogenase (SSADH) deficiency, in which the clinical presentation has shown autistic behaviors [113, 114]. In ASD patients, other metabolites such as pyroglutamic acid have been found increased. Pyroglutamic acid is a metabolite of the antioxidant glutathione formed non-enzymatically from glutamate, glutamine, and gamma-glutamylated peptides via enteric bacteria enzyme [74].

Some five-carbon sugars and related compounds were found increased in urine of a group of ASD patients: ribose, arabinofuranose, polyols, arabitol, xylitol, and the four-carbon sugar threitol [74]. Ribose, a metabolite involved in pentose phosphate pathway (PPP), was found elevated in an inborn error of metabolism, the ribose-5-phosphate isomerase deficiency. The increment of ribose in urine of ASD patients may be due to a deficiency of vitamin B1 (thiamin), a PPP enzymatic cofactor [74]. However, arabitol and xylitol may be produced through a gut-bacterial enzyme from a five-carbon sugar dietary called lyxose [115, 116]. Furthermore, elevated

levels of D-arabitol was related with the most pathogenic *Candida* spp., which may suggest a strong connection with yeast overgrowth in the GI tract [81, 117, 118].

Furthermore, incremented levels of D-mannitol, an indigestible carbohydrate, such as opioid peptides were found in ASD patients. These findings show evidences of intestinal permeability alteration in ASD patients that support "the leaky-gut hypothesis" [102, 119–121]. Therefore, opioid peptide (beta-casomorphin and gliadorphin) profiles are useful to predict and for monitoring responses to a casein and gluten-free diet [122, 123].

In conclusion, metabolomics profiles have implications for comprehending complex interactions between dietary status, gut microbiota, and genes, to characterize individual phenotypes in ASD. The study of metabolite profiles could provide a better understanding of the pathophysiology and promise to treat diseases with nutritional management of the gut microbiome and its metabolites as a way to "nutrimetabolomics" [23, 40, 124]. Novel specific nutritional intervention programs may become available and will potentially help to alleviate the burden on neurodevelopmental disorders [23, 40, 124].

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44 Brain Magnetic Resonance Imaging in Attention-Deficit/Hyperactivity Disorder (ADHD)

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Introduction

The attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by some specific behavioral patterns and cognitive dysfunctions, which could progress, causing difficulties in the school and/or work environment.

Although ADHD syndrome is characterized by attention deficit, hyperactivity, and impulsivity, not all these symptoms have to be present, as many subtypes have been described. Multiple previous subtypes have been modified (combined, with attention deficit dominance or hyperactive/impulsivity dominance), and currently the term "clinical presentations" is accepted, with the caveat that these presentations can vary during the patient's life [1].

According to the World Health Organization, ADHD is diagnosed in approximately 7% of the children population and 5% of the adult population [2].

ADHD is hereditary in 80% of the cases. In approximately 70–80% of children with ADHD, the disorder would persist into adolescence; and in approximately 30–65% cases, it would persist into adulthood. However, the manifestation of the disorder will change during life time, particularly in response to treatment.

Current evidence regarding the etiology points to multiple causes. There appears to be a series of biological disorders interacting among them as well as environmental and psychosocial factors. Early concepts, such as "minimal brain dysfunction," have evolved into different microbiological, pathophysiological, imaging, and genetic implications. Similarly, although psychosocial factors are not considered a dominant cause, many studies have found important association between a dysfunctional family and the development of symptoms. This is also the case for other behavioral disorders. Therein, this factors should be considered in the design of therapeutic interventions.

Overall, 62 clinical trials across the world have proven improved efficacy of methylphenidate in the treatment of ADHD, as compared with

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other drugs. However, there is consensus by experts in the field that following pharmacologic treatment, a multidisciplinary approach to include psychological, educational, and social support is warranted.

Among the neurochemical factors, there is a well-known dysregulation in the production of neurotransmitters, primarily dopamine and noradrenaline. Thus, an accurate initial diagnosis is important, followed by pharmacological treatment to normalize the production of neurotransmitters.

The diagnostic is clinical. As pointed before, an adequate clinic approach with an accurate diagnostic hypothesis is important, as it would lead to a pertinent complementary workup. ADHD is diagnosed by the DSM-IV and V criteria [3, 4]. The ICD-10 criteria, by the World Health Organization, can also be used for diagnosis and follow a similar approach.

In approximately 40–60% of children with ADHD, the symptoms would persist to adulthood [5, 6]. The prevalence of ADHD in adults – following the DSM-IV criteria – is estimated as 1–2% [7]. In adulthood, hyperactivity is decreased and less conspicuous than attention deficit, leading to increased overlook of symptoms and under diagnosis [8]. However, in patients with persistent hyperactivity, there is frequently a detriment in their work performance, progressive social issues, low self-esteem, and increased likelihood of motor vehicle accidents with increased risk of substance abuse and psychiatric disorders [9]. Due to its high prevalence, associated global detrimental, as well as its chronicity, ADHD was recognized as a significant public health problem in 2002. In 1991, Biederman et al. [10] recognized many disorders commonly associated to ADHD. Oppositional defiant disorder (ODD), mood and affective disorders, learning disabilities, and social misbehavior are some of them. They found that more than 50% of children and adolescents with ADHD will have at least one associated comorbidity/disorder. Also, approximately 40–60% of patients with ADHD presented ODD and approximately 20–40% social misbehavior, which traduce in increased risk of substance abuse, violence, and bribery, as well as increased risk for teenage pregnancy.

Most adults with ADHD have an associated comorbid disorder. One of the most frequent is antisocial personality (14–24%). Others include borderline personality (14%), affective disorders with depression (20%), bipolar disorder (20%), anxiety (up to 50%), social phobia (32%), panic attacks (15%), obsessive-compulsive disorder (20%), and drug abuse (20–30%).

Any improvement in the capacity to predict ADHD natural history is important, not only for patient counseling but also for taking appropriate therapeutic decisions, as well as to define or stratify an adequate population where pharmacological treatment and clinical trials would be most beneficial.

Cerebral Neuroimaging Techniques

The structural alterations related to behavior and the cognitive deficit model described in children with ADHD suggest a compromise of frontal areas similarly described in adults with detrimental executive functions and working memory. Thus, a dysfunctional frontal cortex – and/or its functionally related regions of the brain – is most likely related to the neuropsychological alterations underlying ADHD. Neuroimaging studies have been used to evaluate this model, with not only structural but also functional assessment.

Most structural studies (on computed tomography or magnetic resonance imaging) have found some evidence of anomalies in the frontal cortex or basal ganglia, supporting the idea of a frontal-subcortical syndrome. Many functional studies (PET, SPECT, fMRI) have found similar abnormalities in the metabolism or blood flow of the mentioned brain areas.

Development and validation of minimally invasive biomarkers for ADHD is an important step. It would help in early diagnosis, evaluation of disease progression, and also in defining an

adequate target population for clinical trials and new therapeutic agents.

One of the main advantages of MRI studies is the lack of ionizing radiation, as opposed to computed tomography and other imaging techniques. Moreover, MRI has greater versatility combining anatomic images with functional techniques such as perfusion, diffusion, or spectroscopy.

Morphometric MRI

The gold standard for volumetric measurements in most prior MRI studies is the manual delineation of anatomic structures. However, it requires significant training and is very time-consuming. For these reasons, it is not widespread and is not possible in studies with large number of subjects. As an alternative, many semi-automatic methods of segmentation have been proposed, reducing operator cost. These methods differ in the level of automation. One group requires manual intervention to delineate anatomic landmarks, to select initial regions of interest, or to localize seeds within the anatomic structure with the subsequent automatic delineation of the borders. Other group of methods are based on an atlas from previously segmented patients, the software deforms the atlas to fit each new case, and the labels are migrated to the segmented portions [11].

In addition to volumetric studies of anatomical structures as above, others have proposed the analysis of the spatial distribution of the anatomical differences, targeting usually atrophic regions. To achieve this goal, different maps of regional atrophy throughout the evolution of the disease are created.

Significantly decreased volume of the dorsolateral prefrontal cortex – as well as related regions such as caudate nucleus, anterior cingulate gyrus, and cerebellum – has been found in neuroimaging studies of pediatric populations. The volumetric abnormalities of the brain and cerebellum persist with time, whereas the caudate nucleus abnormalities tend to disappear [12, 13].

MR imaging has proven useful to obtain necessary information of the brain regions compromised by ADHD. There is general agreement regarding decreased volume in areas involving executive function and attention (prefrontal and striated areas). However, there are some inconsistencies, particularly regarding laterality differences in the brain volume (right versus left hemispheres).

Decreased volumes have been found in the right frontal lobe as well as the cingulate and striate region [4]. MRI studies with an appropriate sample ($n > 30$) have demonstrated decrease in volume of the right caudate nucleus. One study evaluating 50 children with ADHD found decreased mean volume of the right caudate nucleus, in comparison with a control group, suggesting abnormalities in the frontostriatal circuitry [14]. Decreased total brain volume – without asymmetric differences – was found in 50 girls with ADHD [15]. The same author also found decreased volume of the right globus pallidus and right anterior frontal region in 50 boys with ADHD [16].

In the pediatric population with ADHD, decrease in the volume of the striatum is consistently reported in voxel-based morphometric (VBM) studies, reinforcing the probable importance of this structure in the pathophysiology of this disorder [17, 18]. Early during childhood, ADHD is associated consistently with decrease in volume of the striatum.

On the other hand, striatum anomalies are rare in imaging studies using voxel-based morphometry (VBM) in adults with ADHD. It is possible that this discrepancy is related to the normalization of the striatum morphology with age and with long-term treatment. If this is the case, striatum anomalies seem to be related to symptoms in childhood. However, they would not explain the persistency of symptoms during adulthood [19].

Developmental coordination disorder (DCD) is a prevalent childhood movement disorder, impacting the ability to perform movement skills at an age-appropriate level. Recently, 44 children aged 7.8–12 years have been studied with VBM, including 22 children with DCD and 22 typically developing controls. In *DCD children*, they found a significant, large, right lateralized reductions in gray matter volume in the medial and middle

frontal and superior frontal gyri compared to controls. Decreased volume of the gray matter in the pre-motor frontal region might be related to the characteristic motor deficits in DCD. Thus, functional improvement in children with DCD can be accomplished by directed interventions in motor planning, attention, and functional executive processes associated with the regions of decreased gray matter volume [20].

Neuroimaging studies provide vital information related to the neurobiology of ADHD, but there still exists a wide gap in relevant information. A voxel-based cortical thickness and voxel-based morphometry study were performed to examine neuroanatomic distinctions in 18 children/adolescents aged 7.5–13 years diagnosed with DSM-IV TR as ADHD (nonmedicated). They were compared with 18 healthy matched controls. Voxel-based cortical thickness findings in ADHD children/adolescents revealed reduced cortical thickness in the left superior frontal, left orbitofrontal, and left dorsal anterior cingulate cortex. Voxel-based morphometry findings confirmed decreased gray matter volume in the left orbitofrontal, left middle frontal/dorsolateral prefrontal, left middle temporal, and left cerebellum in comparison to control group. A decrease in white matter volume was also observed in the left inferior frontal and left calcarine region of ADHD children/adolescents. Findings might relate to possible abnormal neuroanatomical development patterns in ADHD children [21].

In an extensive study that included 307 patients with ADHD, 169 unaffected siblings and 196 typically developing controls (mean age 17.2 [range 8–30] years) showed that brain areas involved in decision-making, motivation, cognitive control, and motor functioning (precentral gyrus, medial and orbitofrontal cortex, and cingulate cortices) had significantly smaller gray matter volume in participants with ADHD than in controls. Investigation of unaffected siblings indicated familiarity of four of the structural brain differences, supporting their potential in molecular genetic analyses in ADHD research [22].

Using gray matter morphometry and probabilistic tractography combined with multivariate statistical modeling (partial least squares regression and support vector regression), anomalies have been identified within the fronto-accumbal circuit in childhood ADHD, which were associated with increased aggression. More specifically, children with ADHD showed reduced right accumbal volumes and frontal-accumbal white matter connectivity compared *with* HC. The magnitude of the accumbal volume reductions within the ADHD group was significantly correlated with increased aggression, an effect mediated by the relationship between the accumbal volume and impulsivity. Furthermore, aggression, but not impulsivity, was significantly explained by multivariate measures of fronto-accumbal white matter connectivity and cortical thickness within the orbitofrontal cortex [23].

