Melatonin, Neuroprotective Agents and Antidepressant Therapy

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Editors
Francisco López-Muñoz
Faculty of Health Sciences
Camilo José Cela University
Neuropsychopharmacology Unit
Hospital 12 de Octubre Research Institute (i+12)
Madrid
Spain

Cecilio Álamo
Department of Biomedical Sciences (Pharmacology Area)
Faculty of Medicine and Health Sciences
University of Alcalá
Madrid
Spain

Venkataramanujam Srinivasan
Sri Sathya Sai Medical Educational and Research Foundation
Coimbatore
India

Takahiro A. Kato
Department of Neuropsychiatry
Graduate School of Medical Sciences
Kyushu University
Fukuoka
Japan

Domenico de Berardis
NHS, Department of Mental Health
Psychiatric Service of Diagnosis and Treatment
Hospital "G. Mazzini"
Teramo
Italy

Department of Neurosciences and Imaging
University "G. D'Annunzio"
Chieti
Italy

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Preface

At some point the editors of this book made the decision to work together to consider the relationship between two very classic issues, such as the pineal gland and the old melancholy, today known as depression, with most modern topics as neuroprotection in mental illness. Each of these issues separately has generated numerous scientific and clinical studies that have justified a myriad of publications. However, our interest is focused on the possible interrelationship between these topics.

Conventionally, in the research field of depression, a focus on monoamine neurotransmitters, especially serotonin and noradrenaline, has historically underpinned to understand the pathophysiology of depression and its therapeutic mechanisms. This book is a composition of cutting edge research on brain science and clinical depression with a strong focus on melatonin. In the first half of the text, we cover topics from the discovery of melatonin to its physiology, pharmacology, and expanding pathophysiology. The latest findings regarding drug discoveries that have targeted melatonin are also included. In the second half of this book moving beyond melatonin, we have included writing on the most up-to-date research topics on depression along with discussions on pathophysiology and treatment.

A complex illness, depression requires deeper understanding from a variety of perspectives. Understanding depression necessitates understanding not only of monoamine neurotransmitters, but also of neural systems with glial networks, genetic features including methylation, and other molecules focusing on neurotrophic factors, inflammatory cytokines, and free radicals. These various elements are correlated in a complex manner, creating various conditions of depression, and this is where melatonin is a crucial factor. When it comes to treatment, understanding of these various aspects is absolutely essential.

The pineal gland, the "enigmatic organ," as it was called by Van Gehuchten, is an organ that has been studied thoroughly from a historical perspective. The first chapter of the book refers to his scientific and philosophical evolution throughout human history. Since Classical Antiquity, numerous authors have linked the origin of some mental disorders to physical and functional changes in the pineal gland because of its attributed role in humans as the connection between the material and the spiritual world. Descartes proposed that it was the seat of the human soul and controlled communications between the physical body and its surroundings, including emotions. But the link between the pineal gland and psychiatric disorders was definitively

highlighted in the twentieth century. The use of glandular extracts in patients with mental deficiency, and finally the discovery of melatonin in 1958, reawakened interest in the relationship between the pineal gland and mental disorders, fundamentally the affective and sleep disorders. In the past 25 years, scientific production, both from a basic perspective as clinic, in relation to the pineal gland and melatonin has had an exponential development, as reflected in the initial chapters of this book.

Considering the important prevalence of primary insomnia in the general population, neuroimaging studies remain relatively few. In addition, little is known about the relation between pineal volume and insomnia. In another chapter of this book, the contribution of functional and structural neuroimaging to our current understanding of primary insomnia is examined.

An updated description of the basic and clinical aspects of melatonin is given in several chapters, which also discuss the interesting new clinical perspective for melatonin. One of the key contents of this book is the role played by melatonin as a neuroprotective agent, which is treated by different working groups in four chapters. Thus, the involvement of melatonin and its receptors in neuroprotection and the current status of melatonin in neurodegenerative processes are reviewed in detail. In addition, the concomitant use of melatonin with other pharmacological agents in the treatment of neurodegenerative diseases is described in detail. Furthermore, we explore the mechanism of melatonin that is associated with the role of stress as a key factor to precipitate depression and as a factor altering neurogenesis, supporting the notion that melatonin possesses anti-stress and neurogenic actions. On the other hand, melatonin shows a pleiotropic character and it is a versatile and ubiquitous antioxidant molecule with low toxicity and high efficacy in reducing oxidative damage. Furthermore, melatonin has anti-inflammatory effects by regulation of multiple cellular pathways and properties to prevent excitotoxicity, among others. In this book, melatonin's beneficial effects in hepatic injury and its marked potential for improving human health against the most widely used chemical weapons are reviewed.

The relationship between melatonin, sleep and circadian rhythms is highly known. Alterations in cascade of these parameters can lead to an alteration of mood associated with the emergence of depressive disorders. In fact, a growing literature suggests that the melatonergic system may be involved in the pathophysiology of mood and some core symptoms of depression show disturbance of the circadian rhythm in their clinical expression. In addition, alterations have been described in the circadian rhythms of several biological markers in depressed patients. These aspects are developed in different chapters of the book, highlighting that melatonin through its receptor can modulate the survival of newborn neurons in the adult hippocampus, making it the first known exogenously applicable substance with such specificity.

Fibromyalgia syndrome is a complex chronic condition with an unknown etiology and pathophysiology, causing widespread pain and a variety of other symptoms. However, abnormality in circadian rhythm of hormonal profiles and cytokines has been observed in this disorder. Several studies have reported a common comorbidity between depression and fibromyalgia syndrome. Thus, study of the role of melatonin in fibromyalgia seems justified.

Taking into account the possible pharmacological role of melatonin in different processes, various mechanisms that improve its clinical applicability have been studied. In this book some authors discuss new galenic formulations of melatonin, the possibility of using agents that act directly on melatonergic receptors, as ramelteon, and the antidepressant role of the agomelatine. This agent behaves both as a potent agonist at melatonin MT_1 and MT_2 receptors and as a neutral antagonist at 5-HT_{2C} receptors. Accumulating evidence in a broad range of experimental procedures supports the notion that the psychotropic effects of agomelatine are due to the synergy between its melatonergic and 5-hydroxytryptaminergic effects.

Another important group of chapters of this book concerns the study of some lesser known characteristics of mood disorders. The contribution made by leading experts in each of the items collected in the different chapters is remarkable. In this sense, interesting aspects are analyzed as neuropsychological models of depression, the chronobiology, sleep abnormalities, hormonal dysfunction and affective temperaments of mood disorders, or bipolar disorders and biological rhythm, circadian clock gene dysfunction, and neurocognitive deficit.

Finally, a large section related to drugs for the treatment of depression—antidepressants—is discussed. This section covers from the history of antidepressants to the future therapeutic targets for the treatment of depression, as vasopressin, opioids, brain-derived neurotrophic factors, glutamatergic mechanisms, and corticotropin-releasing factor antagonists as antidepressants. We also collect particular aspects of the use of antidepressants, for example, the combination strategies in patients with treatment-resistant depression, or the use of antidepressants in elderly or in patients with chronic pain and the use of these agents and suicide risk. In addition, there is a pair of chapters related to the pharmacogenomics of antidepressant drugs.

Melatonin is an important mediator of a wide range of physiologic functions, and alterations in the melatonergic systems may be implicated in the etiopathogeny of some diseases. Several investigations have been conducted on melatonin in different psychiatric/neurologic diseases, and these preliminary positive findings with respect to disease pathology and treatment mark only the beginning of a fruitful new era in psychopharmacology.

The etiologic heterogeneity of depressive disorders does not allow to conclude on a general relationship between melatonin deficiency and depression. Nevertheless, this connection may exist in some subforms, and melatonin receptors can be a target for antidepressants. Although available drugs are effective, they also have substantial limitations. Recent advances in our understanding of the fundamental links between chronobiology and major mood disorders, as well as the development of new drugs that target the circadian system, have led to a renewed focus on this area. This book emphasizes these aspects and aims to provide a new grain of sand in the broad field of depression and its treatment.

For this purpose, we were invited to participate in the preparation of this book to an international roster of prestigious authors, who are experts in these topics. The positive response received pleasantly exceeded our expectations. This book should be characterized as a work of an integrative nature where

the context is as important as the text itself and where it is possible to contrast different visions and approaches to this field of study. This would have been impossible without an international and multidisciplinary conception of this project, in which 145 authors from 25 countries have collaborated on the preparation of the 51 chapters of this book. We thank all authors for their efforts, which have served, in a decisive manner, to make this project a reality, as well as the personnel in Springer for the interest shown in this work.

This book is dedicated to the memory of Professor Venkataramanujam Srinivasan, MSc, PhD, MAMS, whose untimely death has saddened his colleagues and the scientific community. We express our condolences to the late Professor Srinivasan who envisioned this book. Professor Srinivasan was an eminent neuroscientist, psychopharmacologist, and professor of physiology at multiple prestigious academic institutions. He founded and chaired the Sri Sathya Sai Medical Education and Research Foundation, where he donated his time and effort in the pursuit of knowledge. He devoted his life researching the physiology of sleep, chronobiology, psychoimmunology, and endocrinology. He was an internationally recognized teacher of physiology and biological psychiatry, a noted investigator of melatonin's physiological function, and a leader in the development of melatonin drugs for the treatment of medical conditions. A prolific writer, he published a plethora of his research findings in highly respected journals and books. Professor Srinivasan provided guidance, patience, and stimulus in the completion of this, his final work, allowing an encyclopedic review of melatonin's therapeutic uses and neuroprotective qualities. This book follows his previous important review of the use of melatonin and its congeners in clinical practice. Professor Srinivasan's knowledge and contributions to medical science will be greatly missed by his colleagues and medical scientists all over the world. Based on the intention of Professor Srinivasan, in addition to us four editors, and with the cooperation of the late professor's daughter Dr. Veda Padmapriya Selvakumar and family, it has finally reached completion. To all those involved, we would like to express our gratitude.

By reading this book, it is possible to grasp not only the research related to melatonin but also an overall picture of the latest research into depression. This work makes an important contribution to the development of future research of depression. We have worked with intensity on the development of "Melatonin, Neuroprotective Agents, and Antidepressant Therapy." After months of hard editorial work, this book sees the light and reaches the hands of the reader. It is hoped that this work will help to better understand the intricate mechanisms of depression and its therapeutic approach. If it benefits only one of the patients treated by the reader, we would be fully satisfied.

Madrid, Spain — Francisco López-Muñoz
Chieti, Italy — Domenico de Berardis
Madrid, Spain — Cecilio Álamo
Fukuoka, Japan — Takahiro A. Kato
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Contents

Contributors

Sarfraz Ahmad Aimcan Pharma Research and Technologies Inc., Guelph, ON, Canada

Cecilio Álamo Department of Biomedical Sciences (Pharmacology Area), Faculty of Medicine and Health Sciences, University of Alcalá, Madrid, Spain

Gamze Altun Department of Histology and Embryology, Medical School, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

Berrin Zuhal Altunkaynak Department of Histology and Embryology, Medical School, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

Mario Amore Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), Section of Psychiatry, University of Genova, Genova, Italy

Vanessa Areco "Dr. Cañas" Laboratory, Biochemistry and Molecular Biology, Faculty of Medical Sciences, Universidad Nacional de Córdoba, INICSA (CONICET-UNC), Córdoba, Argentina

Cynthia L. Arfken Department of Psychiatry and Behavioral Neurosciences, Wayne State University, Detroit, MI, USA

Nazem Atassi Neurological Clinical Research Institute, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Pınar Atukeren Department of Medical Biochemistry, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

Sofia Avissar Department of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, Ben Gurion University of the Negev, Beersheba, Israel

Livia Avvisati Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Naples, Italy

Dominika Berent Department of Psychiatry, Medical University of Warsaw, Warszawa, Poland

James D. Berry Neurological Clinical Research Institute, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Parichart Boontem Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University, Salaya, Thailand

Nash N. Boutrous Department of Psychiatry, University of Missouri – Kansas City, Kansas City, MO, USA

Amnon Brzezinski Department of Obstetrics and Gynecology, Hadassah Medical Center, The Hebrew University, Jerusalem, Israel

J.M. Bumb Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Ramón Cacabelos Chair of Genomic Medicine, Camilo José Cela University, Madrid, Spain

EuroEspes Biomedical Research Center, Institute of Medical Science and Genomic Medicine, Bergondo, Corunna, Spain

Caroline Caddy Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK

Heather K. Caldwell Laboratory of Neuroendocrinology and Behavior, Department of Biological Sciences, Kent State University, Kent, OH, USA

Agata Carpentieri "Dr. Cañas" Laboratory, Biochemistry and Molecular Biology, Faculty of Medical Sciences, Universidad Nacional de Córdoba, INICSA (CONICET-UNC), Córdoba, Argentina

Biological Chemistry, Faculty of Dentistry, Universidad Nacional de Córdoba, Córdoba, Argentina

Valentina Casetti Crisis, Inpatient, and Rehabilitation Services, Oxleas NHS Foundation Trust, London, UK

Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College, London, UK

Zhuoyou Chen Department of Neurology, Changzhou Second People's Hospital, Changzhou, Jiangsu Province, China

Paul Chu-Sin-Chung Centre National de la Recherche Scientifique (CNRS)-UPR 3212, Institute of Cellular and Integrative Neurosciences, Strasbourg, France

Merit E. Cudkowicz Neurological Clinical Research Institute, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Salvatore Cuzzocrea Department of Biological and Environmental Sciences, The University of Messina, Messina, Italy

Emmanuel Darcq Douglas Mental Health University Institute, McGill University, Verdun, QC, Canada

Débora d' Ávila Reis Department of Morphology, Cell Biology Institute, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Gabriela Díaz de Barboza "Dr. Cañas" Laboratory, Biochemistry and Molecular Biology, Faculty of Medical Sciences, Universidad Nacional de Córdoba, INICSA (CONICET-UNC), Córdoba, Argentina

Marina de Barros Pinheiro Arthritis and Musculoskeletal Research Group - AMRG, The University of Sydney, Sydney, NSW, Australia

Andrea de Bartolomeis Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Naples, Italy

Domenico de Berardis NHS, Department of Mental Health, Psychiatric Service of Diagnosis and Treatment, Hospital "G. Mazzini", Teramo, Italy

Department of Neurosciences and Imaging and Clinical Sciences, University "G. D'Annunzio", Chieti, Italy

Omur Gulsum Deniz Department of Histology and Embryology, Medical School, Ondokuz Mayıs University, Samsun, Turkey

Massimo di Giannantonio Department of Neuroscience, Imaging and Clinical Science, Chair of Psychiatry, University "G. D'Annunzio", Chieti, Italy

Dimos Dimelis Department of Psychiatry, 424 Military Hospital, Thessaloniki, Greece

Marco di Nicola Institute of Psychiatry and Psychology, Catholic University of Sacred Heart, Rome, Italy

Peter Dome Department of Psychiatry and Psychotherapy, Semmelweis University, Budapest, Hungary

Laboratory of Suicide Research and Prevention, National Institute for Psychiatry and Addictology, Budapest, Hungary

Alan B. Douglass Royal Ottawa Mental Health Center, University of Ottawa Institute of Mental Health Research, Ottawa, ON, Canada

Manuel Durán Acute Inpatients Unit, Dr. R. Lafora Psychiatric Hospital, Madrid, Spain

Kasima Ekthuwapranee Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University, Salaya, Thailand

Agata Faron-Górecka Department of Pharmacology, Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland

Valeria Feinshtein Department of Clinical Biochemistry and Pharmacology, Ben Gurion University of the Negev, Beersheba, Israel

Alberto Fernández Department of Psychiatry, Faculty of Medicine, Complutense University, Madrid, Spain

Michele Fornaro Polyedra Clinical Group, Teramo, Italy

Department of Education Science, University of Catania, Catania, Italy

Judith Fosbraey Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK

Anastasios Fotinos Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Konstantinos N. Fountoulakis Division of Neurosciences, 3rd Department of Psychiatry, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

Doron Fux Department of Clinical Biochemistry and Pharmacology, Ben Gurion University of the Negev, Beersheba, Israel

Pilar García-García Department of Biomedical Sciences (Pharmacology Area), Faculty of Medicine and Health Sciences, University of Alcalá, Madrid, Spain

Valentina Gilardi Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Naples, Italy

Moran Golan Department of Clinical Biochemistry and Pharmacology, Ben Gurion University of the Negev, Beersheba, Israel

Xenia Gonda Department of Psychiatry and Psychotherapy, Semmelweis University, Budapest, Hungary

Laboratory of Suicide Research and Prevention, National Institute for Psychiatry and Addictology, Budapest, Hungary

Robert Gonzalez Department of Psychiatry, Texas Tech University Health Sciences Center El Paso, El Paso, TX, USA

Piyarat Govitrapong Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University, Salaya, Thailand

Center for Neuroscience and Department of Pharmacology, Faculty of Science, Mahidol University, Salaya, Thailand

Yingjun Guan Department of Histology and Embryology, Weifang Medical University, Weifang, Shandong, China

Claudio Guarneri Department of Clinical Experimental Medicine, Section of Dermatology, The University of Messina C/O A.O.U. Policlinico "G. Martino", Messina, Italy

José Antonio Guerra Department of Pharmacology, Pharmacy Faculty, Complutense University, Madrid, Spain

Solange Guizzardi "Dr. Cañas" Laboratory, Biochemistry and Molecular Biology, Faculty of Medical Sciences, Universidad Nacional de Córdoba, INICSA (CONICET-UNC), Córdoba, Argentina

Kenji Hashimoto Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba, Japan

Hideki Horikawa Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Felice Iasevoli Laboratory of Molecular Psychiatry and Psychopharmacotherapeutics, Section of Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Naples, Italy

Polyedra Clinical Group, Teramo, Italy

Sâmia R.L. Joca Department of Physics and Chemistry, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, São Paulo, Brazil

Rosa Jurado Department of Basic Psychology II, Faculty of Psychology, Complutense University, Madrid, Spain

Faculty of Health Sciences, Psychology Department, Camilo Jose Cela University, Madrid, Spain

Shigenobu Kanba Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Arife Ahsen Kaplan Department of Histology and Embryology, Medical School, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

Suleyman Kaplan Department of Histology and Embryology, Medical School, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

Takahiro A. Kato Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Brain Research Unit, Innovation Center for Medical Redox Navigation, Kyushu University, Fukuoka, Japan

Sefa Ersan Kaya Department of Histology and Embryology, Medical School, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

Brigitte L. Kieffer Douglas Mental Health University Institute, McGill University, Verdun, QC, Canada

Eiji Kirino Department of Psychiatry, Juntendo University Shizuoka Hospital, Shizuoka, Japan

Department of Psychiatry, Juntendo University School of Medicine, Tokyo, Japan

Siyona Kolatkar Department of Clinical Biochemistry and Pharmacology, Ben Gurion University of the Negev, Beersheba, Israel

Gianmarco Latte Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Naples, Italy

Yu-Li Liu Center for Neuropsychiatric Research, National Health Research Institutes, Zhunan, Miaoli County, Taiwan

Department of Pharmacology, China Medical University, Taichung, Taiwan

Francisco López-Muñoz Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal

Pierre-Eric Lutz McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Verdun, QC, Canada

Lilly L.J. Mao Aimcan Pharma Research and Technologies Inc., Guelph, ON, Canada

José Luis Marco-Contelles Medicinal Chemistry Laboratory, Institute of General Organic Chemistry (CSIC), Madrid, Spain

Fernando Marín Department of Cellular Biology, Faculty of Medicine, Complutense University, Madrid, Spain

Giovanni Martinotti Department of Neuroscience, Imaging and Clinical Science, Psychiatry, University "G. D'Annunzio", Chieti, Italy

Monica Mazza Polyedra Clinical Group, Teramo, Italy

Department of Health Science, University of L'Aquila, L'Aquila, Italy

Louise Mercer Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, The Institute of Psychiatry, King's College London, London, UK

Yoshito Mizoguchi Department of Psychiatry, Faculty of Medicine, Saga University, Saga, Japan

Juan D. Molina Acute Inpatients Unit, Dr. R. Lafora Psychiatric Hospital, Madrid, Spain

Faculty of Health Sciences, Camilo José Cela University, Madrid, Spain

Akira Monji Department of Psychiatry, Faculty of Medicine, Saga University, Saga, Japan

Julio Cesar Morales-Medina Research Center for Animal Reproduction, CINVESTAV-Universidad Autónoma, Tlaxcala, México

Stephan Moratti Department of Basic Psychology I, Faculty of Psychology, Complutense University, Madrid, Spain

Ingo S. Nölte Department of Neuroradiology, University Hospital Mannheim, Mannheim, Germany

Nootchanart Ruksee Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University, Salaya, Thailand

National Institute for Child and Family Development, Mahidol University, Salaya, Thailand

Trevor R. Norman Department of Psychiatry, University of Melbourne, Austin Hospital, Heidelberg, VIC, Australia

Masahiro Ohgidani Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Laura Orsolini Academic Department of Experimental and Clinical Medicine, United Hospitals, Polytechnic University of Marche, Ancona, Italy

Villa S. Giuseppe Hospital, Hermanas Hospitalarias, Ancona, Italy

Polyedra Clinical Group, Teramo, Italy

School of Life and Medical Sciences, University of Hertfordshire, Hatfield, Herts, UK

Mehmet Emin Onger Department of Histology and Embryology, Medical School, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

Zahiruddin Othman Department of Psychiatry, School of Medical Sciences Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

Timo Partonen Department of Health, National Institute for Health and Welfare, Helsinki, Finland

Andrei Pereira Pernambuco Cell Biology Institute, Department of Morphology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Department of Health Sciences, University Centre of Formiga (UNIFOR-MG), Centre for Research and Extension (CEPEP), Formiga, MG, Brazil

Department of Health Sciences, University of Itaúna, Itaúna, MG, Brazil

Giampaolo Perna Department of Clinical Neurosciences, Hermanas Hospitalarias, FoRiPsi, Villa San Benedetto Menni, Como, Italy

Department of Psychiatry and Neuropsychology, University of Maastricht, Maastricht, The Netherlands

Department of Psychiatry and Behavioral Sciences, Leonard Miller School of Medicine, University of Miami, Coral Gables, FL, USA

Monica Piersanti NHS, Department of Mental Health, Psychiatric Service of Diagnosis and Treatment, Hospital "G. Mazzini", ASL 4, Teramo, Italy

René Pita Chemical Defence Department, CBRN Defence School, Madrid, Spain

Giovanni Polimeni Sicilian Regional Centre of Pharmacovigilance, C/O Unit of Pharmacology, A.O.U. Policlinico "G. Martino", Torre Biologica, Messina, Italy

Francisco J. Povedano Faculty of Health Sciences, Camilo José Cela University, Madrid, Spain

Emiliano Prinzivalli Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Naples, Italy

Gerardo Bernabé Ramírez-Rodríguez Laboratory of Neurogenesis, Division of Clinical Investigations, National Institute of Psychiatry "Ramón de la Fuente Muñiz", México, DF, México