Another study with voxel-based morphometry (VBM) using the DARTEL approach has shown significantly smaller gray matter volume in subjects with ADHD compared to their matched controls within the anterior cingulate cortex, the occipital cortex, bilateral hippocampus/amygdala, and in widespread cerebellar regions. Further, reductions of the anterior cingulated cortex gray matter volume were found to correlate with scores of selective inattention [24].

A large number of structural neuroimaging studies have used voxel-based morphometry to identify gray matter abnormalities in youths with conduct problems, but the findings have been disparate and few have been replicated. Anisotropic effect-size signed differential mapping was used for voxel-based meta-analyses. Statistical parametric maps comparing gray matter differences between youths with conduct problems and typically developing youths were available for 11 of the studies, with peak coordinates available for the remaining studies. Youths with conduct problems had decreased gray matter volume in the left amygdala (Fig. 44.1) (extending into anterior insula), right insula (extending ventrolaterally into the prefrontal cortex and inferiorly into the superior temporal gyrus), left medial superior frontal gyrus (extending into the right anterior cingulate cortex), and left fusiform gyrus. Subgroup meta-analysis assessing age-at-onset effects identified reduced gray matter volume in

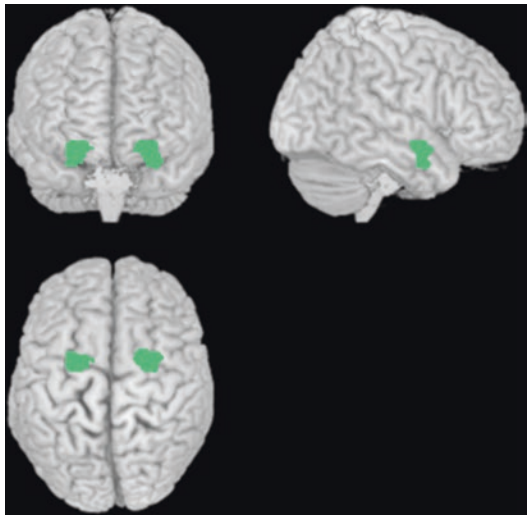

Fig. 44.1 3D reconstruction of right and left amygdala

the left anterior insula (extending into amygdala). Meta-regression analyses revealed that greater scores on measures of callous-unemotional traits were associated with a lower reduction in gray matter volume in the left putamen. The proportion of male and female youths in the sample was associated with decreased gray matter volume in the left amygdala and increased gray matter volume in the right inferior temporal cortex. While there was no association with comorbid attention-deficit/hyperactivity disorder or intelligence quotient, age range was associated with gray matter differences in the left amygdala [25].

Girls have been underrepresented in past studies on ADHD [26], probably due to the predominance of male subjects in clinical settings [27]. Females with ADHD have fewer hyperactive/impulsive symptoms and more inattentive symptoms, present more commonly with the predominantly inattentive subtype, and tend to be underdiagnosed when compared to boys with ADHD.

ADHD in adulthood is a serious and frequent psychiatric disorder with the core symptoms, inattention, impulsivity, and hyperactivity. Increased cortical thickness in the left occipital cortex may represent a mechanism to compensate for dysfunctional attentional networks in male adult ADHD patient [28].

Some studies have pointed to the relation of limbic structures in the pathogenesis of ADHD. Recently, using manual segmentation on MR images, Nickel et al. compared the volume of the amygdala and hippocampus in 30 patients with ADHD and 30 control subjects. They did not find a significant difference in the volumes. However, patients with significantly increased hyperactivity demonstrated decreased volume of the left amygdala. This suggests that limbic alterations might be significantly related to hyperactive symptoms in ADHD [29].

In other study, voxel-based morphometry (VBM) was applied to 44 adults with ADHD, combined subtype, aged 18–54 years, and 44 healthy controls matched for age, sex, and IQ. Here, ADHD patients showed reduced gray matter volume in the right supplementary motor area. Using more lenient thresholds, they also observed reductions in the subgenual anterior cingulate and right dorsolateral prefrontal cortices and increases in the basal ganglia, specifically in the left caudate nucleus and putamen. There was a positive correlation between the cumulative stimulant dose and volume in the right supplementary motor area and dorsolateral prefrontal cortices clusters, suggesting that adults with ADHD show brain structural changes in regions belonging to the so-called cool executive function network. Thus, long-term stimulant medication may act to normalize these gray matter GM alterations [30] (Fig. 44.2a, b).

131 patients with ADHD, whom had stopped the previous use of stimulant medication for 6 months, and 95 healthy control subjects (18–58 years of age) were studied with VBM. The results showed that ADHD in adulthood is associated with global rather than regional volumetric abnormalities and that previous use of stimulant medication does not seem to modify subsequent brain volumes in a significant way [31]

Recently, radiomics were used. It consist in the extraction from magnetic resonance imaging scans of a large amount of quantitative information from digital imaging features. Using radiomics, they can build and evaluate classification models based on pathological subtyping. The researchers examined 83 children aged 7–14 with newly diag-

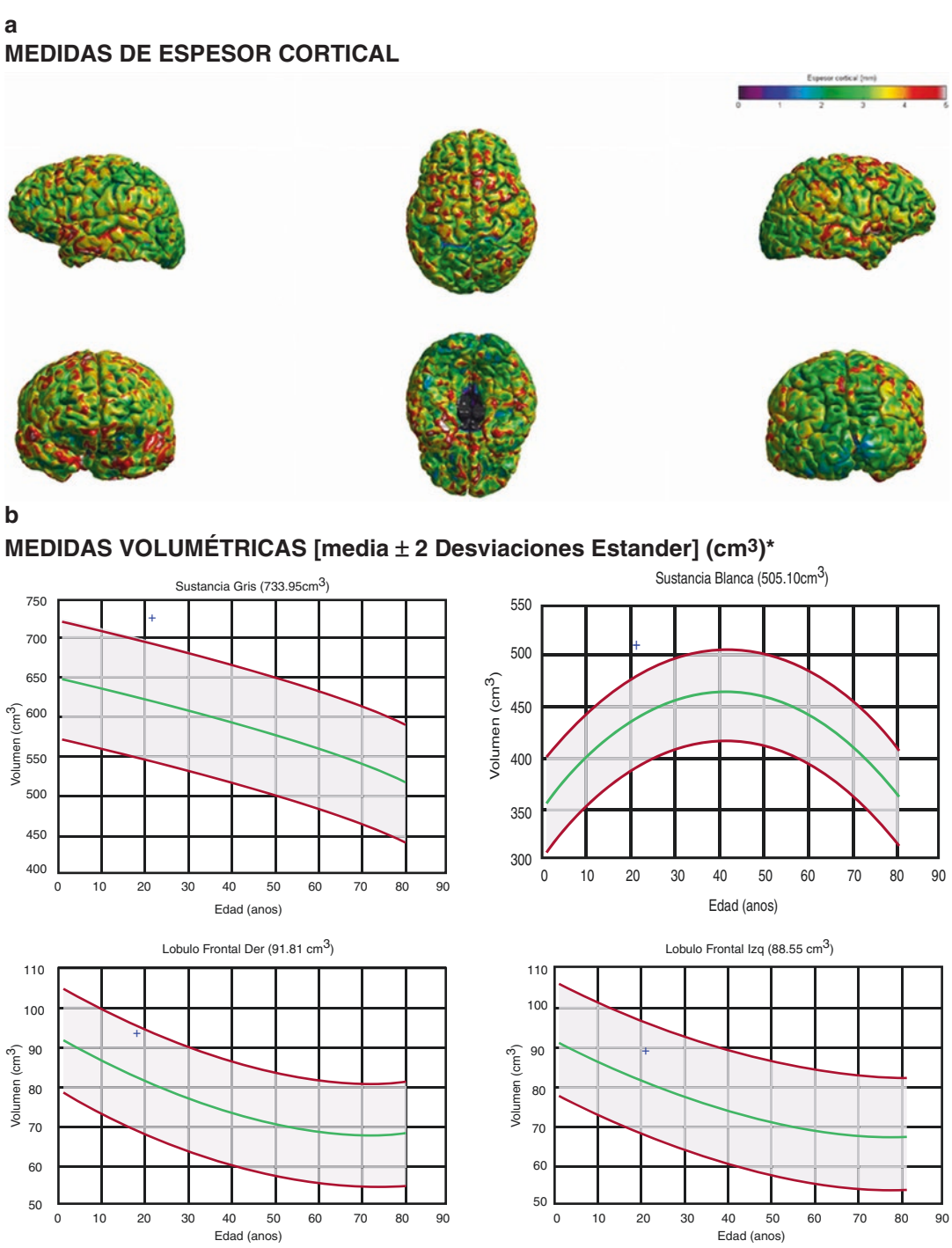

Fig. 44.2 (**a**, **b**) Measurements of cortical thickness and standard deviation in adult patient with ADHD compared with group of healthy patients of the same sex and age

nosed and never-treated ADHD, including children with the inattentive ADHD subtype (ADHD-1) and the combined subtype (ADHD-C). The scientists compared these MRI results with those of a control group of 87 healthy children of the same age and screened relevant radiomics signatures from more than 3,100 quantitative features extracted from the gray and white matter.

They found alterations in the shape of the left temporal lobe, bilateral cuneus, and areas around left central sulcus, and these differences contributed significantly to distinguishing ADHD from typically developing controls. Within the ADHD population, features involve in the default mode network and the insular cortex significantly contributed to discriminating the ADHD inattentive subtype from the combined subtype. They could discriminate patients with ADHD with control subjects with 73.7% accuracy and discriminate ADHD-1 from ADHD-C patients with over 80.1% accuracy [32]

Attention-deficit/hyperactivity disorder + comorbid oppositional defiant disorder (ADHD+ODD) and ADHD-only were associated with volumetric reductions in brain areas crucial for attention, (working) memory, and decision-making. Volumetric reductions of frontal lobes were largest in the attention-deficit/hyperactivity disorder + comorbid oppositional defiant disorder (ADHD+ODD) group, possibly underlying observed larger impairments in neurocognitive functions. Previously reported striatal abnormalities in ADHD may be caused by comorbid conduct disorder rather than oppositional defiant disorder (ODD) [33]

Functional MRI

Relations between structural anomalies and functional deficits have also been studied in ADHD. For 756 human participants in the ADHD-200 sample, they produce gray and white matter volume maps with voxel-based morphometry, as well as whole-brain functional connectomes. Joint independent component analysis was performed, and the resulting transmodal components were tested for differential expression in ADHD versus healthy controls. Four components showed greater expression in ADHD. They have been observed reduced default mode network (DMN) and task-positive networks (TPN) segregation co-occurring with structural abnormalities in dorsolateral prefrontal cortex and anterior cingulate cortex, two important cognitive control regions. They also observed altered intranetwork connectivity in default mode network, dorsal attention network, and visual network, with co-occurring distributed structural deficits. There was strong evidence of spatial correspondence across modalities: for all four components, the impact of the respective component on gray matter at a region strongly predicted the impact on functional connectivity at that region. Overall, these results show that ADHD involves multiple, cohesive modality spanning deficits, each one of which exhibits strong spatial overlap in the pattern of structural and functional alterations [34]

Studies performed with fMRI and MEG have found hypoactivation of the prefrontal cortex, predominantly in the right hemisphere as well as the caudate nucleus and anterior cingulate, and differences in the activation of the basal ganglia [35, 36].

Compared to the healthy controls, the ADHD in children showed significantly decreased functional connectivity that primarily involved the default mode network and frontoparietal network regions and cross-network long-range connections. Further graph-based network analysis revealed that the ADHD in children had fewer connections, lower network efficiency, and more functional modules compared with the healthy controls. The attention-deficit/hyperactivity disorder-related alterations in functional connectivity but not topological organization were correlated with clinical symptoms of the attention-deficit/hyperactivity disorder children and differentiated the patients from the healthy controls with a good performance. Taken together, these signs suggest a less integrated functional brain network in children with ADHD due to selective disruption of key long-range connections, with important implications for understanding the neural substrates of ADHD, particularly executive dysfunction [37].

A study explored age-related brain network differences between ADHD patients and typically developing (TD) subjects using resting state fMRI (rs-fMRI) for three age groups of children, adolescents, and adults. They collected rs-fMRI data from 184 individuals (27 ADHD children and 31 TD children; 32 ADHD adolescents and 32 TD adolescents; and 31 ADHD adults and 31 TD adults). The

Brainnetome Atlas was used to define nodes in the network analysis. They compared three age groups of ADHD and TD subjects to identify the distinct regions that could explain age-related brain network differences based on degree centrality, a well-known measure of nodal centrality. The left middle temporal gyrus showed significant interaction effects between disease status (i.e., ADHD or TD) and age (i.e., child, adolescent, or adult). Additional regions were identified at a relaxed threshold. Many of the identified regions (the left inferior frontal gyrus, the left middle temporal gyrus, and the left insular gyrus) were related to cognitive function, suggesting that aberrant development in cognitive brain regions might be associated with age-related brain network changes in ADHD patients [38].

Studies with functional imaging in children are rare. Positron emission tomography offers information of the brain glucose metabolism, globally and in selected areas. Studies performed with small samples in adolescence have found no abnormalities in the brain glucose metabolism [39, 40].

Magnetic Resonance Spectroscopy

The most frequently used spectroscopy is that originated from hydrogen nucleus. The most frequently evaluated metabolites are N-acetyl-aspartate (NAA), myo-inositol (mI), choline (Cho), creatine (Cr), and glutamate + glutamine (Glx) (Fig. 44.3).

NAA is a marker of neuronal and axonal viability and density. Myo-inositol has been regarded as glial marker located in the astrocytes, as product of myelin degradation, and the most important osmolyte or cell volume regulator. Choline is a marker of the phospholipid metabolism and cellular membrane turnover marker, reflecting cellular proliferation. Creatine is used as internal reference value, since it is the most stable cerebral metabolite. It has a role in the energetic system of the brain and in the osmoregulation. Lactate peak indicates anaerobic glycolysis in tumors. Lipid peak indicates necrosis and/or disruption of myelin sheath. Glutamate is an important neurotoxic brain marker. Excess of glutamate can produce neuronal death through excitotoxic processes [41]. It is also assumed that glutamate in the frontal circuits is an important regulator of dopamine [42–44], and through a feedback mechanism, the concentration of dopamine can influence the concentration of glutamate [45, 46].

Magnetic resonance spectroscopy may be necessary to obtain information from living tissues [47]. A previous study of 23 ADHD patients does not find significant differences in the neurometabolites of the dorsal-lateral frontal region [48]. There are three studies that show an

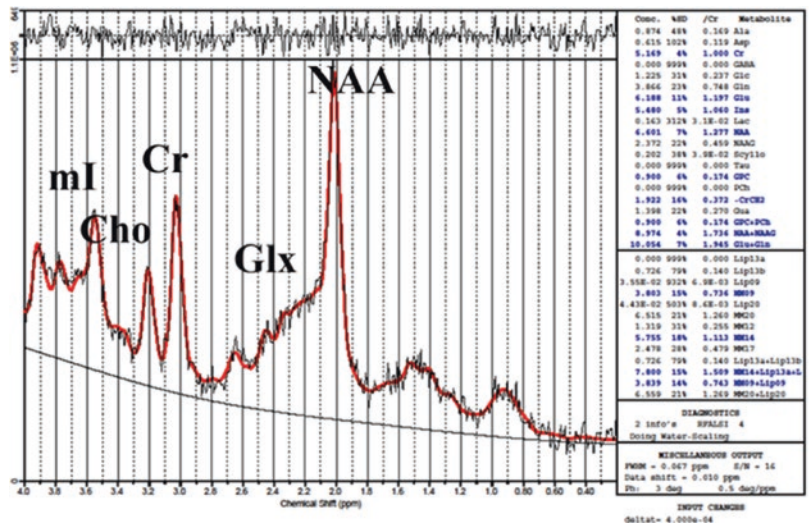

Fig. 44.3 A typical in vivo example of linear combination model spectrum in the same area with metabolite peaks. *mI* myo-inositol, *Cho* choline compounds, *Cr* creatine, *Glx* glutamate + glutamine, *NAA* N-acetyl-aspartate

increase in N-acetylaspartate/creatine ratios in children with ADHD in the right frontal region [49] and in the left semioval center, respectively [50, 51].

In vivo phosphorus 31 magnetic resonance spectroscopy (31P MRS) has shown lower bilateral membrane phospholipid (MPL) precursor levels in the basal ganglia and higher MPL precursor levels in the inferior parietal region (primarily right side) in 31 psychostimulant-naive children with ADHD (mean [SD] age, 8.1 [1.2] years; range, 6.1–10.0 years) as compared with 36 healthy control children (mean [SD] age, 8.1 [1.3] years; range, 6.1–10.4 years), and these results are suggestive of possible progressive, nonlinear, and sequential alterations implicating a bottom-up developmental dysfunction in parts of the cortico-striato-thalamo-cortical network in ADHD [52].

Conclusion

ADHD is a social problem and not just a problem for the sick, their families, and educators. It impacts society and the general policy of nations. It is still necessary to carry out public education campaigns to make people aware of the reasons why everyone, as a society, should be seriously concerned about ADHD and we must support research to conquer this disease. The diagnosis is clinical, but neuroradiological techniques can be used in research studies.

In the future, imaging-based disease classification will gain importance versus the diagnostic and statistical manual of mental disorders. Standardization will be necessary to clinically validate these technique, but they will prove useful as they advance, in particular in data acquisition and analysis. Interventional psychoradiology will also be developed.

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45 Research Contributions of Magnetic Resonance Spectroscopy in Psychopathology

Nicolás Miguel Fayed, Carlos Torres, Humberto Morales, Luisa F. Ángel Ríos, and Alicia Fayed Coca

Introduction

Brief History of Neuroradiology and Magnetic Resonance Imaging

The term Neuroradiology was first used in Great Britain in 1938 as a link between Radiology and Neuropsychiatry; however, it was not officially established in Europe until the Symposium Neuroradiologicum of 1949 in Rotterdam, chaired by Ziedses des Plantes. Around the same time in the United States, Dyke established Neuroradiology as a specialty and he was one of its most outstanding pioneers. In the 1950s and 1960s, several radiologists distinguished themselves in Europe and in the United States in the development of Neuroradiology.

MRI scanners are based on the discovery of nuclear magnetic resonance, a phenomenon that was detected independently by Bloch [1] and Purcell [2] in 1946. Magnetic Resonance Imaging (MRI) was introduced to clinical medicine in 1981, for which Lauterbur [3] and Mansfield [4] were awarded the Nobel Prize in Medicine in 2003. In the short space of time that has elapsed since then, MRI has assumed an especially important role in medical diagnosis. It likely represents the most important advance in imaging since the introduction of the X-ray in 1895. MRI, without any doubt, is the best imaging modality to study the central nervous system including the spinal cord.

In recent years, neuroscience has experienced a growing interest in applying its methods for understanding psychopathologic disorders, and neuroimaging techniques represent one of the fundamental tools to achieve this goal. Techniques that allow the metabolic (spectroscopy) and functional (perfusion, diffusion, diffusion tensor, tractography) assessment of multiple pathologies are currently used.

Magnetic Resonance Spectroscopy (MRS)

Magnetic resonance spectroscopy (MRS) is a noninvasive method that studies cellular bio-

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chemistry in vivo. When MRS is used in living tissues, it is possible to measure their chemical composition, assess the characteristics of some metabolic processes, and identify in advance the chemical or metabolic relationship of the disease. It could be used to detect abnormalities in anatomical regions that appear normal in conventional MR images, or to better assess a visualized pathology in a conventional MRI. Several studies have used MRI for the evaluation of cerebral structural anomalies associated with psychiatric disorders; however, the use of MRS to characterize chemical or metabolic tissue abnormalities in specific psychiatric disorders continues to increase.

Richard Ernst was awarded the Nobel Prize in Chemistry in 1991 for his contribution to the development of the high-resolution Magnetic Resonance Spectroscopy methodology in 1975.

Magnetic Resonance Spectroscopy is the only noninvasive technique able to measure the chemical products present in the body. The rapid proliferation of MRI magnets with the capability of MRS throughout Europe and the United States has increased the interest in using MRS as a clinical diagnostic tool.

Although MRI and MRS share similar fundamental principles, many important differences can be highlighted between these two techniques. For the clinician, one of the main differences is that MRI produces a visual image, while MRS obtains chemical information that can be expressed with numerical values.

MRS is a technique that allows characterizing of certain cerebral metabolites and interpreting them qualitatively and/or quantitatively. It can be considered as a biochemical photograph of a certain volume, in our case, of the brain. It aims to improve the sensitivity and specificity in routine neurodiagnoses, and although its interpretation (physiological and biochemical) is totally different from the conventional images we are used to observe, MRS results may determine new treatment strategies that allow controlling of disease progression, treatment response, or evaluating of prognosis [5].

Fundamental Principles of MR Spectroscopy

MRS technique requires short radiofrequencey (RF) energy pulses (B1) to excite the nuclei, followed by a period of signal acquisition. The acquired signal represents the free induction drop (FID) that is converted by the Fourier transform into spectral values. The horizontal axis (abscissa) of a spectrum represents the resonance frequency. MRS uses different MR field intensities and therefore, the abscissa is usually expressed in parts per million (ppm), which represents a small frequency interval proportional to the total resonance frequency used in the experiment.

Chemical deviations in parts per million are positive toward the left, and the zero point [tetramethylsilane (TMS) is used for 1H] is placed at the resonance frequency of each compound [6]. The interpretation of the results of MRS is greatly simplified if the volume of tissue that produces the signals is localized, so that it corresponds to a limited volume of interest (VOI) (Fig. 45.1).

As we know, water is abundant in our body, and its quantity is much greater than that of the

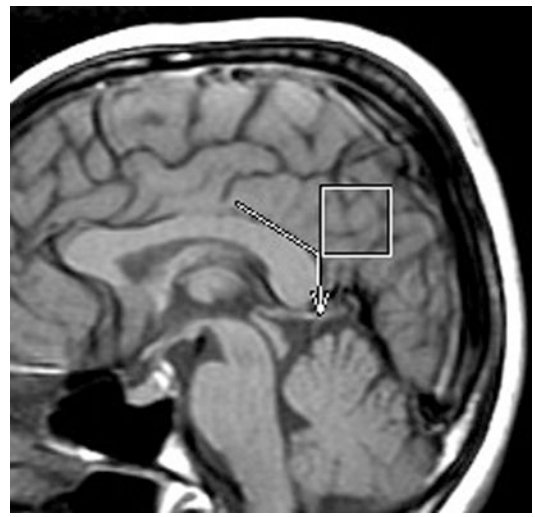

Fig. 45.1 Sagital T1-weighted magnetic resonance imaging with the voxel placed in the bilateral posteromedial parietal cortex (posterior cingulate gyrus and inferior precuneus)

metabolites we want to evaluate; thus, in obtaining and visualizing the spectra the resonance frequency of hydrogen in water must be saturated or canceled [7, 8].

The location or resonance of the most relevant compounds in parts per million (ppm) are: lipids, between 0.8 and 1.3 ppm; lactate, at 1.33 ppm (doublet) and a second peak at 4.1 ppm (quadruple); alanine, at 1.48 ppm; N-acetil-aspartate (NAA), at 2.02, 2.5 and 2.6 ppm; glutamine y glutamate, at 2.1 and 2.55 ppm; creatine (Cr), at 3.02 and 3.94 ppm; choline (Co), at 3.22 ppm; myo-inositol (mI), at 3.56 and 4.06 ppm.

Myo-inositol is considered a glial marker, a possible degradation product of myelin and osmolyte or cell volume regulator. Choline is a constituent of the phospholipid metabolism of cell membranes and reflects cell proliferation. Creatine plays an important role in the cerebral energy system (ATP), as a marker of cerebral metabolism. NAA is a marker of viability, density, and neuronal and axonal function. Lactate is visualized in oxygen deficiency states and therefore is a product of anaerobic glycolysis. Lipids reflect a necrotic process as well as products of microbial metabolism [9]. Glutamate and glutamine are difficult to separate at 1.5 Tesla field strength, and thus this separation is only achieved in high magnetic fields. Glutamate, a product of ammonium metabolism, is neurotoxic and a marker of hypoxic injury.

Currently, brain spectra can be obtained with single- or multi-voxel techniques. The single-voxel technique has the advantage of a more exact spatial localization, greater homogeneity, better water suppression, and shorter acquisition time, but only one spectrum can be obtained for each data acquisition. The multi-voxel technique, on the other hand, has the advantage of obtaining multiple spectra simultaneously during a data acquisition and is useful to evaluate a larger area of brain parenchyma or a larger lesion; however, the spectral resolution is reduced and there is increased voxel contamination. The single-voxel technique is still the most reproducible [10–13].