Eva Ramos Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain

Zoltan Rihmer Department of Psychiatry and Psychotherapy, Semmelweis University, Budapest, Hungary

Laboratory of Suicide Research and Prevention, National Institute for Psychiatry and Addictology, Budapest, Hungary

María Angélica Rivoira "Dr. Cañas" Laboratory, Biochemistry and Molecular Biology, Faculty of Medical Sciences, Universidad Nacional de Córdoba, INICSA (CONICET-UNC), Córdoba, Argentina

Timothy Roerhrs Henry Ford Sleep Disorders and Research Center, Henry Ford Health System, Department of Psychiatry and Behavioral Neurosciences, Wayne State University, Detroit, MI, USA

Alejandro Romero Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain

Arann R. Rowe Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, The Institute of Psychiatry, King's College London, London, UK

Gabriel Rubio Psychiatry Service, 12 de Octubre University Hospital, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Department of Psychiatry, Faculty of Medicine, Complutense University, Madrid, Spain

Gabriel Schreiber Division of Psychiatry, Ben Gurion University of the Negev and Barzilai Medical Center, Beersheba, Israel

Mamuel Sebastián-Aldeanueva Primary Care Administration of Soria, Soria, Spain

Gianluca Serafini Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), Section of Psychiatry, University of Genova, Genova, Italy

Kyra-Verena Sendt Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK

Sukhwinder S. Shergill The National Psychosis Unit, South London and Maudsley NHS Foundation Trust, London, UK

Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, The Institute of Psychiatry, King's College London, London, UK

Winston W. Shen Department of Psychiatry, Wan Fang Medical Center, Taipei Medical University, Taipei, Taiwan

Department of Psychiatry, College of Medicine, Taipei, Taiwan

Nicola Smallcombe Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK

Paola Solano Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), Section of Psychiatry, University of Genova, Genova, Italy

Venkataramanujam Srinivasan Sri Sathya Sai Medical Educational and Research Foundation, International Medical Sciences Research Study Center "Prasanthi Nilayam", Kovai Coimbatore, Tamil Nadu, India

Kinga Szafran-Pilch Department of Pharmacology, Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland

Murat Terzi Department of Neurology, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

Farah Tiwana Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK

Francisco Toledo-Romero Psychiatry Service, "Virgen de la Arrixaca" University Hospital, Murcia, Spain

Nori Tolosa de Talamoni "Dr. Cañas" Laboratory, Biochemistry and Molecular Biology, Faculty of Medical Sciences, Universidad Nacional de Córdoba, INICSA (CONICET-UNC), Córdoba, Argentina

Carmine Tomasetti Polyedra Clinical Group, Teramo, Italy

Laboratory of Molecular Psychiatry and Psychopharmacotherapeutics, Section of Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Naples, Italy

Clara Torrellas EuroEspes Biomedical Research Center, Institute of Medical Science and Genomic Medicine, Bergondo, Corunna, Spain

Derek K. Tracy Crisis, Inpatient, and Rehabilitation Services, Oxleas NHS Foundation Trust, London, UK

Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, The Institute of Psychiatry, King's College London, London, UK

Aysın Pınar Turkmen Department of Histology and Embryology, Medical School, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

Hafize Uzun Department of Medical Biochemistry, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

Alessandro Valchera Polyedra Clinical Group, Teramo, Italy

Villa S. Giuseppe Hospital, Hermanas Hospitalarias, Ancona, Italy

Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Naples, Italy

Glenn R. Valdez Department of Psychology, Grand Valley State University, Allendale, MI, USA

Gustavo H. Vazquez International Consortium for Bipolar and Psychotic Disorder Research, McLean Hospital, Belmont, MA, USA

Department of Neuroscience, Palermo University, Buenos Aires, Argentina

Xin Wang Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Marcus Ward Royal Ottawa Mental Health Center, University of Ottawa Institute of Mental Health Research, Ottawa, ON, Canada

Gregers Wegener Translational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Centre for Pharmaceutical Excellence, School of Pharmacy, North West University, Mahikeng, South Africa

Shannah K. Witchey Laboratory of Neuroendocrinology and Behavior, Department of Biological Sciences, Kent State University, Kent, OH, USA

Ana Isabel Wu-Chou Department of Psychiatry, Wan Fang Medical Center, Taipei Medical University, Taipei, Taiwan

Ying Xu Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, State University of New York, Buffalo, NY, USA

Mohd Jamil Yaacob Department of Psychiatry, Faculty of Medicine, Asian Institute of Medicine, Science and Technology, Bedong, Kedah Darul Aman, Malaysia

Xifei Yang Toxicology Laboratory, Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong Province, China

Murat Yuce Department of Histology and Embryology, Medical School, Ondokuz Mayıs University, Samsun, Turkey

Kıymet Kubra Yurt Department of Histology and Embryology, Medical School, Ondokuz Mayıs University, Samsun, Turkey

Rahimah Zakaria Department of Physiology, School of Medical Sciences, University Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

Han-Ting Zhang Department of Behavioral Medicine and Psychiatry, and Department of Physiology and Pharmacology, West Virginia University Health Sciences Center, Morgantown, WV, USA

Edward W. Zhou Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Yongjin Zhu Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

History of Pineal Gland as Neuroendocrine Organ and the Discovery of Melatonin

1

Francisco López-Muñoz, Fernando Marín, and Cecilio Álamo

1.1 Introduction

The pineal gland is one of the anatomic organs that have generated most controversy and speculation throughout history. Its anatomical localization in the crossroads of the central nervous system (CNS) and its uneven nature in an environment of double structures together with its morphological appearance have attracted the attention of numerous scientists. Thorough and complex physiological theories have been proposed connecting this structure with the human body functionality, including philosophical postulates that relate to its spiritually. In fact, this cephalic gland, also known as epiphysis or "superior excrescence" in order to distinguish it from the hypophysis or "inferior excrescence," has gone through historic periods of absolute oblivion, deemed as a mere rudimentary vestige, but also through splendorous periods in which it even came to be considered the anatomic jail of the human soul.

In any case, until the middle of the twentieth century, the pineal gland was regarded as an "enigmatic organ," as Arthur van Gehuchten (1861–1914) rightly said [1]. This "enigmatic organ" was historically ascribed important responsibilities, as that of being the link between the body and the spirit in the human body. As a matter of fact, this function had already been considered in the Hindu philosophy and its Vedic literature from ancient times. One of the most popular legends in this culture tells how Parvati, God Shiva's wife, covered his eyes, submerging the world in the darkness. Fortunately, a third eye appeared on his forehead, thus saving the world from an inevitable disaster (Fig. 1.1) [2]. In this regard, according to the ancient Hindu traditions, the human beings would have a "third eye" or mystic organ (the pineal gland), corresponding to

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F. López-Muñoz (✉)
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal
e-mail: francisco.lopez.munoz@gmail.com; flopez@ucjc.edu

F. Marín
Department of Cellular Biology, Faculty of Medicine, Complutense University, Madrid, Spain

C. Álamo
Department of Biomedical Sciences (Pharmacology Area), Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain

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Fig. 1.1 Artistic impression of one of the legends about the appearance of Shiva's third eye

the sixth *chakra* (*ajna*), which would provide them with some sort of window to their own spiritual life and which would hold the key to their own mental power [3]. It would then be the organ of clairvoyance and meditation [4].

This mediatory role of the pineal gland between the material and spiritual worlds reached its highest relevance in the seventeenth century, the time when Modern Science was born, thanks to one of its most prominent prime drivers, René Descartes (1596–1650) who hypothesized that this anatomic structure was home to the soul. For Descartes, the pineal gland was not only the material seat of the divine spirit but was also responsible for the appropriate communication between the human machine and its environment, being the intimate spring that controlled the exact functioning of the human body [5].

Finally, from the second half of the nineteenth century on, the definitive breakdown of this prescientific stage of "pinealogy" started, and the period of scientific analysis (as we know it today) on the nature of this organ began, culminating in the obvious confirmation of its endocrine nature following the isolation of melatonin, in 1958, by team of Aaron B. Lerner (1920–2007). To deepen the historical development of the pineal gland, see López-Muñoz et al. [6, 7].

1.2 Galen's *Conarium*: The Pineal Gland in the Classic Antiquity

In Western culture, the first express mention of the pineal gland must be looked for in the classic Hellenic antiquity and, more specifically, among the members of the so-called Alexandrian School. This medical trend of thought emerged in Egypt during the Ptolemaic times and resulted in a new anti-Hippocratic physiology that would serve as basis for the doctrinal body of Galenism. Its two most eminent representatives, Herophilos of Chalcedon (325–230 BC) and Erasistratus of Ceos (310–250 BC), considered, respectively, by some authors as "the Father of Anatomy" and "the Father of Physiology," took up the stoic legacy of the Neumatism, promoted centuries back by Diogenes of Apollonia (fifth century BC) and theories of Anaximenes of Miletus (585–524 BC) on the air as a vital principle. They elaborated their physiological theory about animal spirits, in which the pineal gland would play an important role. For these authors, the air, once inside a living being, would be transformed into *pneuma* (*spiritus*, in Latin). Erasistratus tells how the air (cosmic *pneuma*) once transported from the lungs to the heart is changed in the cardiac organ into *pneuma zootikon* (*spiritus vitalis*, in Latin), in order to be, subsequently, carried through the bloodstream to the brain, where it would be then transformed inside the brain ventricles into *pneuma psychikon* (*spiritus animalis*, in Latin) [8]. In this historic frame, according to Ariëns-Kappers' [4] opinion, Herophilos of Chalcedon could well have been the true discoverer of the pineal gland, ascribing its functions in valvular control, as a sphincter, regulating the flux of the *pneuma psychikon* from the third ventricle to the posterior ventricle [9]. However, there is no direct evidence backing up these claims, given the fact that the writings from the Alexandrian anatomist

were completely lost and we only find reference to them in the works of Galen, who stated that the "ancient anatomists" knew about the pineal organ.

It is precisely Claudius Galen (131–200) who completes the first detailed description of this organ that has survived until modern times [10]. Galen gathered the philosophical-physiological Greek legacy, modified the pneumatic theory, and elaborated a physiological doctrine that would last for more than fifteen centuries [11]. Thus, according to the master of Pergamo, the blood that pneumatized in the heart would be conducted to the *rete mirabile* of the brain, originating in the lateral ventricles (considered by Galen to be only one paired ventricle, named anterior ventricle) the psychic pneuma or *spiritus animalis*. This pneuma, comprised of very subtle material substances, would then pass to the spinal cord and the nerves (regarded empty spaces) as a *dynamis psykhiké*-inducing agent, resulting in a muscular action [12].

Even though Galen did never dissect human corpses, he used to humanize by analogy the results from the study of the body of multiple animal species, mainly pigs. The name *Konareion*, with which the pineal gland was designated, comes from his quill (*kônos*, pinecone in Greek), due to the similarity between this fruit and the epiphysis he studied (*conarium*, in Latin). Galen described in deep detail the conarium's anatomy in his *De anatomicis administrationibus*, but consigned its functional role to a mere pseudoglandular lymphatic organ that would serve as fastening to the mass of cerebral veins that go all over the posterior and dorsal faces of the diencephalon. He defended this hypothesis in the eight book of his work *De usu partium*. Galen considered that, in its flux through the ventricular system, the superior cerebellar vermis and not the pineal gland, as Herophilos used to think, was the anatomic structure that acted as a sort of valve able to close the aqueduct of Sylvius avoiding the passing of the psychic pneuma to the fourth or posterior ventricle, seat of memory [13]. A source of confusion inherent to this theory could be the synonym employed by Galen to designate the *vermis superior cerebelli*, which is interchangeably referred to as *epiphysis*, a term designated to the pineal gland in modern times. According to Galen, the pineal gland would be an extracerebral organ lacking self-motility, and therefore, it could not exercise valvular work. For that reason, he would name those defending the pineal theory "stupid" and "ignorant." While he gives no names, he probably refers with these qualifiers to Hippolytus of Rome (ca. 170–235), later Saint Hippolytus, who in his work *Refutatio omnium haeresium* also discusses the role of the pineal gland and the spirits flux [14].