Psychopathology

Etymologically, psyche (psyche): soul or reason; pathos (pazos): disease; and logos, which means discussion or rational discourse, may be used in three contexts:

As an area of study within healthcare that describes behavioral changes that are not explained by the maturation or development of the individual, nor as a result of learning processes, also known as psychological disorders or mental disorders.

As a descriptive term: a specific reference to a sign or symptom that can be found as part of a psychological disorder.

As an area of study in psychology that, contrary to the health status (as defined by the World Health Organization: social, psychological, and biological), focuses on studying the processes that can induce “unhealthy” states in the mental process. In this way, the role of learning and behavioral analysis (behavioral psychology) or any other cognitive process, can explain people’s “unhealthy” states, and can guide potential treatment approaches. In this sense, mental disorders are not strictly a synonym of psychopathology, since there are ways to explain the absence of health. For example, the learning processes related to phobia are well known, whether or not this clinical entity falls within the psychiatric category of phobia.

Different professions can become involved in the study of psychopathology. However, mainly psychiatrists and psychologists are interested in this area, since they are involved in research related to the origin of the clinical pictures, their manifestation and development, and they also participate in the management. On a more general level, many other specialties can participate in the study of psychopathology. For example, professionals in the field of neuroscience can focus their research efforts in studying the brain changes that occur in mental disorders.

We intend, in this chapter, to make a compilation of the evolution of our most relevant clinical and applied research work in the field of psychopathology that we have been developing with

Magnetic Resonance Spectroscopy for 13 years (2005–2018), and whose results have been published in various national and international scientific journals.

The first step before starting a research study is to perform a reproducibility study of the metabolites, measured in the same area of the brain with two consecutive acquisitions without removing the patient from the scanner. According to the alpha coefficients, we can assume an average variation of approximately 8% for the myo-inositol/Creatine (mI/Cr) index, and around 10% for N-acetil-aspartate/Creatine (NAA/Cr), Choline/Creatine (Co/Cr) and Glutamate/Creatine (Glx/Cr) [14]. The next step would be to obtain metabolite values in the gray and white matter of healthy brains in individuals of different age [5].

Normal Development

In the newborn, myo-inositol is the predominant metabolite in the spectrum while choline increases in the first days of life. The concentrations of NAA and creatine are lower than in adults. During the first weeks of life, an increase of creatine and NAA is observed, as well as decrease in choline and myo-inositol. Spectral abnormalities in this age can be of great help in diagnosis and monitoring, especially after myelination is completed.

A progressive increase of NAA is observed with age in the white matter of normal individuals, predominantly in the first 2–3 years of life, and continuing until the age of 20–25, after which it starts to decrease. These change patterns may reflect neuronal maturation due to increased synaptic, dendritic, and axonal projections. NAA decreases progressively in the cerebral cortex from birth to old age.

Creatine levels in the brain also change during development, increasing from birth to approximately 2 years of life. During the first year of life, and parallel to myelination, the concentration of choline decreases rapidly. Lactate is an important source of brain energy and is often elevated in congenital errors of metabolism or hypoxia but also in conditions of moderate reduction in cerebral blood flow and/or increase in cerebral metabolism, such as in response to hyperventilation or caffeine ingestion. Small elevations of lactate, in conjunction with other chemical changes such as glutamate elevation may also reflect subtle bioenergetic changes of the brain. It is a marker in specific psychiatric disorders such as bipolar disorder. Glutamate and GABA, the most important neurotransmitters, stimulant to nerve cells and cell inhibitor respectively, are of substantial interest in some clinical conditions, such as epilepsy.

A prospective study of 207 patients with Neuropsychiatric Disorders (148 women and 59 men; age 29–90 years; mean age 56) and 193 Healthy Controls group (117 women and 76 men; age 29–90 years; mean age 53.2) showed levels of NAA and NAA/Cr are significantly lower in patients with Neuropsychiatric Disorders than in the Healthy Controls HC group, whereas levels of Glx and Glx/Cr are significantly higher in those with Neuropsychiatric Disorders than in healthy people. When analyzing metabolite levels in the whole sample while controlling for age, gender, and psychiatric disorders, we observe that all metabolites are correlated with age. Glu, Glx, and NAA and their ratios to Cr show a negative correlation (increase in age with a decrease in metabolite levels and vice versa), whereas the remaining metabolites, such as mI and Cho, show a direct correlation. A decrease in Glu and Glx 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 [15–18].

Aging is associated with a general reduction in the level of NAA. Several independent studies have found reductions in the absolute concentration of NAA and in the ratios NAA/choline and NAA/Cr in the cortical gray matter [19], ranging from a few percent to >10% over an age range spanning the second to the eighth decade.

Applications of Magnetic Resonance Spectroscopy in the Study of Psychopathology

Attention-Deficit/Hyperactivity Disorder (ADHD). Autism

Research with magnetic resonance spectroscopy has recently been applied to ADHD. Some research groups have already investigated neurometabolic differences between patients and healthy controls using this noninvasive method. We carried out studies on 41 children with ADHD and autistic, with a mean age of 9 years, and we compared them with a group of healthy children in order to find out if there were metabolic changes in the white matter of the left centrum semiovale and/or in the cortical-subcortical right prefrontal region [20, 21]. We found an increase in the NAA/Cr ratios in the right prefrontal region as well as a more significant increase of these ratios in the white matter of the left centrum semiovale, when compared with autistic children and controls. We postulate that this increase in NAA levels may be related to mitochondrial hypermetabolism in patients with ADHD or could be due to an increase in the axonal transport of neurotransmitters, as seems to occur in Asperger syndrome; however, what happens to these patients remains unclear. The role of NAA in white matter axons is unknown; however, it is possible that they participate in the synthesis of neurotransmitters. Such information may allow us to add new knowledge in the understanding of this complex disorder. Paradoxically, although the anatomy, function, and biochemistry of this disorder are well known, we remain far from consolidating these data and obtaining a coherent explanation. In hyperammonemia, glutamine is reportedly elevated as is NAA in Canavan disease [9, 22].

In familial leukodystrophies, such as adrenoleukodystrophy and metachromatic leukodystrophy, choline compounds may be elevated due to the presence of inflammation [23].

In autistic children a decrease of NAA was reported in the temporal cortex and in the cerebellar white matter [24]. In other studies with autistic children, variable levels of choline and creatine have been observed in different cortical areas [25]. We did not find changes in the white matter of autistic children, in the centrum semiovale, in comparison to healthy controls.

Delayed Psychomotor Development

Isolated psychomotor development is a fairly common disorder in preschool and school-age children, with an estimated prevalence greater than 10% [26]. It has been suggested that more than 15% of school-age children have mild developmental delay [27, 28].

Recent neuroimaging studies have not shown any abnormality in conventional magnetic resonance in 53% of patients, even when delayed myelination is suspected, either as a cause or as a factor associated with this pathology.

Given that NAA is considered a marker of neuronal and axonal integrity, we tried to determine if MRS was a valid technique for the detection of white matter, abnormalities that would support the hypothesis of delayed myelination in children with isolated psychomotor delay and normal MRI [29]. The study included 12 children with an average age of 7 years who presented some delay in normal development related to speech or language, motor or learning skills (Categories F80 to F82 of the International Code of Diseases), and 11 normal children of the same age and sex. We found a significant decrease of NAA/Cr, NAA/Co, and NAA/mI ratios in the white matter of the left centrum semiovale in children with isolated psychomotor delay in comparison to the controls, and we observed how the NAA/Cr ratio progressively increased with age in the two groups; it however remained lower in the psychomotor delayed group when compared to the controls. The decrease of NAA/Cr could be a promising indicator of this disorder based on the hypothesis of changes in normal myelination, caused either by delayed myelination, hypomyelination, or myelin damage. Some authors hypothesize that the decrease in NAA in the brain of children with developmental delay may be due to hypomyelination or decreased

synaptic density. It is evident that MRS is a tool that can offer important information in children with neurodevelopmental delay, allowing for an early diagnosis and institution of appropriate therapies.

Mild Cognitive Impairment and Alzheimer's Disease

Mild cognitive impairment (MCI) is an intermediate cognitive state between normal aging and dementia. Patients with amnestic MCI have a deterioration of secondary memory, or memory for information but can still lead a normal life. A large proportion of patients with amnestic MCI progress to Alzheimer's disease (AD), dementia with Lewy bodies (DLB), or vascular dementia, while a fraction remain stable in their cognitive capacity, and a few even improve. The preclinical phase of Alzheimer's disease is being subject of numerous investigations with the goal of obtaining an early diagnosis.

The general objective of our research has been to develop and evaluate imaging analysis techniques using magnetic resonance spectroscopy for early prediction of patients with mild cognitive impairment likely to progress to Alzheimer's disease.

For this purpose, we conducted spectroscopy studies in the left hippocampus, left occipital cortex, and right parietal cortex of 53 patients with MCI who were clinically followed for an average of 3 years; and we were able to demonstrate, to the best of our knowledge for the first time in the world, that spectroscopy can predict the conversion of MCI to probable AD with enough years in advance. A ratio of NAA/Cr less than or equal to 1.61 in the left occipital cortex predicts with 100% sensitivity and 75% specificity [30]. The positive predictive value was 83% and the negative predictive value was 100%, correctly classifying 88.7% of the patients (area under the curve = 0.91, confidence interval 95% = range between 0.80 and 0.97). Values in the hippocampus and in the parietal cortex had no significant predictive value. In addition, spectroscopy proved superior to neuropsychological tests.

In a subsequent study, we performed MRS in the posterior cingulate gyrus and in the left occipital cortex of 71 patients clinically diagnosed with MCI who were followed and clinically controlled for an average of 2 and a half years; which were compared with a control group of 35 patients of the same sex and similar age but without neuropsychological abnormalities [31]. We observed that the ratio of NAA/Cr in the left occipital lobe was able to differentiate patients with MCI from controls and that the ratios of NAA/Cr in the posterior cingulated gyrus and in the left occipital lobe were able to differentiate the converters to probable Alzheimer from non-converters with a very significant p. A ratio of NAA/Cr equal to or less than 1.43 in the posterior cingulate predicts with a sensitivity of 74% and a specificity of 84%, correctly classifying 82% of patients. The cross-validated accuracy of classification was 82%, reaching 85% when the APOE genotype and memory test were included in the analysis.

Glutamate (Glu) is the most abundant excitatory neurotransmitter in the central nervous system (CNS) and is involved in the pathophysiology of Alzheimer's disease (AD) in which there is an increased excitotoxicity. Biochemical composition of living tissues including the levels of Glu was analyzed by magnetic resonance spectroscopy (MRS) [16]. Previous reports point to decreased levels of Glu in AD. As Glu plays an important role in memory, we hypothesize that Glu levels are decreased in patients with AD when compared with controls. A consecutive sample of 30 patients with mild-to-moderate AD underwent H-MRS with the voxel placed in the bilateral posterior cingulated gyrus. For comparison purposes, we carried out the same technique in 68 patients with mild cognitive impairment (MCI) and in 26 controls. The healthy controls had higher metabolite levels of N-acetyl-aspartate (NAA) than patients with MCI and AD. In turn, patients with MCI and the controls had higher levels of Glu than in patients with AD. The differences were significant in the analysis of variance (ANOVA) test model corrected for age. In the post hoc analysis, the most remarkable differences were seen between patients with AD and

the rest (patients with MCI and the controls). In AD, the levels of Glu and NAA are decreased in comparison with MCI and normality, which reflects loss of neurons.

Similarly, in another study with 119 patients, we used MRS in the posterior cingulate and in the left occipital cortex to try to discriminate patients with AD from patients with MCI, MCI associated to vascular pathology, depression, anxiety, Lewy bodies, etc. and to assess early patients with MCI that progress to AD. We confirmed that the NAA/Cr and NAA/mI ratios in the posterior cingulate can differentiate AD from MCI patients, as well as AD from MCI patients with associated depression. We also observed how the NAA/Cr ratio in the left occipital cortex and predominantly in the posterior cingulated can predict the progression of MCI to probable AD, with high sensitivity and specificity [32] (Fig. 45.2).