The galenic organization of the brain functionality suits a model that could be called "pneumatic-ventricular model," with a notorious hydraulic nature [15], as the brain is considered as some kind of bomb that distributes the psychic pneuma coming from the sensitive nerves from the lateral ventricles towards the fourth ventricle, in order to propel them later on through the motor nerves [16]. As Spillane [17] highlights, Galen's theory of the spirits will be the longest-lasting throughout the whole history of science.

1.3 The Valvular Function of the Pineal Organ and the Three Cell Medieval Theory

Galen's psychophysiological postulates were slightly modified by the following authors, mainly in relation to the ventricular localization of the psychic functions. This way, Posidonius of Byzantium (ca. 370), by the end of the fourth century, established the seat of imagination in the anterior part of the brain, memory in the posterior part, and reasoning in the third ventricle, while Nemesius (ca. 390) positions these three faculties in the anterior, third, and posterior ventricles, respectively, giving rise to the "Three Cell Theory" [15], very much in vogue throughout the Middle Ages (Fig. 1.2). Likewise, Augustine of Hippo (Saint Augustine) (354–430), in his neuropsychological writings, takes the nervous system organization defend by Erasistratus: "*Et aer*, *qui nervis infusus est*, *paret voluntati*, *ut membra moveat*, *non autem ipse voluntas est*" ("The air infused into the nerves is obedient to the will, and

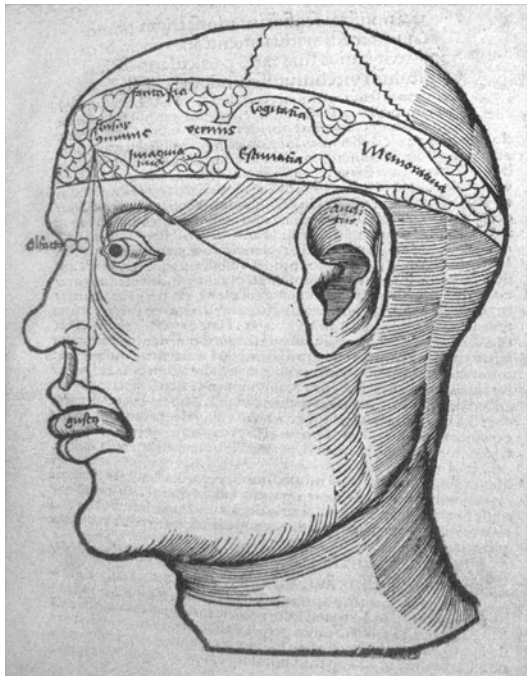


Fig. 1.2 Illustration of the work *Anathomia* by Mondino de Luzzi, in which the vermis is shown controlling the passage between the anterior and third ventricles. This drawing is, in turn, one of the most popular representations of the three ventricular cells theory and the localization of brain functions

makes the limbs move in the absence of the aquiescence of the very will") (*De Genesi ad Litteram*, 401–415). Finally, the Arabian author Qusta ibn Luqa (Costa ben Luca or Constabulus) (864–923) combined Galen's and Nemesius' theories in his work named *De differentia inter animam et spiritum*, in which he defended the existence of a sort of "memory valve" (the vermis and not the pineal gland) as a kind of sphincter that would regulate the flow between the third and the posterior ventricles (Fig. 1.3) [18].

In this sense, and although the theory giving the pineal gland a valvular role in the ventricular flux of the spirits had already been discarded by Galen, this hypothesis regained strength in the late Medieval times. This could have been the result of a new misconception, as several medical texts from that time, such as the *Liber de oblivione* by Abu Ja'far Ahmad bin Abi Khalid Ibn al-Jazzar (ca. 900–980), or the *Speculum Majus* de Vincent de Beauvais (1190?–1267?), would employ the term "*pinea*" in order to designate the

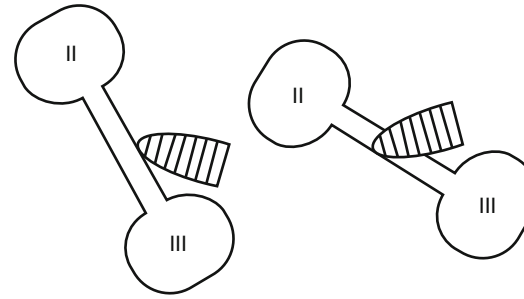


Fig. 1.3 Schematic reconstruction of the theory by Qusta ibn Luqa on the role of the vermis as a valve between the third ventricle (II) and posterior ventricle (III), seat of memory, preventing the flow of the animal spirits (Reprinted with permission from the online Stanford Encyclopedia of Philosophy (Descartes and the pineal gland)).

cerebellar vermicular appendix to which Galen attributed the control of the spirits flow to the posterior ventricle [19].

1.4 The Pineal Gland in Its Anatomical Context

Renaissance thought, mainly platonic, allowed the comeback of modern science and the abandonment of the Medieval Scholastics, still robustly anchored among the university faculties [20]. In the field of medicine, Andreas Vesalius (1514–1564), Father of Modern Anatomy, supposed an inflection point. Vesalio still considers the cephalic organ as the side to the classic *dynámeis* and defends the previous neurophysiological aspects, as the conduction of the animal spirits through the nerves [21]. However, Vesalio refuted all the classical theories about the ventricular localization of the psychic functions, as well as the ability of the *rete mirabile*, the net of blood vessels located at the base of the brain, to produce the animal spirits [22].

With respect to the pineal organ, the theory stating its role as the "guardian" of the flux of the animal spirits continued to be defended during the Renaissance period by authors of the standing of Giacomo Berengario da Carpi (c.1460–c.1530), Jean Fernel (1492–1558), and even, later on, William Harvey (1578–1657) himself in his work *Praelectiones Anatomiae Universalis* (1626). Berengario published in 1522 his *Isagogae breves*, in which he describes the brain ventricles,

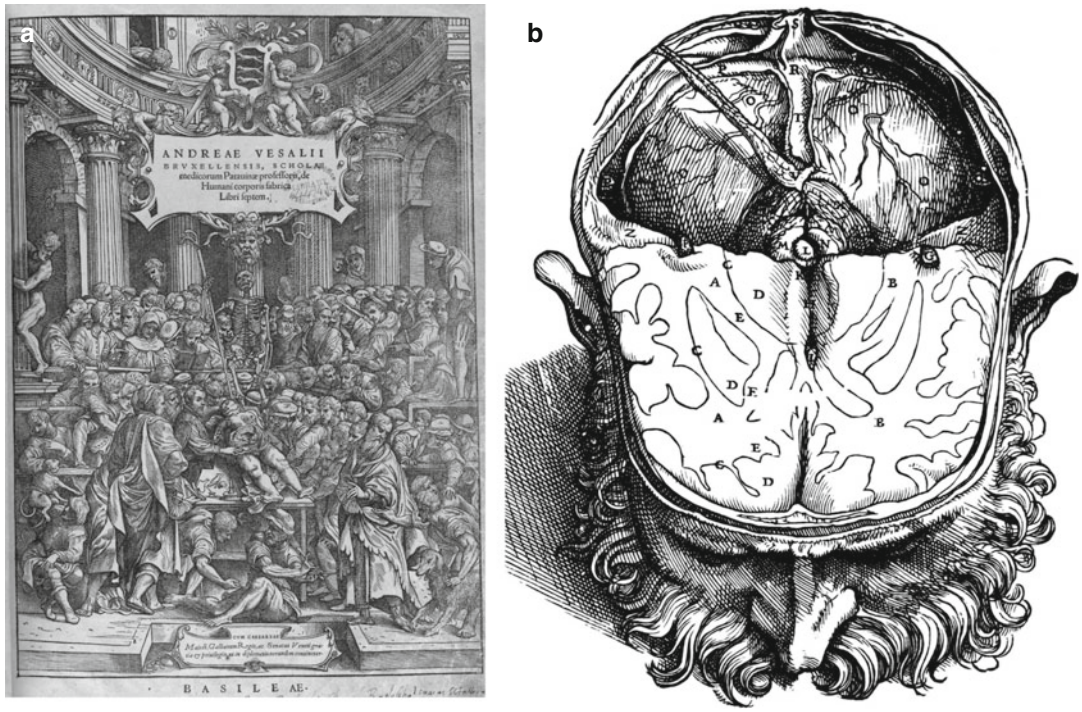


Fig. 1.4 Frontispiece of the famous work by Andreas Vesalius *De Humani Corporis Fabrica…* (Basel, 1543) (**a**), and illustration of the brain (**b**) corresponding to the second edition of this work (1555), in which the location of the pineal gland (L) is shown, right in the center of the cranial cavity

the choroid plexuses, and the pineal gland, which he denominated "the appendix of thought" [23]. The great contribution of Berengario with respect to the *conarium* was to also ascribe it a role as a filter of cerebrospinal fluid. On the other hand, Fernel, modern exponent of the galenic medical system (*Universa Medicina*, 1554), also defends the valvular concept of the epiphysis, although Lokhorst and Kaitaro [14] are of the opinion that the anatomic structure to which Fernel refers is not the pineal gland itself but rather the cerebellar vermis (as Galen and Ben Luca postulated). In the same way, the Italian physician Girolamo Fracastoro (1483–1553) pointed out that the existence of an odd-numbered brain organ was required in order to integrate and coordinate the sensitive perceptions received by the organism. For Fracastoro, this organ should be the *conarium*, in his opinion seat of reasoning. Nevertheless, from a functional perspective, some Renaissance authors considered the conary organ to be just a mere anatomical support to the neighboring vascular structures.

All these authors' contributions were pointing to a change in the prevailing ideological conception in relation to the pineal organ, which was embodied in the person of Andreas Vesalius. In his masterpiece, *De humani corporis fabrica* (Basel 1543), he ridicules every preceding anatomy treatise, and in his VII book (Fig. 1.4a), in which he analyzes the cephalic organs, a detailed description of the human epiphysis can be found (Fig. 1.4b). According to Bargmann [11], the first graphical representation of the history of the pineal gland is due to Vesalius. Two years later, Charles Estienne (1503–1564), one of the members of the famous family of publishers of that name in Paris, aptly illustrated the epiphysis relationships in his book *De dissectione partium corporis humani* (1545). From a physiological perspective, Vesalio definitely rejected the valvular conception of the pineal gland, as well as that of other anatomic structures, such as the *vermis superior cerebelli*, according to Galen and Qusta ibn Luqa's proposal, or the choroid plexus, whose valvular role was proposed by Mondino de Luzzi

(1275–1326) in his *Anathomia* (1316). Still, the mechanical hypothesis of the spirits flux regulation during Renaissance continued to be stood up for and, once assimilated to the cerebrospinal fluid, lasted even to the times of the genial François Magendie (1783–1855), who, in a piece of work published in 1828 (*Mémoire physiologique sur le cerveau*), affirmed that the pineal gland was "a valve that opened and closed the brain aqueduct" [25]. Possibly, in the opinion of Ariëns-Kappers [26], the last author defending the role of the pineal organ as a regulator of the ventricular flux of cerebrospinal fluid at the level of the aqueduct of Sylvius was Élie de Cyon (1842–1912), nothing less than in the year 1907.

1.5 The Pineal Gland as the Seat of the Soul in the Philosophical and Physiological Cartesian Approaches

The role of the pineal gland in the human physiology gained great significance in the seventeenth century, the time of the birth of modern science, thanks to one of its leading proponents, René Descartes (Fig. 1.5a), who said that the seat of the soul resided within it [5, 27]. On the basis of the Greek philosophical analysis, although within the borders of the Catholic faith he always professed, Descartes, who cultivated not only philosophy but also mathematics, physics, astronomy, music, and physiology [28], unleashed the Platonic idea of a human duality, that is, body-soul [29, 30]. Thus, Descartes distinguishes the soul and intelligence (*res cogitans*), as well as a transcendent and free God, from the rest of the cosmos, which includes the human body (*res extensa*) and the animals, which would be subject to the laws of mechanics and mathematics.

Although Descartes always defended the originality of his philosophical hypotheses, when it comes to physiological and anatomical terms, he adopted much of the prevailing theories from classical antiquity, mainly the proposals of the Pneumatic Alexandrian school in relation to the so-called animal spirits (*copula animae* cum *corpore*), later "christianized" by St. Augustine [31]. In this sense, according to Descartes psychophysiological approaches, the placid harmony

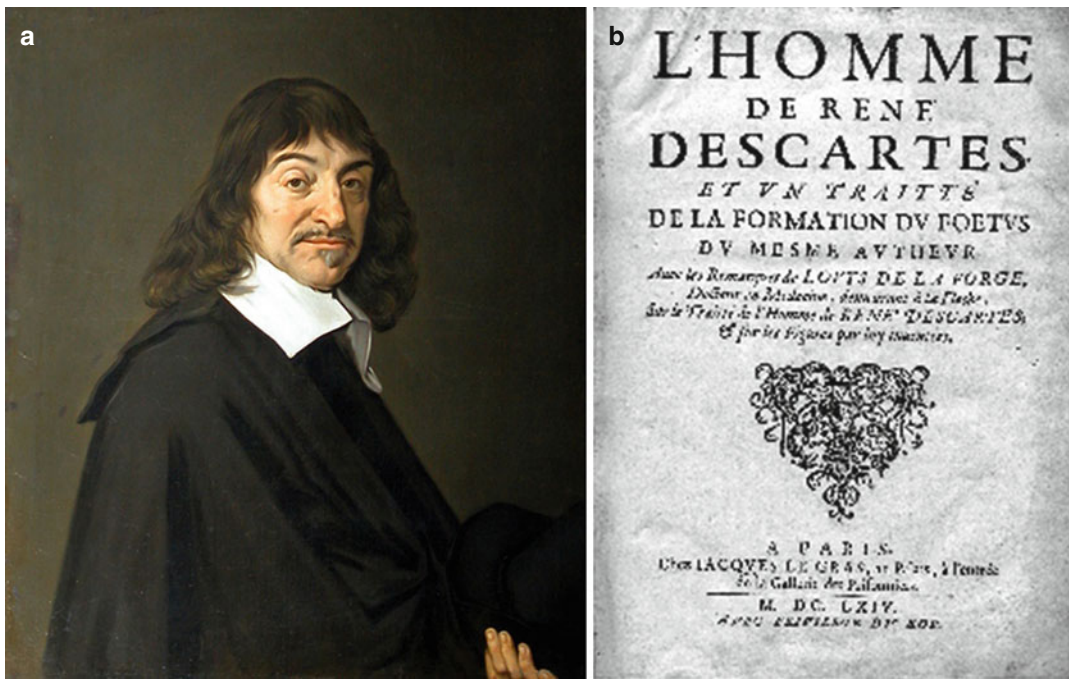


Fig. 1.5 Portrait of René Descartes, painted by Frans Hals, in 1648 (Musée du Louvre, Paris) (**a**) and cover of the second edition of the *Treatise of Man* (first in French), entitled *L'Homme de Rene Descartes et un Traité de la formation du foetus* (Paris, 1664) (**b**)

between the will of the mind and the movement of the body would require a perfect communication, which would be the responsibility of the galenic *spiritus animalis*, subtle fluids that would occupy the interior of the brain ventricles and the nerves, like tiny fast-moving particles, in short, a sort of "quintessence," caused by rarefaction of body fluids. Finally, for this harmonious relationship to take place, it would require that the *res cogitans* or human soul had a corporeal and physical seat, where that mysterious communication was possible. Thus, Descartes sets the seat of the soul within "the innermost parts of the brain," i.e., the pineal gland (*epiphysis cerebri* of the classics) [5, 19, 27].

Much of the Cartesian physiological doctrine was included in the *Treatise of Man* (1664) (Fig. 1.5b), probably the work that most influenced the conception of human psychophysiology throughout the seventeenth century and which is considered the first European book on physiology [32]. However, the first mention of the pineal gland in a work published by Descartes is found in his *Dioptrica* (1637), in which in its fifth discourse he refers to "a certain small gland [located] in the middle of the ventricles" and that is the seat to *sensus communis*. Nevertheless, we must point out that Descartes never used the adjective "pineal" to designate the epiphysis. He says "small gland," "gland H" [96]. Occasionally he uses the term *koonareion* (or *conarium* in Latin) proposed by Galen centuries before as its morphology reminded him of a pineapple cone [8]. Descartes, in particular, placed the pineal gland in the rostral portion of the *sulcus lateralis cerebri*, which connects the third cerebral ventricle with the fourth ventricle [21], "a well-protected location, which is almost immune to disease" [22]. In this anatomical framework, the pineal gland is topographically placed as if it were hung from a series of arterioles and unbound to the brain substance (Fig. 1.6). Although the illustrations in *The Treatise of Man* provide evidence of a clear shape error, showing the pineal gland located inside the ventricles, they might not reflect a deficit in Descartes anatomical knowledge, since these illustrations were commissioned by Claude Clerselier (1614–1684), French editor of the treatise and the philosopher's brother-in-law, 14 years after the death of the author, to Louis de la Forge (1632–1666), doctor in medicine from La Flèche, and Gerard van Gutschoven (1615–1668), professor of anatomy in Louvain [33, 34]. The inner structure of the pineal gland, as that of the rest of the brain, consists, according to Descartes, of thin threads separated by pores through which blood from the choroid plexus and the epiphyseal arterioles penetrates [35]. To perform its function, the pineal gland would distill fine particles suspended in the sanguineous fluid and generated in the heart's left ventricle, in the heat of the myocardium ("some very subtle wind or rather a lively and very pure flame " in the scientist words) [36] and transformed into the *sprits animaux*.

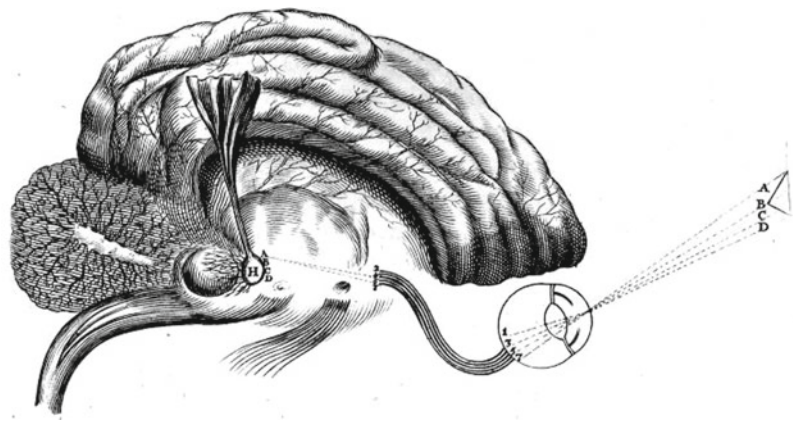


Fig. 1.6 Anatomical location of the pineal gland, according to the ideas of Descartes and the interpretation of the illustrator, Florent Schuyl (Figure XXXIV from *De Homine*, 1662)

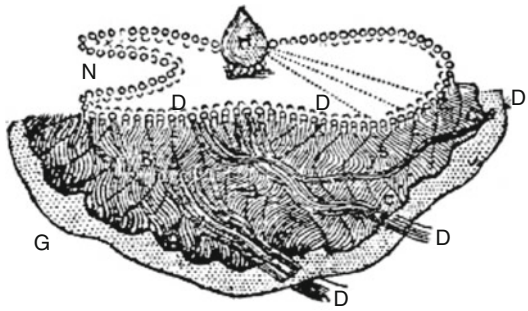


Fig. 1.7 Engraving by Gerard van Gutschoven, with regard to the 64th Article of the *Treatise of Man* (1667), entitled "*On the formation of the objects' ideas in the place intended for imagination and common sense*"

In the Cartesian description of physiology, the pineal gland would receive sensory impressions from the outside and would instigate distal muscle movements, through the animal spirits [19, 27, 37]. These would be driven by active movements of the gland, towards the ventricular brain system (Cartesian brain cavities) and hence reach the periphery of the body, through the myriad pores that allegedly cover the ventricular walls. Once these spirits reach the muscle, they change its shape in a way that induces muscle movement. With the help of a drawing (Fig. 1.7), the whole process is outlined in the *Treatise of Man*: "You can see in the figure the spirits coming out of the gland having enlarged the part of the brain marked A and covered every pore, flowing from there to B, then to C, and finally to D, from where they will be distributed to all members and so the thin filaments that build the nerves and the brain have such tension, that the actions, even when the strength to move those filaments is little, communicate easily from one end to the other, without the detours of the paths they pass by hindering them from doing so" (Art. 65) [26]. In order to provide this mechanical explanation of the physiological phenomenon, Descartes takes advantage of presumed motility of the pineal gland [35], because: "… it is made of a soft material and is not fully attached to the brain substance, but only pinned to a few small arteries, whose walls are quite weak and flexible; the gland is suspended as a weighting scale as a result of the force with which the heat of the heart pumps the blood towards it" (Art. 72) [26]. This movement ability to regulate the flow of the animal spirits that the epiphysis has could be assimilated, in mechanical terms, to the role of a valve.

But also, the pineal gland would play an important role in the human psychophysiology [39–41], since this organ is the seat of the rational human soul. Thus, "all the action of the soul is that, by the mere fact of wanting one thing, it makes the small gland, to which it is closely bound, move in the needed way in order to produce the effect that corresponds to the will" (Art. XLI of the *Treatise Passions of the Soul*) [42], so that it would cause muscle movements bend it, in a way that the spirits could slide through the pores of the ventricular walls. For Descartes, in sum, every change in the position of the pineal gland corresponds to a different perception of the soul, and this, in turn, could move the gland by the mere fact of perceiving [29]. The epiphysis is, in short, and following the words of the author himself, "the organ of common sense and imagination," the storage of past experiences and responsible for "the appetites and passions."