In another study with 87 patients, we demonstrated that decreased NAA/Cr and NAA/mI in the occipital cortex and in the posterior cingulated correlates with the clinical dementia rating (CDR) scale and with the global deterioration scale (GDS) [33]. Given MR spectroscopy is able to predict the progression from MCI to AD and can differentiate one type of patient from the other, the next working hypothesis was to try to evaluate the efficacy of the different drugs currently available for the treatment and/or prevention of mild-to-moderate AD. Although mild improvement was noted in the 24 patients treated with Rivastigmine [34] with respect to 15 untreated patients, (we observed a modest short-term improvement of neuronal function in the frontal and parietal cortex, related to an increase in NAA/Cr); no significant changes were observed in the cognitive and non-cognitive scales between the baseline and posttreatment studies in another study with 64 patients who received Donepezil and Memantine [35]. Furthermore, no significant changes were observed when comparing ADAS-cognitive and NAA/Cr ratios between the studies at baseline and 6 months after treatment.

The importance of these latest investigations, apart from showing that the current treatments have a very limited effect on this type of pathologies, is to highlight the possibilities of MR spectroscopy as a useful tool for early diagnosis and for monitoring the response of future treatments in Alzheimer's disease. We can conclude that Magnetic Resonance Spectroscopy is a valuable tool to predict which patients will progress to more deteriorating pathologies. Our team has carried out several spectroscopic studies in patients with MCI and AD, reaching the conclusion that the location of the voxel for spectroscopy is relevant, and that its placement in the posterior cingulate and in the left occipital cortical region has a greater predictive robustness than the hippocampus. We also conducted several studies in cognitively intact at-risk elderly individuals (e.g., subjective cognitive decline patients and APOE4 carriers) in order to identify biomarkers associated with increased risk of developing AD.

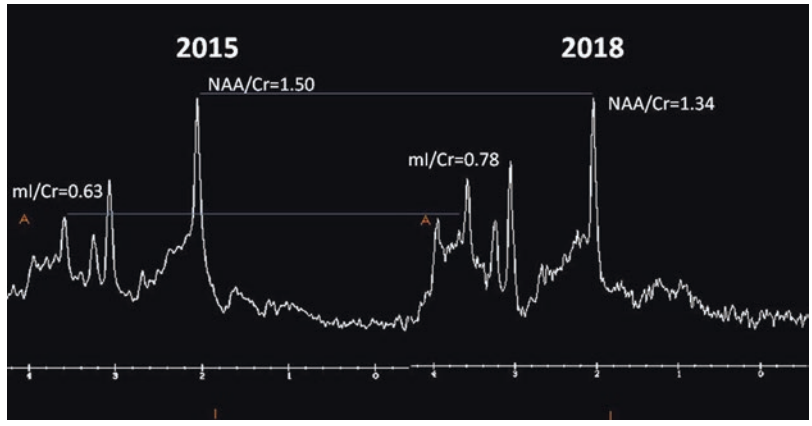

Fig. 45.2 Spectroscopy in patients with mild cognitive impairment in 2015 (myo-inositol/creatine = 0.63 and N-acetylaspartate/creatine = 1.50) and probable Alzheimer's disease in 2018 (myo-inositol/creatine = 0.78 and N-acetylaspartate/creatine = 1.34)

The development of treatments is still a priority for future research to prevent, delay, slow down, or halt the degenerative process. Innovative strategies should be developed, new paths should be considered, and pharmacological therapeutics should take into account the multifactorial dimension of the disease instead of treating one of the elements [36, 37].

Parkinson's Disease

To date and with the exception of PET, there are no specific radiological markers of the disease. However, it is necessary to have a marker in order to monitor the progression of the disease and the response to treatment.

The results of the spectroscopy studies in patients with Parkinson's disease have been variable and the only repetitive finding in all these studies has been the lack of consistency. This is due, in part, to the fact that many studies have been performed with few patients. The first study with 151 patients did not show significant reduction of NAA/Cr in the basal ganglia [38]. Other studies using spectroscopy have also shown no differences in NAA/Cr at the basal ganglia level. Some authors have described a 24% decrease in Cr concentration in the substantia nigra [39]. In some studies performed with 3 and 4 Tesla magnets, an increase in the GABA/Glu ratio has been observed in the basal ganglia in comparison with the cerebral cortex [40].

In our study, we recruited patients with Parkinson's disease in different stages of the disease according to the Hoehn and Yahr scale and different degrees of clinical severity measured with the Unified Parkinson's Disease Rating Scale (UPDRS) who had been treated with drugs. The aim of the study was to determine whether MR spectroscopy or diffusion tensor imaging could be markers of disease progression. We found a significantly negative correlation between the UPDRS scale and glutamate (Glu), glutamate + glutamine (Glx) and its ratios with creatine, which may suggest an increase in glutamatergic metabolism in early stages and a decrease in metabolism or effect of the medication in the late stages of the disease [41].

Fibromyalgia and Somatoform Disorders

Clinical heterogeneity of fibromyalgia is one of the greatest limitations for clinical research and for the development of effective treatments.

Our first objective was to conduct a pilot study in patients with fibromyalgia using psychological (depression and anxiety), cognitive (catastrophism), and biological (pain threshold) variables as well as neuroimaging techniques.

Understanding the neurophysiology of the pain process using different neuroimaging methods has gained increased interest in recent years. Brain imaging has confirmed the increased pain reported by patients with fibromyalgia, during experimental painful stimulation. In addition, thalamic activity, which contributes significantly to the processing of pain, is diminished in fibromyalgia. However, the central mechanisms of pain in fibromyalgia may not exclusively depend on neuronal activity. It has been found that neuroglial activation plays an important role in the induction and maintenance of chronic pain, which may have important implications for future research and for pain treatment in fibromyalgia.

Based on all these data, we decided to investigate with a cross-sectional study whether there are biochemical brain differences between 10 patients with fibromyalgia (FM) and 10 healthy controls of the same sex and age, as well as whether there is a correlation between cerebral metabolite abnormalities and clinical variables [42]. In order to do this, we placed spectroscopy voxels in both hippocampi, thalami, posterior cingulated and left primary sensory-motor region, the latter after being appropriately localized with functional magnetic resonance imaging (fMRI) while performing right-hand movements. We observed that in patients with FM, there was a significant increase in glutamate + glutamine and glutamate + glutamine/creatine levels in the posterior cingulated, as well as significantly lower levels of myo-inositol in both hippocampi and in the sensory-motor area. We also observed that the increase in glutamate plus glutamine in the posterior cingulated correlates with depression, pain, and a questionnaire on the global impact of fibro-

myalgia. Glutamate is therefore an excitatory amino acid; and its increase indicates that the metabolic function of patients with fibromyalgia differs from controls, can cause permanent brain damage and may be a potentially pathological factor in FM. In fact, it has been recently described that these patients improve clinically by subjecting them to a diet low in glutamate. Basic research studies have shown that 30 min after the administration of glutamate, massive acute edema of the neuronal cell bodies and dendrites is seen under electron microscope. Consequently, water moves into the extracellular space resulting in astrocyte edema [43]. Relevant clinical manifestations are related to this edema [44]. To compensate for the increase in cellular osmolarity, myo-inositol and choline move into the extracellular space, reducing their concentration within the astrocyte. As a result, glutamate is an exciter amino acid, and its increase indicates that the metabolic function of patients with fibromyalgia differs from the controls; its elevation can cause permanent brain damage and can be a factor potentially pathological in fibromyalgia.

The posterior cingulated is an important component of the "brain's default circuit," an area of association between different brain regions that participates in the consciousness of pain. In patients with Fibromyalgia, functional connections with brain regions that process pain seem to be increased and this increase in connectivity is directly related to patients' spontaneous pain. Thus, an increase in excitatory neurotransmitters could lead to neuronal hyperexcitability. We cannot confirm, however, if the observed increase in glutamate is related to the increase in neuronal activity for the following reasons: first, spectral features may be contaminated since MRS measures the association of glutamate + glutamine; second, we do not know if the obtained measurement is from the metabolic or from the neurotransmitter source; third, the spectral signal is obtained from a heterogeneous area of the brain where gray and white matter are included and the measurement is an average of different cell types that include astrocytes, glia and neurons. There are two other recent studies that have shown an increase in glutamate in the amygdala and posterior insula in patients with fibromyalgia which place glutamate as a key neurotransmitter behind the molecular processes involved in fibromyalgia. This also opens the door for MRS studies in other functional chronic pain syndromes that may also be related to central factors.

Finally, these data also suggest that pharmacological interventions that act specifically on glutamatergic neurotransmission may be effective in these complex patients. Investigation of these questions is necessary.

Our study also confirms a hippocampal dysfunction with myo-inositol reduction that may be partially responsible for depressive symptoms. This deficit could be explained by reduced synthesis or, more interestingly, by an increase in the consumption of this component. Patients with clinical depression usually have decreased myo-inositol levels in their cerebrospinal fluid and preliminary studies with high-dose supplements of myo-inositol show promising results in people with disorders such as bulimia, panic, obsessive-compulsive disorder, agoraphobia, and bipolar depression. We speculate that myo-inositol, via its conversion to glucuronic acid, is consumed as a protective detoxifying reaction of the brain and can be associated with depression.

The role of the amygdala in the emotional response and the differences found with MRS in the hippocampus of patients with FM suggests the presence of some sort of abnormal emotional process that could partially explain anxiety and depression [45].

In a subsequent study, we performed spectroscopy in patients with fibromyalgia and with somatization disorder and we compared it with healthy controls. Patients were given questionnaires on pain catastrophizing, anxiety, depression, pain, quality of life, and cognitive impairment [46]. This study demonstrated a significant increase in the levels of Glx, a combined measure of glutamate (Glu) and glutamine (Gln), within the posterior cingulated cortex (PCC) of patients with fibromyalgia (FM) and, to a lesser extent in the patients with somatization disorder (STD) compared to controls. Levels of Glx correlated with pain catastrophizing suggesting that elevated levels of Glx in the posterior cingulated

cortex (PCC) are associated with increased pain catastrophizing. Another study from our group confirmed that there is an increase in glutamate/creatine and of glutamate + glutamine/creatine in the posterior cingulate of patients with fibromyalgia as well as in somatizers, migraine, and trigeminal neuralgia, indicating dysfunction of the posterior cingulated [47]. It also opens the door for Proton MRS (1H-MRS) and suggests that reducing glutamatergic activity through pharmacological treatment could improve the outcome of patients with FM and STD. Memantine acts as a neuroprotectant by decreasing glutamate excitotoxicity and is known to increase levels of brain-derived neurotrophic factor (BDNF), thus influencing synaptic plasticity in rats and reducing b-amyloid-induced apoptotic death and neuroinflammation in the hippocampus [48]. The NMDA receptor antagonist memantine has been used to treat Parkinson's disease, spasticity, convulsions, vascular dementia, and Alzheimer's disease and has an excellent clinical safety record spanning more than 20 years. Our group has also demonstrated memantine's good tolerance and usefulness in 63 patients with fibromyalgia [49].

In order to evaluate memantine's efficacy in modifying brain metabolites in patients with fibromyalgia, we conducted a randomized double-blind study with 25 patients who received 20 mg of memantine per day for 6 months. The secondary aim of the study was to assess the efficacy of memantine for the treatment of pain and other key FM symptoms such as cognitive dysfunction, depression, anxiety, and quality of life in a pre–post study [50]. We also expected that the differences in metabolite ratios would correlate with the degree of clinical improvement. Despite the limitation of a small sample size, it can be concluded from our study that memantine may induce some shorter recovery of neuronal function in the posterior cingulated cortex and in the posterior insula of patients with chronic FM. On the basis of our results, the posterior cingulated cortex and posterior insula should be included in the areas to be explored in FM patients. This is a positive trial in which memantine demonstrated remarkable spectroscopic effects, and we conclude that memantine increases brain metabolism in patients with FM. Given the significant correlation found in the right posterior insula between metabolite values and clinical scales, MRS may be useful to monitor progression and response to treatment in FM.

Conclusion

MR spectroscopy is an extremely successful metabolic imaging modality that continues to develop and aims for even greater achievements. There is no doubt that magnetic resonance spectroscopy has a bright future in both medical diagnosis and in the ongoing development of medicine. The use of nuclei other than hydrogen, such as sodium, fluorine, and phosphorus, will continue to be investigated and will probably be useful for medical diagnosis in the near future. In theory, a high magnetic field provides an increase in the signal-to-noise ratio of spectroscopy, leading to significant increase in the spectral resolution, which allows for a more extensive quantification of chemical substances. Improvement of MR antennas and decreased time of acquisition of the sequences will continue to improve the quality of the studies as well as the comfort of the patient.

The use of magnetic resonance spectroscopy indicates an advance in the research strategies for its application in psychopathology and offers a unique possibility to understand the pathophysiology of psychopathological disorders through a noninvasive study.