The reasons that lead Descartes to consider the pineal gland as the control center of the body, house of the *sensorium commune* (convergent point of all sensations in the brain), and seat of the soul (*siège de l'àme*) are, certainly, of a purely anatomical nature (see Table 1.1, in the opinion of Finger [43]), although other mathematical reasons in his election should not be ruled out, since Descartes opts for an organ located precisely at the geometric center of the brain. At this point, we must bear in mind Descartes great interest in medical disciplines: while residing in Leiden he witnessed autopsies, and he practiced dissections in slaughterhouses (Fig. 1.8) and took an Anatomy course taught at the University of this same city by Professor Adriaan van Valkenburg (nd) [31, 44], so he possibly knew the work of the famous professor of Anatomy at Utrecht University, Ijsbrand van Diemerbroeck (1609–1674), contemporary to the French philosopher, who proposed the pineal gland as the possible location of the *sensorium commune*. Thus, Descartes believes that all cephalic and sensorial organs are double, except that small and lonely

Table 1.1 Descartes ten reasons to select the pineal gland as the seat of the soul and controller element of the body machinery

Reason 1	The pineal gland is part of the brain
Reason 2	The pineal gland is a unique and odd body
Reason 3	The pineal gland is located in the midline of the brain
Reason 4	The pineal gland is an anatomically protected structure
Reason 5	The pineal gland controls the ventricular flow
Reason 6	The pineal gland is provided with mobility
Reason 7	The pineal gland is a small organ
Reason 8	The pineal gland is able to generate the animal spirits
Reason 9	The pineal gland is an orphan organ from a functional perspective
Reason 10	There exist classic psychophysiological models that support the proposed role for the pineal gland

Modified from Finger [43]

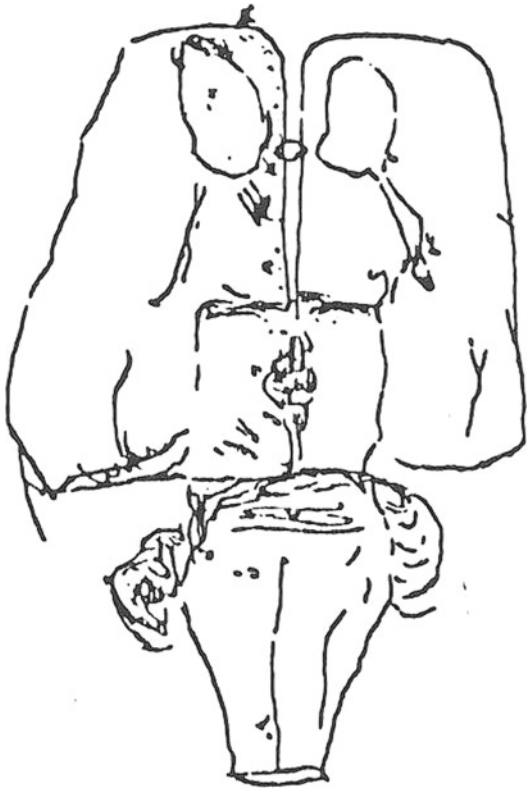


Fig. 1.8 Drawing of a sheep brain by Descartes himself during the time he resided in Amsterdam (Collection Leibniz, Hannover)

little gland, geometrically located in the center of the brain (*primus inter pares*) and suspended over the channels containing the animal spirits (Figs. 1.6 and 1.8) [45]. Its central location would allow it to receive, with the same intensity, any stimulus from peripheral organs, whereas its unitary nature would enable the integration process of perceptions and feelings coming from duplicated bodies.

The Cartesian hypothesis that states "the pineal gland as the seat of *sensus communis*" was quickly adopted by many contemporary authors to French philosopher Lokhorst and Kaitaro [14] and Jean Cousin (nd), who defended his Thesis (*An kônarion sensus communis sedes*?) at the École de Médecine in Paris on the 24th of January, 1641 (Fig. 1.9), or the professor Medical Theory at Utrecht University, Henricus Regius (1598–1679), who also defended this theory in June 1641 (*Die frühe Naturphilosophie*). However, the Cartesian hypotheses also had significant detractors, as Christophe de Villiers (1585–1650), who believed that the pineal gland was too tiny an organ to exercise the important mission that Descartes gave it [22]. Even one of the French philosopher's best friends, his mentor, Father Marin Mersenne (1588–1648), believed that the Descartes choice was not correct, as some autopsies performed on people with fully preserved mental faculties revealed the presence of "stones" and other abnormalities in the pineal organ [46]. Also, serious discrepancies can be found among the followers of the mechanistic approach in the Cartesian postulates. Such is the case of the Danish Niels Steensen or Stenon (1638–1686), mechanistic physiologist in the purest sense of the word, or Thomas Willis (1621–1675) himself. Already in 1665, Stenon, in a lecture at Monsieur Thevenot's house, said that the Cartesian theory was physiologically too speculative and meaningless. Later, in his *Dissertatio de cerebri anatome* (1671), he severely criticizes Descartes and refutes his theory of a rational soul seated in the pineal gland. Stenon says, not without reason, that this gland (which he calls "superior gland") is a motionless body, attached to the meninges and dorsal to the ventricular system, and lacking the Cartesian pores, preventing its role in the


DEO OPTIMO MAXIMO VNI ET TRINO.
VIRGINIQVE DEIPARÆ.
ET SANCTO LVCÆ ORTHODOXORVM MEDICORVM PATRONO.
QVÆSTIO MEDICA.
QVODLIBETARIIS DISPVTATIONIBVS MANE DISCVTIENDA
IN SCHOLIS MEDICORVM DIE IOVIS XXIV. IANVARII.
M. IOANNE DE MONSTROEIL Doctore Medico, Præside,
Proponebat Lutetiæ IOANNES COVSIN Patisinus. A. R. S. H. M. DC. XLI.
DOMINI DOCTORES DISPVTATVRI.

Fig. 1.9 Advertisement of Jean Cousin doctoral thesis defense (Paris, January 24, 1641) concerning the question whether the pineal gland was really the seat of *sensus communis* (Bibliothèque Nationale de France, Paris)

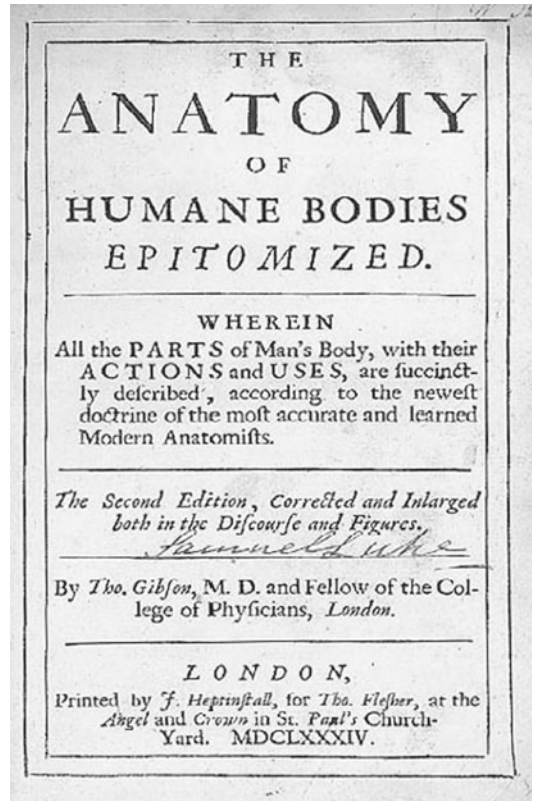
convection the "animal spirits" [47]. The Danish anatomist Thomas Bartholin (1616–1680), in addition to the size of the gland, also adduced other specific reasons to reject that specific location in his work *Anatome veterum omnium* … (1673), as its hypothetical and uncertain mobility or the absence of ventricular and intraglandular pores through which the spirits would spread, along the lines of Stenon's critical comments [48]. Willis, meanwhile, argues that it is hardly credible that the pineal gland is the seat of the soul and the seat of reasoning, given that animals, lacking the superior faculties of the soul, such as memory or imagination, are endowed of pineal organs even more evolved than those of humans (*Cerebri anatome cui accessit nervorum descriptio et usus*, 1664). For Willis, the epiphysis, like other glandular organs located in the vicinity of major vascular beds, would merely serve a function of absorption of secreted fluids from arterial vessels [15].

1.6 A Century and a Half of Decline in the Scientific Understanding of the Pineal Gland

In spite of the fact that Cartesianism continued to inspire scientific movements in the eighteenth century, as the main life force that inspired the vitalistic current during the Enlightenment [34], the role given by Descartes to the pineal gland found little scientific support. For example, Claude-Nicolas Le Cat (1700–1768), professor of Anatomy at Rouen University, who won the Royal Prussian Academy of Sciences prize in 1753 with an essay on the qualities of the animal spirits [49], proposed that the "material" that flowed inside nerves was not a known "material" (water, blood, steam, electricity, light, fire, etc.), but a so-called universal fluid resulting from blood leaking in the cerebral cortex, where he claims that the human soul settles, and not in

the pineal gland. Other prominent physicians from the eighteenth century, such as Julien Offray de la Mettrie (1709–1751), Pierre Jean Georges Cabanis (1757–1808), Etienne Bonnot de Condillac (1714–1780), Charles Bonnet (1720–1793), and Baron Paul von Holbach (1723–1789), also joined this discredit to the Cartesian theory about the pineal gland. However, it would only be during the first half of the nineteenth century that Descartes' theory regarding the physiological role of the epiphysis was definitely discarded. In the *Dictionnaire des Sciences Médicales* published in 1829 by Antoine Jacques Louis Jourdan (1788–1848), it is said of the pineal gland: "… with respect to the function of the pineal organ, nothing from Descartes' fiction can be accepted, a theory conceived in a time of rationalism abuse and natural science imperfection… Today, we do not need these chimeras, although we do not know yet the conarium functions…" [50]. Thus, the spiritual role of the pineal gland, from the standpoint of science, came to an end.

On the other hand, progresses in the understanding of the pineal organ during the eighteenth century, particularly from a physiological perspective, were rather scarce. Only anatomic descriptions of this gland continued to appear, increasingly accurate. Among the many postcartesian anatomists and in relation to the epiphysis, the English author Thomas Gibson (1647–1722) should be mentioned, who in his book *The Anatomy of Humane epitomized Bodies* (1682) (Fig. 1.10) reaffirms the current name of the pineal gland, describing it as a "*penis*" suspended on and between the inferior colliculi or "*testes*" [51]. This gradual loss of interest in the conary organ further increased during the first half of the nineteenth century, a period in which anatomists, perhaps influenced by the German romanticist philosophical current, which questioned the physiological experiment scientific evidence, dwindled the functional role of the pineal gland [10]. In fact, Karl Friedrich Burdach (1776–1847) stated that this anatomic body lacked any specific function. Figure 1.11 shows a drawing of a sagittal section


THE
ANATOMY
OF
HUMANE BODIES
EPITOMIZED.

WHEREIN
All the PARTS of Man's Body, with their ACTIONS and USES, are succinctly described, according to the newest doctrine of the most accurate and learned Modern Anatomists.

The Second Edition, Corrected and Inlarged both in the Discourse and Figures.

By *Tho. Gibson*, M. D. and Fellow of the College of Physicians, *London.*

LONDON,
Printed by *J. Heptinstall*, for *Tho. Flesher*, at the *Angel* and *Crown* in St. *Paul's* Church-Yard. MDCLXXXIV.

Fig. 1.10 Cover of the work by Thomas Gibson *The Anatomy of Humane Bodies Epitomized*, printed in London in 1684, by John Heptinstall (1657–1732). In this anatomical text, the study of all organs of the human anatomy and their functions are tackled in the light of scientific knowledge and new physiological doctrines of the late seventeenth century

of the human brain, from an anatomical atlas widely spread during the first half of the nineteenth century, in which the morphology and location of the pineal gland can be fully appreciated.

However, thanks to the Cartesian postulates on the seat of rational thought in the pineal organ, some authors of the eighteenth and nineteenth century tried to find the origin of certain thinking disturbances in physical and functional abnormalities in this gland, basically the presence of calcifications, an observation, on the other hand, devoid of novelty, to the extent that it was just an adaptation of the medieval legends about the "stone of madness" (remember that

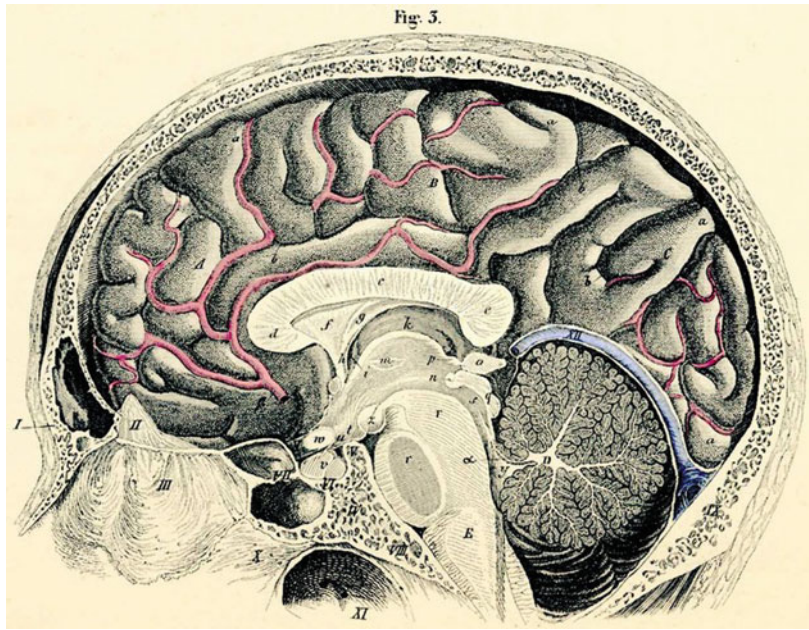


Fig. 1.11 Anatomical print from the atlas *Handbuch der Anatomie des Menschen*, published in 1841, in Leipzig, by Professor Carl Ernest Bock (1809–1874). In this sagittal section of the human brain, the pineal gland can be observed, marked with the letter "o" in its central location

the stones of madness extraction was a recurring theme for different Flemish school painters during the seventeenth century). During the Middle Ages, it was common to think, from the prevailing fraud in the medical field, that one of the causes of insanity was the production of "stones" in the brain, which, as kidney stones, would press the brain tissue, causing mental disorders or dementia. Nevertheless, from a scientific perspective, and through the development of pathological anatomy from the eighteenth century, it became possible to observe the existence of "grit" or small stones in the pineal gland during autopsies executed on some patients, which enabled the revival of the hypothesis of a link between the two. However, one of the pioneers of this new medical discipline, Giovanni Battista Morgagni (1682–1771), in his *De sedibus et causis morborum per anatomen indagatis* (1761), expressed his skepticism about the alleged relationship between the existence of acervulus in the pineal organ and the existence of mental deficiency or mental retardation.

1.7 The Resurgence of Interest in the Pineal Gland and the Knowledge of Its Structure and Inner Nature

Finally, we can say, according to Ariëns-Kappers [4], that the second half of the nineteenth century marks the final break with that prescientific stage in pinealogy, based on antropophylosophical speculations and mythological metaphorization, and the beginning of the studies aiming to elucidate the true physiological role of the pineal gland. This period marked the triumph of Friedrich Gustav Jakob Henle's (1809–1885) and Jean Léo Testut's (1849–1925) comparative anatomy, which later evolved following the current established by Charles R. Darwin (1809–1882), towards a comparative anatomy with an evolutionary orientation. Already as early as in 1816, the professor of Anatomy and Physiology at Heidelberg University, Friedrich Tiedemann (1781–1861), conducted an embryological study on the pineal gland, comparing the epiphysis from fetuses with that from some reptiles. But it

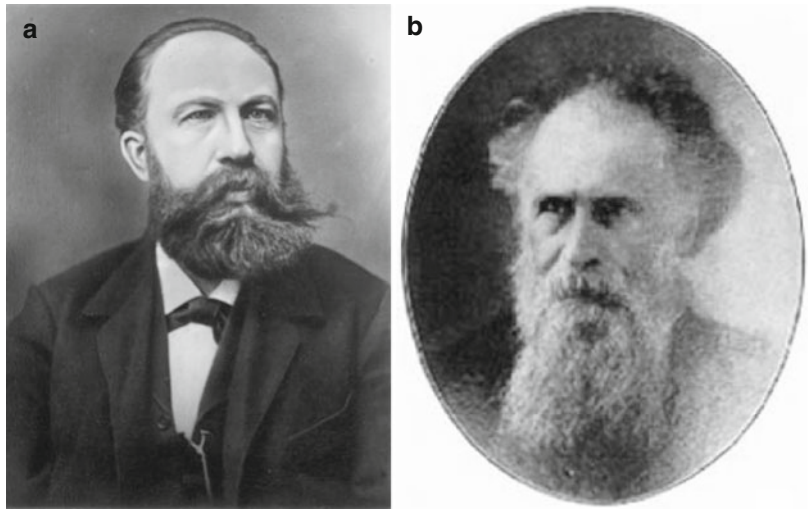


Fig. 1.12 Pioneers of the anatomic study of the pineal gland, from the evolutionary doctrine: (**a**) Stieda Ludwig, director of the Institute of Anatomy at University of Koenigsberg, Germany. (**b**) Franz von Leydig, Professor of Zoology and Comparative Anatomy at University of Tübingen and later professor of Comparative Anatomy at University of Bonn and director of the Institute of Anatomy and the Museum of Zoology of this institution

would be a follower of this evolutionary current, Stieda Ludwig (1837–1918) (Fig. 1.12a), director of the Koenigsberg Institute of Anatomy, who first described a pale stain in the frontal region of the head of frogs, which he called "*Stirnfleck.*" Stieda discovered that this stain was composed of a small, solid, and round mass of cells ("*Subkutane Stirndrüse*") [10], but failed to interpret its functional significance. In 1868, Franz von Leydig (1821–1908) (Fig. 1.12b) described in detail the frog parietal gland discovered by Stieda. Finally, Alexander Goette (1840–1922) would be the one who, in 1872, related phylogenetically this frog "*Stirnorgan*" with the pineal gland [52]. Later, during the 1880s, many scientists would have an impact on this field of research, postulating that in predecessor species of current vertebrates, the pineal gland could have been a third uneven-numbered eye. With all these data, the "theory of the saurians' third eye" by Julien and Peytoureau was configured.

The study of epiphyseal structure in lower vertebrates unveiled the photoreceptor role of the pineal gland in these animals. Yet in mammals this gland was classified as a rudimentary vestigial organ, the remainings of the lacertids or cold-blooded vertebrate's third eye [23], among which the lamprey and the iguana stand out. This research culminated in the publication by František Karel Studnička (1870–1955) of a long chapter entitled "The Parietal Organ" in the *Textbook of Comparative Microscopic Vertebral Anatomy* by Albert Oppel (1905). To Studnička, the "parietal organ" would be a kind of outgrowth of the primitive brain wall, consisting of ependymal cells capable of generating glandular and glial cells, or even neurons or photoreceptors, which has remained in some species. Specifically he highlights the case of the lamprey, whose "parapineal body" he called "paraphyses" [98]. The consideration of the pineal gland as a vestigial organ with no physiological function in mammals lasted until the late 1950s. Notwithstanding, this documentation generated, from this moment on, a growing interest among the scientific community.

Moreover, the progress in optical technology and the breakthrough of the micrographic technique enabled, since the mid-nineteenth century, the boom of microscopic anatomy and, therefore, the understanding of the histological structure of the pineal organ. In this light, it was Giulio Bizzozero (1846–1901), professor of Pathology at University of Turin, the first scien-

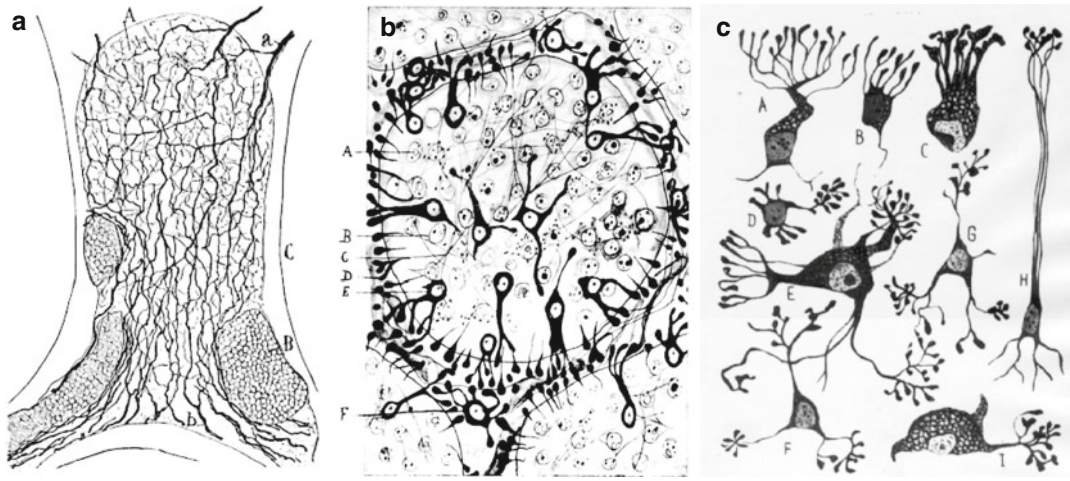


Fig. 1.13 Histological drawings of the pineal gland made by some members of the prestigious Spanish School of Histology, emerged around the figure of Santiago Ramón y Cajal during the first third of the twentieth century: (**a**) Drawing by Cajal himself, representing the sympathetic innervation of the mouse epiphysis [60]. "*A* interstitial plexus, *a* sympathetic fibers that come from the top, *b* arriving fibers with lower arteries, *B* section of a venous vessel, *C* interhemispheric cerebral cortex." (**b**) Diagram of the structure of the adult human pineal gland, by Achúcarro [61]: "*A* neuroglial cell with intranuclear ball, *B* neuroglial cell, *C* neuroglial fibers, *D* nerve endings in the perivascular connective spaces, *E* nervous cell from inside the lobule, sending piriform appendices into the perivascular space, *F* nerve cell in the perivascular connective spaces." (**c**) Different morphological types of pineal parenchymal cells according schemes by Río-Hortega [62]

tist, who described, in 1868, several cell types in the pineal parenchyma. This Italian author distinguished between first and second class epiphyseal cells, which he, respectively, attributed a nervous and a connective nature [54]. Building on the ideas of Bizzozero, some later authors, among which G. Hagemann (1872) stands out, believed that the conarium would be kind of nervous ganglion. Other researchers, taking as a starting point the assumption that the epiphysary organ had a glandular nature, approach ontogenically its parenchymal cells with other secretory epithelial cells, such as the parathyroid gland acidophilic cells [55] or the ependymal cells and those of the choroid plexus [56]. In fact, the early work in the scientific literature addressing the histological constitution of the epiphyseal body makes it a lymph node [57], in which one can find "abundant round cells lymphocyte-like." In the early years of the twentieth century, the idea of an epiphyseal body with a neuroglial nature was also defended. So, Jean Verne [58] considers the pineal gland as a predominantly glial organ whose cells constantly produce new fibers at the expense of the nuclear chromatin.