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Prescribed Psychotropic Drugs in the Elderly

46

Michel Bourin

Introduction

The definition of the concept of the elderly has evolved over the last two decades, since it is considered that it is no longer the age of retirement (65 years) but rather that of 75 years which applies as being on average the age when one perceives aging in most subjects. The most conventional biological parameters can be modified without pathology. Significant changes in the effect of certain medications may occur as age increases but in fact it is primarily polypathology that leads to medication and therefore to problems of drug interactions [1]. Conventionally, "elderly" has been defined as a chronological age of 65 years old or older, while those from 65 through 74 years old are referred to as "early elderly," and those over 75 years old as "late elderly" [2]. Geriatricians and gerontologists often use the following construct to subdivide the population: 60–74 years "young old," 75–85 years "old old," and 85 years "very old" or "oldest old."

The aim of this article is therefore to discuss the main problems encountered when prescribing psychotropic drugs to the elderly person and the pitfalls that can be avoided.

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Pharmacokinetic Changes

The age most often changes the pharmacokinetic parameters of psychotropic drugs, so it is necessary to think about it before prescribing.

Absorption

A few major changes in resorption are related to age [3], but sometimes the rate of resorption associated with changes in gastric emptying is often slower in elderly patients that remain supine for prolonged periods of time.

Distribution

The elderly person has a reduced lean body mass, the amount of body water is reduced in absolute value and in percentage, and thus a relative increase in percentage of the fat mass is observed [4]. There is also usually a decrease in serum albumin that fixes many drugs, particularly weak acids (e.g., aspirin and some anti-inflammatory drugs). There may be a concomitant increase in the serum orosomucoid alpha-glycoprotein that fixes many basic drugs. Thus, the ratio of the drug to the free drug can be appreciably modified and thereby artificially increase the effectiveness of the prescribed drugs.

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Metabolism

There is no clear evidence that drug metabolism itself (the biologically assisted chemical alteration of the administered parent molecule) is less efficient in healthy old age than at younger ages, whereas a decreased capacity may be associated with ill-health and frailty.

The ability of the liver to metabolize drugs does not appear to be significantly diminished with age for many of them. It would appear that the greatest changes occur in Phase I reactions, that is, those that are performed by the microsomal system that has a mixed oxidative function. However, elderly individuals do show a reduced enzyme induction capability and are less able to tolerate overdoses. There is much less change in the liver's ability to perform conjugation reactions (Phase II) [5]. Some of these changes may be caused by decreased hepatic blood flow [6], an important variable in the clearance of drugs that have a high coefficient of liver extraction.

Finally, diseases that affect hepatic function, for example, congestive heart failure, are more common in the elderly [7]. Congestive heart failure can dramatically alter the liver's ability to metabolize drugs and may also decrease hepatic blood flow.

Elimination

Since the kidney is the major organ of drug elimination, the decrease in the functional capacity of the kidney related to age compared to what it was before can be very important. There is indeed an age-related decline in creatinine clearance in about two-thirds of patients. It is important to note that this decline is not reflected by an equivalent increase in serum creatinine as creatinine production. It is also reduced due to decreased muscle mass related to age. The decrease in clearance is fairly repeatable, so direct measurements of creatinine clearance are only necessary if suspected renal involvement or a disorder of sodium hydroxide metabolism, for example, if severe dehydration is suspected. The practical result of this modification is a significant prolongation of the plasma half-life of many drugs and the possibility of accumulation up to toxic concentrations if the dosage is not reduced in quantity or frequency. Dosage recommendations for the elderly are often reduced due to decreased renal clearance.

If only the dosage of a drug for the young adult is known, a correction can be made using the Cockcroft-Gault formula [8], which is applicable to patients between the ages of 40 and 80:

$$\mathrm{CCr} = \left\{\left((140 - \mathrm{age}) \times \mathrm{weight}\right) / \left(72 \times \mathrm{SCr}\right)\right\} \times 0.85 \ \left(\text{if female}\right)$$

Abbreviations/Units

- *CCr (creatinine clearance) = mL/minute*
- *Age = years*
- *Weight = kg*
- *SCr (serum creatinine) = mg/dL*

Pharmacodynamic Changes

All areas of the brain do not age at the same speed: some are more affected by age. This results in behavioral or performance changes in certain cognitive or sensorimotor tasks. This atrophy causes a drop in attention and concentration. Cognitive abilities related to attention and concentration are also affected: working memory decreases, language becomes more difficult, alertness decreases, etc. The memory is more fragile, with however a strong enough variability according to the person: some have the memory of a young person, while other subjects have more marked deficits. Motor skills and sensory performance also tend to decrease gradually: food loses its taste, movements are slower and rarer, and hearing is worse, and so on. Other disorders, such as behavioral disturbances (apathy, social withdrawal, mild emotional dullness) usually occur later, when the frontal cortex begins to lose volume. This happens when the areas of the brain below the frontal cortex begin to atrophy.

The brain areas in charge of memory (the hippocampus, as well as the temporal cortex) are among the most fragile. This atrophy plays a role in the appearance of memory disorders, the most visible during aging. The fact is that aging-related memory loss is about recent memories, old memories being relatively preserved by normal aging. This is because atrophy affects the hippocampus more strongly, a brain area responsible for memorizing recent memories. The hippocampus usually loses much of its volume and atrophies during aging. It should be noted that the atrophy of the hippocampus is mild in normal aging, but becomes much more marked in Alzheimer's disease, hence the memory loss characteristic of this disease. It should be pointed out, however, that memory disorders are quite variable depending on the individual: some elderly people keep a young memory, while other people of the same age will have more or less marked deficits. We do not really know what the origin of such a disparity is.

The question is: What are the consequences of brain aging on the efficacy of psychotropic drugs prescribed? The conditions of cerebral aging are not very well known. It has long been believed that elderly patients were much more "sensitive" to the action of many drugs, implying a change in the pharmacodynamic interaction of drugs with their receptors. It is now known that many, possibly most, of these apparent changes are the consequence of a modified pharmacokinetics or a decrease in homeostatic responses. However, it seems that aging alters the brain dopaminergic system but also the serotonergic system and especially the 5-HT 1B receptors [9].

The interaction between the cholinergic and dopaminergic systems is amply documented [10]. These two systems interact in the striatum and nucleus accumbens. It has been shown that blockade of D1 receptors inhibits the release of acetylcholine while their stimulation increases cholinergic transmission [11]. On the other hand, the stimulation or blocking of the D2 receptors decreases the release of the acetylcholine.

Acetylcholine controls dopaminergic transmission via nicotinic receptors [12]. The interaction between nicotinic and dopaminergic systems is important for cognitive functions. The interaction between these two systems can contribute to improved learning. The mesolimbic dopaminergic pathway is controlled by the nicotinic system. Control of the dopaminergic system via nicotinic receptors, especially those composed of subunits α42β and β7, seems to play an important role in memory functions, addiction, and neuroprotection [13].

Although there is no evidence of a presynaptic location of nicotinic receptors on serotonergic endpoints [14], many studies have demonstrated interactions between these two systems, including stimulation of serotonin release following activation of nicotinic receptors [15]. However, the serotonergic neurons of the dorsal raphe nuclei express nicotinic receptors in their cell bodies. Moreover, the cholinergic neurons from the Broca band emit extensions toward the nucleus of the dorsal raphe. An immune cytochemical study [16] revealed the colocalization of the alpha 4 subunit and the 5-HT3 serotoninergic receptors in the rat striatum. There is a complex bidirectional interaction between the two systems. Indeed, when the serotonergic activity of a cerebral region increases, cholinergic activity decreases and, vice versa [17], 5-HT would have a double effect on nicotinic receptors. Indeed, at low concentrations, 5-HT inhibits the activation of nicotinic receptors [18]. The cholinergic and glutamatergic systems interact in the inter-peduncle nucleus and in the lateral piriform nucleus. The activation of presynaptic nicotinic receptors localized on the glutamatergic endpoints stimulates the excitatory transmission of the neurons of the supra-optical nucleus of the hypothalamus [19]. Acetylcholine modulates glutamatergic transmission at the synapses of habenula medial [20]. The glutamatergic terminal axons of human neocortex possess hetero receptors alpha 7 involved in the release of glutamate [21].

The interaction between these two systems is not only essential to memorization and learning processes, but also to neuronal plasticity. Modulation of glutamatergic transmission by the cholinergic system was demonstrated during postnatal development [22]. In addition, the

interaction between the two systems appears to play a role in neuroprotection [23]. It is suggested that 5-HT may be an endogenous allosteric modulator.

All these considerations allow us to understand that the cerebral aging of a system of neurotransmission such as the dopaminergic system can be more or less compensated by the activation of other types of receptors.

In the cardiovascular system, the increase in cardiac output due to mild or moderate exercise is fully ensured up to at least 75 years (in subjects without obvious cardiac involvement), but the increase is mainly due to an increase in systolic ejection in the elderly and not in tachycardia, as in the young adult. Mean arterial pressure increases with age (in most Western countries), but the incidence of symptomatic orthostatic hypotension also increases significantly, as it can be increased by psychotropic drugs that have antagonistic level of alpha adrenergic receptors such as tricyclic antidepressants or certain neuroleptics [24]. Similarly, the mean postprandial blood glucose (2 h after meals) increases by about 10 mg/l for each year of age above 50 years. Thermal regulation is also affected, and hypothermia is poorly tolerated in the elderly.

Hypnotics

Insomnia is the most common sleep disorder in the elderly. It is often comorbid and its management includes the treatment of a possible underlying pathology. Nondrug psychobehavioral therapies are preferred in the elderly, who are more likely to develop an undesirable effect of hypnotic sedative treatments [25]. Non-benzodiazepine drugs and melatonin are of interest for the short-term treatment of insomnia in the elderly. Behavioral disorders in REM sleep may be due to treatment with low dose clonazepam or melatonin. The treatment of symptomatic obstructive sleep apnea syndrome involves weight loss and positive pressure ventilation. In any case, sleep hygiene contributes to improving the sleep disorders of the elderly subject [26].

Benzodiazepines and analogues such as Z drugs have essentially a transient role in the treatment of sleep disorders. These substances all have similar effects by decreasing the delay in falling asleep, the number and duration of awakenings and increasing the duration of sleep, especially for those under 60 years of age. They differ mainly in their pharmacokinetics [27]. Benzodiazepines with a preferred intermediate duration of action in early awakening, for example, oxazepam or temazepam, may have a residual action that favors diurnal sedation and confusion. Benzodiazepines with a long half-life, the use of which has been suggested in the presence of anxiety, are of a delicate use in the elderly and should not be introduced without a specialized psychogeriatric opinion, given their risks and the very polymorphic nature of anxiety disorders in this population [28]. The plasma half-life of many benzodiazepines increases from 50% to 150% between the ages of 30 and 70 years. The majority of these changes occur for every decade ranging from 60 to 70 years [27].

The decrease in age-related renal function and the presence of hepatic impairment both contribute to decreased elimination of these products. In addition, there has been an increase in the volume of distribution for some of these drugs. Lorazepam and oxazepam may be less affected by these changes than most benzodiazepines. In addition to these pharmacokinetic factors, it is generally accepted that elderly subjects also have a more variable sensitivity to sedative-hypnotic drugs from a pharmacodynamic standpoint [29]. Among the toxic effects of these drugs, special attention should be paid to ataxia in order to prevent accidents [30].

There is no evidence of superior efficacy of Z-drugs (zopiclone, zolpidem, and zaleplon) compared to benzodiazepines or significant differences in the occurrence of side effects or residual effects.

Zolpidem is eliminated more slowly in women, and the FDA recommends reducing doses by half in women, 5 mg instead of 10 mg for immediate release and 6.25 mg for the delayed forms instead of 12.5 mg [31]. New data have shown that blood levels of zolpidem of 50 ng/ml

decrease the ability to drive enough to cause an accident while people feel awake. This threshold is still exceeded 8 h after taking zolpidem 10 mg in 15% of women and 3% of men, and 8 h after a dose of 12.5 mg delay in 33% of women and 25% of men [32]. Products with a short half-life that are used as sleep inducers, such as zolpidem, produce an acute peak of concentration that may trigger confusion, amnesia, falls, or breathing difficulties in the elderly patient [33].

Hypnotics have a less favorable risk/benefit ratio in the elderly than in adult patients. Adverse effects can disrupt the quality of life of the elderly and weaken their organization at home: drowsiness or daytime fatigue (4 x baseline risk), impaired concentration and memory (5 x), falls (2 x), traffic accidents (2 x), motor coordination disorders, nightmares, confusion, disorientation and aggression ("paradoxical effects"), decreased swallowing frequency. Frequent use of tranquilizers or hypnotics is a risk factor for sleep apnea [34]. Recall that any polypharmacy, more common in the elderly because of multiple disorders, is associated with an increased risk of adverse effects.

In addition, these people often have alterations in body composition, renal and hepatic function, which may result in prolonged half-life and accumulation of active substances.

Prolonged use of hypnotics is addictive. Tolerance (loss of efficacy) and, particularly, physical dependence (withdrawal symptoms at a standstill) develop rapidly with benzodiazepines, in a few weeks or even a few days already. It is difficult to identify characteristics of patients who could predict chronic use of sleeping pills following a first prescription [35]. However, it is thought that there are risk factors for the development of addiction: personality disorders, alcoholism, use of medications without medical supervision.

Sedating antidepressants such as trazodone do not have the treatment of insomnia in their official indications, but their use is conceivable when a sleep disorder is part of a depressive disorder. The available studies are not sufficient to assert a prolonged effect on sleep time and duration of sleep compared to placebo. The risk of anticholinergic adverse effects, residual sedation, or confusion also exists for these drugs, and its use should follow the same rules as for benzodiazepines [36].

H1 antihistamines such as diphenhydramine and doxylamine are over-the-counter medications used for sleep disorders. They have a relatively long duration of action (4–6 hours). They induce anticholinergic side effects, prolongation of the QT interval, rarely paradoxical excitations or convulsions. Although widely consumed by the elderly and considered less likely to be addictive, they are not recommended because of their anticholinergic effects and risk of accumulation, especially with doxylamine (half-life ~10 h), resulting in all the more risk of falling. Tolerance also develops and the effect diminishes with regular use [37].

Melatonin is a hormone produced by the pineal gland, whose secretion is increased as night approaches and contributes to the induction of physiological sleep. It has an indication in monotherapy for the treatment of primary insomnia in patients from 55 years. Duration of treatment is limited to 3 weeks and in exceptional cases up to 13 weeks after medical reassessment [38].

The benefit of melatonin on sleep has been studied in the elderly and the maintenance of sleep has been significantly improved with doses of 0.1–3 mg compared to placebo. There is little fear of addiction or withdrawal problems, but subjective efficacy is not as good as hypnosedatives and price is higher [39]. The effect of renal insufficiency, whatever its degree, on the pharmacokinetics of melatonin has not been studied. Caution should be exercised when administering melatonin to patients with renal insufficiency. No data are currently available on the use of melatonin in patients with hepatic impairment. Published data show that endogenous melatonin levels increase markedly during daytime hours due to reduced clearance in patients with hepatic impairment. Therefore, melatonin is not recommended for patients with hepatic impairment [25].

Herbal medicine. There are few rigorous evaluations of the effectiveness of plants with sedative properties (linden, verbena, lemon balm,

bitter orange, hawthorn, hops, and passionflower). Valerian is the most commonly used plant and some clinical evaluation data report moderate efficacy on sleep quality, though questioned in recent studies [40].

Anxiolytics

Depressive and anxious symptoms are often associated in the elderly. This comorbidity does not lead most often to a co-prescription of an antidepressant and an anxiolytic. The prescription of a benzodiazepine-type anxiolytic, in the medical approach of elderly depression, is not recommended.

First and foremost, the potential benefits are largely offset by many undesirable effects. The first of these is addiction, especially since very quickly effective anxiolytics provide false reassurance to the patient. The consumption of anxiolytics exposes older people to risks because of metabolic changes that promote the accumulation of active metabolites and thus increases the risk of overdose and adverse effects [41].

Among the benzodiazepines most commonly prescribed in anxiety are bromazepam, prazepam, lorazepam, clorazepate, and diazepam. Derived from benzodiazepines, etifoxine may be prescribed in the psychosomatic manifestations of anxiety [42], although its rare but serious adverse effects are its limitation. For benzodiazepines, these undesirable effects are essentially psychomotor, with the increased risk of falls and fractures. For all, there may be an accentuation of cognitive disorders [43].

In the elderly person, a molecule without a muscle relaxant action is preferred to reduce the risk of falling [44] and the doses will be reduced. In rarer cases, paradoxical reactions are observed. Finally, in case of combination with benzodiazepine, it is discussed less effective antidepressant action.

Moreover, antidepressants, in addition to their properties as antidepressants, have sedative or stimulating properties. They occur in the very first days of treatment and are of great interindividual variability.

The first-selected pharmacotherapy for people with most anxiety disorders is selective serotonin reuptake inhibitors or serotonin-norepinephrine reuptake inhibitors [45]. These drugs are more efficient in the elderly as anxiolytics than antidepressants [46]. Caution should be exercised for elderly patients with cognitive and/or malnourished disorders (serotonin syndrome within the first 8 days of treatment).

In cases of severe insomnia and/or anxiety, co-prescription of a benzodiazepine may be warranted. To avoid the risk of dependence, it is recommended to use the minimum effective dose and to stop treatment as soon as the anxiety and/or insomnia have improved because of the antidepressant (average of 15 days). The therapeutic approach of depression and anxiety of the elderly is not confined to a prescription drug (even double). Psychotherapeutic (such as supportive psychotherapy) behavioral, environmental, and/or educational approaches have been proven. These patients must also be able to benefit from these care techniques.

Another option is to use herbal therapy that has some controlled placebo studies showing an efficacy in patients presenting with an adjustment disorder with anxious mood [47].

Antidepressants

Depression is thought to be underdiagnosed and out-treated in elderly patients. The suicide rate in the population aged over 65 supports this view. Unfortunately, apathy, reduced affectivity, and social withdrawal due to major depression can be mistaken with senile dementia. Limited data suggest that elderly subjects are also sensitive to antidepressant medications (both imipraminic and non-specific MAO inhibitors), more than younger patients, but are more sensitive to their toxic effects. This factor – and also the decreased clearance of some of these drugs – underscores the importance of careful dosage adjustment and strict attention to the occurrence of toxic effects. If an imipraminic antidepressant is to be used, a medication with reduced antimuscarinic effects such as moclobemide (specific and reversible

MAO A inhibitor) or an SSRI should be selected, although evidence of efficacy of these drugs has not been formally proven in the treatment of mood disorders in the elderly [36]. The consequences of the heat wave of summer 2003 in France are certainly the fact of the heat itself. But also of the use of anticholinergic drugs that increase sedation and that cause a dryness of the mouth without increasing the sensation of thirst.

The appreciation of the thymic state of the very old person is difficult: What is the part of philosophical reflections related to the proximity of death? What is the impact of a depressive state on the elderly person who is losing their autonomy and who gradually becomes disabled? [48].

Faced with a very old patient, we wonder how was their personality, their way of being, and their relationship to the world throughout their life. These factors of appreciation are important and will intervene when the indication is given to the treatment. The succession of bereavements/losses in the third part of life can lead to sadness. It does not necessarily require treatment as long as the patient has autonomy and interest in the surrounding world.

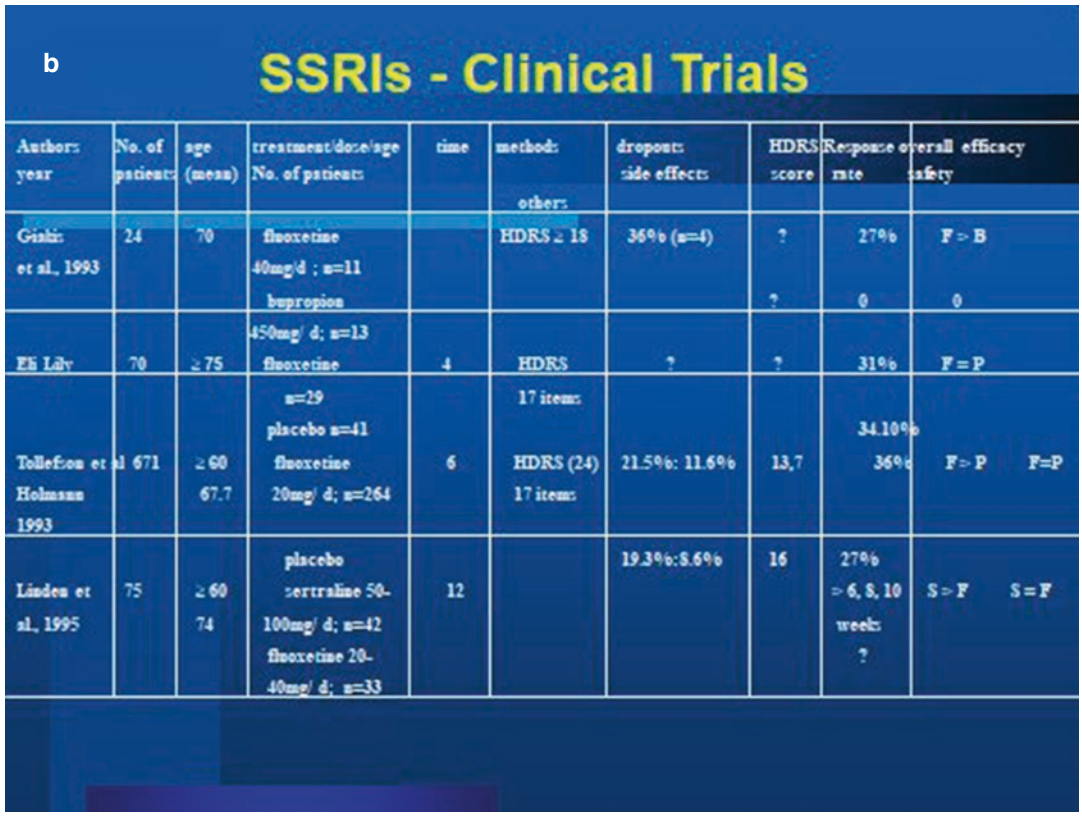
Meta-analyses also show that the effectiveness of antidepressants is questionable and depends on the initial intensity of depressive symptoms. These results indicate that antidepressants are to be reserved for severe depression [49, 50]. Currently, guidelines recommend, as a first intention, the use of selective serotonin reuptake inhibitors (SSRIs) in the depression of the elderly. The effective doses of the antidepressants in the elderly are similar to those published in the young subjects. In the elderly, it is better to start at a lower dose than usual. However, SSRIs have never been shown to be effective in elderly depression (Fig. 46.1a and b) whereas tricyclics are effective in this indication [51, 52]; an animal study corroborates these results [53]. It may be difficult to integrate the biographical data of the

a

SSRIs Clinical Trials

Authors year	No. of patients	age (mean)	treatment/dose/age No. of patients	time	methods	dropouts side effects others	HDRS score	Response rate	overall safety	efficacy
Altamura et al., 1989	28	>65 (68.5)	fluoxetine 20mg/d; n=13	5	HDRS 17 item	2	15	?	A≥F	F≥A
			amitriptyline		score +/- 26	4	10	?		
Geretsegger et al., 1994	106	61-68 (73.7)	fluoxetine 20-60mg/ d; n=52	6	HDRS (28) MADRS; CGI	13	23	16%	P≥F	P=F
Schöne et al.		74.3	paroxetine 20-				20	37%		
Feighner and Cohn, 1985	157	61-90 (<70)	fluoxetine: 20-80mg/ d; n=78	6	HDRS (25) CGI	47%	16	49%	F=D	F>D
			doxepin 50-250; n=79		HDRS (26)	61%	17	48%		
La Pin et al., 1992	40	60-80 71.8	fluoxetine 20mg/ d; n=20	6	HDRS (24)	5%	14	?	F=M	F≥M
			minaxerine 40mg/ d; n=20			20%	16	?		
Altamura	68	60-82	mooclobemide 400mg/ d		MADRS			-56%	M=F	M≥F
			fluoxetine 20mg/ d					-48%		

Fig. 46.1 (**a** and **b**) Results of the clinical trials of SSRIs in the elderly


b

SSRIs - Clinical Trials

Authors year	No. of patients	age (mean)	treatment/dose/age No. of patients	time	method: others	dropouts: side effects	HDRS score	Response rate	overall efficacy safety
Giuli: et al., 1993	24	70	fluoxetine 40mg/d ; n=11 bupropion		HDRS ≥ 18	36% (n=4)	? ?	27% 0	F > B 0
Eli Lilly	70	≥ 75	450mg/ d; n=13 fluoxetine	4	HDRS	?	?	31%	F = P
Tollefson et al Holmann 1993	671	≥ 60 67.7	n=29 placebo n=41 fluoxetine 20mg/ d; n=264	6	17 items: HDRS (24) 17 items:	21.5%: 11.6%	13,7	34.10% 36%	F > P F=P
Linden et al., 1995	75	≥ 60 74	placebo sertraline 50-100mg/ d; n=42 fluoxetine 20-40mg/ d; n=33	12		19.3%:8.6%	16	27% > 6, 8, 10 weeks ?	S > F S = F

Fig. 46.1 (continued)

patient, their usual psychological mode of functioning and the clinical signs of depression into the therapeutic decision. It may happen that the presence of a beginning cognitive disorder, sometimes confused with a depressive state, further complicates the prescriber's decisions. Therapeutic adherence, which is essential for the success of drug treatment, also depends closely on all these factors.

Although tricyclics have revolutionized the treatment of depression, their anticholinergic effects may exacerbate cognitive disorders and promote confusional states. This makes their use tricky in this population. If it is necessary to prescribe a pharmacological treatment to a very old patient for a depressive state, then preference is given to SSRIs, even if they are more anxiolytics than antidepressant in the elderly (Fig. 46.2).

In Practice: What Criteria for Choosing an Antidepressant? What Dosage?

The choice of SSRI will be based on the patient's comorbidities (electrolytes, renal and hepatic function, rhythm disorders, and cardiac ejection fraction) and his or her comedications. The introduction will always be in small doses and the increase in steps. The maintenance dose often corresponds to one-third of the usual doses recommended for young adults. The half-life of each antidepressant determines the optimal timing of its plasma concentration. This assay will be performed at the equilibrium pharmacokinetic accumulation of the drug, that is to say, in the elderly, at the time corresponding to at least five half-lives after the introduction or the last dose change. The expected clinical effect can only be assessed after a mini-

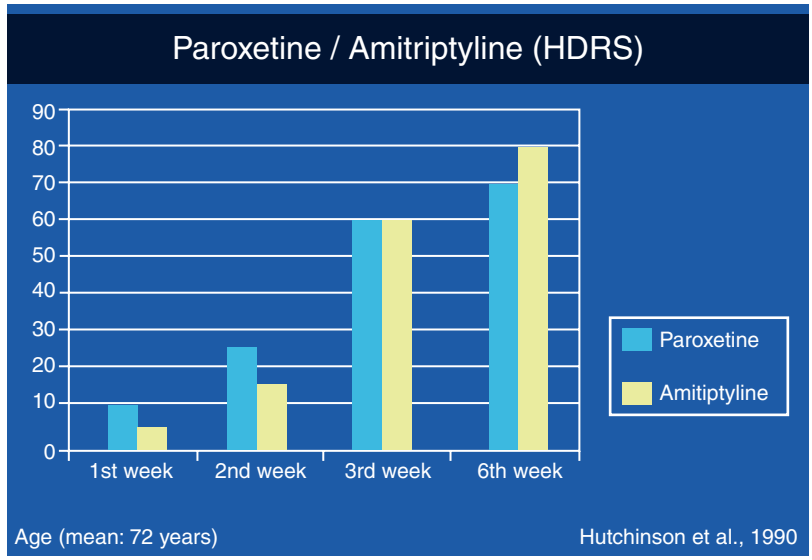


Fig. 46.2 Comparison of the efficacy of Paroxetine versus Amitrityline in the elderly (but the mean age is only 72)

mum of 4–6 weeks. If the evolution is not satisfactory, the control of the plasma concentrations of the antidepressant can make it possible to distinguish between a pharmacological resistance (satisfactory concentration), a lack of exposure (insufficient concentration), or, on the contrary, an ambiguous presentation of toxicity (excessive concentration) [54].

In Practice: Which SSRI for the Very Old Subject?

Citalopram and sertraline are among the SSRIs of choice for the elderly in view of their short half-life, and low potential for drug interactions. Paroxetine would be carefully used due to the more frequent occurrence of extrapyramidal syndrome, its greater weaning prevalence, potential for drug interactions (potent cytochrome P450 (CYP) 2D6 and moderate CYP1A2 inhibitor), and an often high plasma concentration in the very old person for whom dose adjustment is essential. Similarly, fluoxetine is not suitable for the treatment of the elderly because of its very long half-life (in the healthy volunteer, the half-life of fluoxetine is 4–6 days and that of the demethylated metabolite (norfluoxetine) is 4–16 days) as well as its inhibition of many isoenzymes [55].

Depending on the symptomatology of depression, the severity of anxiety disorders, sleep disorders, the importance of apathy, and the decline in vital energy, a choice can be made in favor of venlafaxine or mirtazapine [56]. The cardiac toxicity of venlafaxine requires clinical and electrocardiographic monitoring, as well as control of plasma levels and electrolytes. Reboxetine is of less common use because of its noradrenergic effect. The use of this molecule in the elderly patient is characterized by high plasma levels even in small doses [57].

In Practice: An Antidepressant Alone, Is it Enough?

In the elderly, it is particularly beneficial to associate with the drug treatment of depression complementary approaches: behavioral, changing places, discussion and reorganization of family relationships, psychotherapy, and integration into group activities (Fig. 46.3). Although resistant depressions are a common observation, they do not allow the combination of antidepressants as in adults, because of frequent polymorbidity and more frequent occurrence of a serotonin syndrome. The latter is difficult to diagnose because of its lack of specificity and the absence of paraclinical examinations. Knowledge of interactions allows us to act according to the precautionary principle. Apathy, a drop in vital energy, a feeling of uneasiness evoking the persistence of a depres-

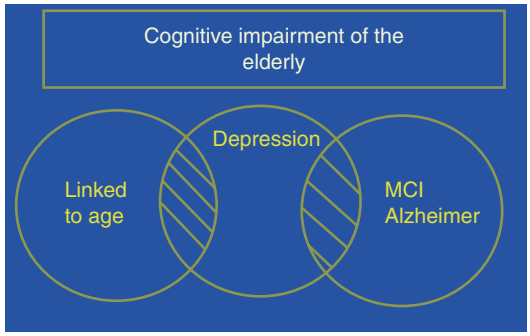


Fig. 46.3 Cognitive impairment in the elderly

sive state may be a reflection of high plasma levels [58]. These clinical signs may improve after adjusting the dose.

Mood Stabilizers

Lithium is often used in the treatment of mania with psychotic form or not in the elderly. Since it is eliminated by the kidneys, dosages must be adjusted. Concomitant use of thiazide diuretics decreases the clearance of lithium and should be accompanied by an additional dose reduction and more frequent measurement of lithium blood concentrations especially in the summer [59].

Tolerability of lithium is poor in advanced age: risk of overdose by decreased renal elimination, interaction with diuretics (increased monitoring, decreased dosage), and cardiovascular, thyroid, renal, cognitive effects could be expected [60]. Titration will be carried out at lower and more gradual doses, by controlling the lithiemia every 15 days until the effective dose is reached (acceptable level of 0.5–0.8 mmol/L) and then every month during 3 months, then every quarter. If lithium has long been prescribed as a mood stabilizer and remains effective and well tolerated, it will be continued by reducing doses, proscribing salidiuretics and carefully monitoring kidney (Cockcroft's formula) and thyroid functions.

In case of reduced tolerance, carbamazepine or valpromide will be substituted; divalproate sodium is rather intended for manic access. Carbamazepine is used at a low dose of 200–600 mg/day (tegretolemia 4–12 mg/L). Its hepatic metabolism, its strong protein binding, the risk of drug interactions, the negative cognitive impact as a sedative, the risk of falling (ataxia if overdose) hyponatremia, and elevation of transaminases limit its prescription in the elderly and require close monitoring: hepatic, renal, electrolyte [61]. Valpromide or sodium divalproate (250 mg/day to increase 250 mg every 3 days) also have very troublesome side effects in this context: confusion, fall, negative cognitive impact in the long run. Their strong protein binding involves reducing dosages in case of malnutrition (1 day out of two) [62].

Since it is eliminated by the kidneys, dosages must be adjusted. Concomitant use of lithium thiazide diuretics decreases and should be accompanied by an additional dose and more frequent measurement of lithium blood concentrations especially in the summer.

Two therapeutic approaches are therefore possible during manic access at present, the use of normothymics and/or anticonvulsants and the use of antipsychotics. Evaluation of the efficacy of chemotherapy during mania shows that the impact of these two classes may be somewhat different [63]. There are very few data regarding the use of lamotrigine in elderly patients [64].

Neuroleptics and Antipsychotics

Confusional states, whose somatic origin is still sought, may require the use of neuroleptics [65]. Among typical antipsychotics, haloperidol blocks dopamine D2 receptors and is devoid of antimuscarinic and antihistaminic activity. Used at the lowest possible dose, it has the advantage of having a short half-life, being low hypotensive, and not causing hyperglycemia. However, it has the disadvantage of inducing a prolongation of the QT interval, or even *torsades de pointe.* The ability to administer intravenously, intramuscularly, and subcutaneously is very useful in difficult situations with major behavioral disorders. Given the polypharmacy of the elderly, its inhibitory effect on CYP450 2D6 [66] must be taken into account. The occurrence of extrapyramidal syndromes and tardive dyskinesia in the short and long term, facilitated by underlying cerebral

lesions, should require us to administer at minimal doses, to limit and especially to circumscribe, over time, its use [67].

The therapeutic alternative of the last 20 years lies in the use of atypical neuroleptics, of which we must always choose those with the shortest half-life. Quetiapine, recommended for behavioral problems in patients with Parkinson's disease or Parkinson's syndrome, is not recommended in patients with hypotension and bradycardia [68]. The combination of quetiapine with SSRIs, anticholinesterase drugs, and of course beta-blockers promotes the occurrence of bradycardia. Quetiapine is a moderate inhibitor of CYP1A2, 2C9, 2C19, 2D6, and 3A4/5.5, which should be considered in patients receiving other drugs. The dosage of quetiapine in the elderly is usually between 12.5 and 50 mg/d. Olanzapine, with a longer half-life, has limited use because of the increased incidence of diabetes and glycemic dysregulation favoring diabetic decompensation in the elderly person. Its orodispersible form is very useful in patients whose compliance is bad at a given moment of their evolution. Extrapyramidal syndromes and tardive dyskinesia have been observed clinically after treatment of certain duration [69].

Risperidone has often been advocated for the treatment of behavioral disorders in patients with dementia [70]. Nevertheless, its use remains limited in old age because of the half-life of 34 h of its metabolite hydroxy-risperidone. In the elderly, risperidone should therefore be prescribed at doses less than or equal to 0.5 mg/day. Its inhibitory effect on CY2D6 should be taken into account in the therapeutic approach. Risperidone causes extrapyramidal syndromes that can in severe cases confine the patient to bed. This medication is also to be avoided in the decompensated diabetic. Risperidone and olanzapine have been associated with more frequent stroke [71, 72]. Excess mortality would have been noted in patients treated with dementia for olanzapine, risperidone, and quetiapine. Aripiprazole (doses of 2, 5, 10 mg/day) was compared against placebo in 487 patients. Aripiprazole 10 and 5 mg/day showed significant improvement compared to placebo at the NPI and BPRS scores. At 2 mg/day, it was not effective [73]. Haloperidol at low doses seems to remain the gold standard in the elderly.

In Practice: What Place to Reserve for Neuroleptics in the Elderly?

The use of neuroleptics in the very elderly is common but requires reflection, as these patients are extremely sensitive to side effects and develop tardive dyskinesia faster than the adult subject. Some neuroleptics have been associated with excess mortality. For these reasons, these drugs should never be used for anxiolytic purposes alone. On the other hand, it is not relevant to associate neuroleptics or antipsychotics with benzodiazepines [74].

Conclusion

The quality of life in the elderly can be greatly improved and life can be prolonged by the intelligent use of drugs. However, there are several practical barriers that the prescriber needs to know about treatment adherence.

Noncompliance may arise from negligence or confusion; clear and precise prescriptions are necessary, avoiding non-strictly necessary medicines that are often taken at the expense of those that are.

The reduction in dosage should not be systematic because the subject is old; in fact, often the ineffectiveness of the drugs in this segment of the population is related to the use of doses that are too low. It is therefore appropriate to increase the doses gradually to evaluate the tolerability, which remains the limit of use of many psychotropic drugs.

The main problem with the use of psychotropic drugs in the elderly, as well as other drugs, is that there are very few placebo-controlled studies in patients over 75 years old. For example, the paradox is that SSRIs are used in the depression of the elderly without satisfactory studies, whereas in the same population tricyclics have proved their effectiveness.

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