But it would be the so-called Spanish School of Histology that, during the first decades of the twentieth century, would shed more light on this topic [59]. So while Santiago Ramon y Cajal (1852–1934), who considers the pineal organ as a "sanguineous vascular gland," describes in detail the innervation of the pineal body of various mammals (Fig. 1.13a) [60], Nicholas Achúcarro (1880–1918) and José Miguel Sacristán (1887–1957) analyze some histological concepts wrongly inherited from previous authors and establish the secretory nature of the human pineal gland (Fig. 1.13b) [61]. Meanwhile, Pio del Rio Hortega (1882–1945) deals with the nature of pineal cell types and applies innovative silver impregnation techniques to the study of the epiphysis of a variety of birds and mammals, including humans. This attraction from the Spanish histologists from the beginning of the century to the intimate understanding of the pineal gland structure and its functional role culminates in the chapter entitled "Pineal Gland," in the pres-

tigious work *Cytology and Cellular Pathology on the Nervous System*, published in 1932, in New York, by Wilder Penfield (1881–1976) [63] of which Río-Hortega takes charge. In this review, Río-Hortega renames the two cell types described in this body, denominating fundamental cells or pineal cells the previously called parenchymal cells (Fig. 1.13c) and interstitial cells the neuroglial cells. All these observations enabled Río-Hortega to say in 1922 that "the parenchymal cells of the pineal … constitute a cellular mode with typical characteristics, which should be studied separately as something new" [62]. This would therefore be the first time in history that this cellular was acknowledged as its own entity, known today as pinealocyte [59]. Río-Hortega's contributions would be endorsed during the 1980s, following the introduction of immunohistochemical techniques to the study of the pineal gland [64–68]. In 1943, Wolfgang Bargmann (1906–1978), from the Department of Anatomy at University of Kiel, collected all histological knowledge about the pineal gland in what would become the first specific monograph on this research field in relation to the epiphysis [24].

Likewise, in the years that framed the turn from the nineteenth to the twentieth centuries, the first scientific data were published on the endocrine nature of the pineal gland. Although G. Galeotti (1897) had already described the alleged presence of secretory granules in pineal cells (cit. [53]), Zaherina Dimitrova [69] would be the first author to take a stance in this respect. In an extensive publication on the pineal gland of humans and several mammals, Dimitrova described the existence of beads or nuclear balls in the epiphyseal cells. These "grains" were initially interpreted as a cell nucleus secretion phenomenon, which would subsequently regenerate. From the clinical perspective, the first publication that linked the pineal functionalism with endocrine disorders is due to R. Gutzeit, who in 1896 published a case of teratoma of the pineal area in a 7.5-year-old child who exhibited a premature and excessive development of the external genitalia [70]. Two years later, Otto Heubner (1843–1926) associated again the existence of pineal tumors to early puberty, when he studied the case of a 4.5-year-old child who died from such a tumor, who developed, during his last year of life, marked primary and secondary sexual characteristics [71]. Meanwhile, Otto Marburg (1874–1948) described the "early genitosomy syndrome" and classified the pineal gland as a sexual development inhibitor or "chastity gland" [72]. However, during the 1940s, some authors, such as Dorothy S. Russell (1895–1983) in 1944 [73] or Nathan B. Friedman (1911–2001) in 1947 [74], observed that most tumors in the pineal area were indeed parapineal tumors, developed from embryonic remains, like seminomas and germinomas, hence their relationship with sexual function. Meanwhile, Carlo Foà (1880–1971) experimentally demonstrated the early development of secondary sexual characteristics and gonadal function in several animal species after several glandular lesions, pinealectomy, for instance [75].

From the therapeutic perspective, and in spite of the lack of relational evidence linking pineal calcifications and mental pathology, at the beginning of the twentieth century, pineal extracts came to be administered in the treatment of subjects with mental deficiency. In fact, in an alleged "controlled" study published in 1914, it is stated that improvements were observed in some children with mental retardation following this therapy and also in 12 cases of senile dementia [76]. Similarly, in 1920, W.J. Becker also administered pineal extracts (called Epiglandol) to psychotic patients [77], a practice that was consolidated in subsequent years. In fact, Kitay and Altschule [9], in their previously mentioned famous work, found 17 studies conducted before 1950, in which pineal extracts had been given to schizophrenic patients obtaining generally positive results.

Finally, it is noteworthy that during the first half of the twentieth century, a new parascientific current arose, with a philosophical-mythological nature. With regard to the Cartesian approaches stating a pineal gland control of the "human spirits" as well as the prevailing evolutionary currents from the late nineteenth century, they assimilated this organ with Hindustani cultures' "Third Eye": the "Door of Brahma," through which people's spirits can fuse together with the

soul of the universe, or the third eye of the Hindu God Shiva (Fig. 1.1). This current reached its peak with the so-called anthroposophy, assimilation between mysticism and modern science developed by Rudolf Steiner (1861–1925), perpetuating such myths, relating, for example, the pineal gland to Polyphemus the Cyclops only eye in the works of Homer or the medieval practice of parietal tonsures by Christian monks. Following this trend, Boie Dietrich (1923–2001) defines the pineal organ as "the physical consolidation of an ethereal center" [78].

1.8 The Pineal Gland as an Endocrine Organ

Towards the middle of the twentieth century, three events of great importance came together, making possible the impressive progress of research on the pineal gland that has taken place since the mid-1960s: the publication of a book entitled *The Pineal Gland* in 1954 by Mark D. Altschule and Julian Kitay, at Harvard University; the isolation of melatonin in 1958 by Aaron B. Lerner and collaborators, at Yale University; and the discovery that this endocrine organ was directly controlled by external environmental factors [79–82]. The sequence of all these important findings in a short period of time (1954–1965) set the basis for the current scientific knowledge about the pineal gland [83], changing the outdated concept of vestigial organ without physiological relevance for the modern and current concept of "neuroendocrine transducer" [84].

In the early 1950s, Altschule and Kitay, who had correlated the administration of pineal extracts with modifications in the size of rats' ovaries, reviewed all the controversial literature on the pineal gland published until 1953 (a total of 1,762 original works) and published in 1954 a book entitled *The Pineal Gland*. From their critical review on the state of the pinealogy at that time, these authors concluded that the three probable pineal organ functions were control of gonadal function, participation in chromatic skin response to changes in ambient light in lower vertebrates, and a potential link to behavioral disorders. With this work, the pineal gland finally entered the academic circles of science.

The next step was the identification of the endocrine factor responsible for the functional activity of the pineal gland. In this sense, already in 1917, Carey P. McCord (1886–1979) and Floyd P. Allen, Johns Hopkins University, were able to confirm that the pineal extracts were able to whiten the skin of tadpoles [85], possibly due to a phenomenon of aggregation of pigment granules in individual melanophores. Forty years later, Aaron B. Lerner (Fig. 1.14a), Yale University, began his studies to identify the responsible factor for this darkening of the skin in amphibians, initially with Yoshitaka Takahashi, an internist at University of Tokyo, which ended in 1958 with the isolation of a small amount (100 μg) of an indolamine (N-acetyl-5-methoxytryptamine), extracted from processing 250,000 bovine pineal glands (100 Kg of material), which he called "melatonin" (Figs. 1.14b, c) (from the Greek *melas*, black or dark) [86]. This discovery was the biggest milestone in the history of the pineal research, because, finally, it was demonstrated that a chemical, synthesized and released by the pineal gland, exhibited an endocrine function [87]. In subsequent years, several authors, mainly from team of Julius Axelrod (1912–2004), Department of Clinical Pharmacology of the National Institutes of Health (NIH), in Bethesda, and group of Virginia M. Fiske (1910–1999), Wellesley College, showed that the synthesis of melatonin in the pineal organ main cell, the pinealocyte, was regulated by ambient light in mammals [79, 82, 88–91], through a neural pathway from the retina, ending in sympathetic neurons of the superior cervical ganglion [92], and that it was a derivative of serotonin [93], a indolamine distributed throughout the body, including the brain.

The neuroendocrine nature of the pineal gland was finally proven in 1965, thanks to the publication of two scientific papers of great importance in this field. On the one hand, Roger A. Hoffman and Russel J. Reiter, at Medical Research Laboratory of Edgewood Arsenal (Maryland), showed that the gonadal changes in rodents

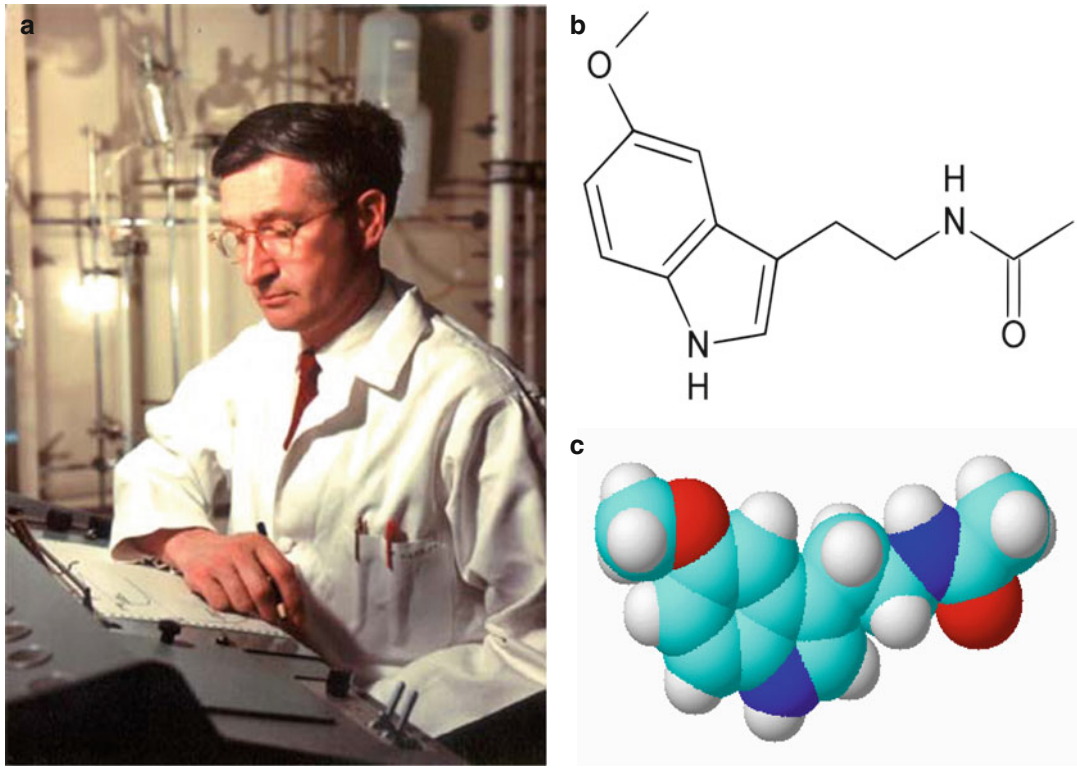


Fig. 1.14 Aaron B. Lerner, first director of the Department of Dermatology at Yale University, in a photograph of 1971 (**a**). Lerner's team was responsible for the isolation of melatonin, the most significant discovery in the history of pineal research. Figures (**b**, **c**) show the chemical structure and three-dimensional structure of melatonin, respectively

exposed to conditions of darkness disappeared completely after making a pinealectomy [80], while Axelrod and his then NIH colleague, Richard J. Wurtman, 20 years later director of the Clinical Research Center at Massachusetts Institute of Technology (MIT), introduced the term "neuroendocrine transducer" to refer to the pineal gland [84]. With this concept, the above authors attempted to explain the principle of the pineal physiology, that is, the transformation of external light signals reaching the gland from the retina through a neural circuit, in an endocrine response, involving the synthesis and release of the hormone melatonin (Fig. 1.15) which, in turn, would act as a potent neurotransmitter in the CNS, turning the pineal organ into a "biological clock" [84]. In this work, published in *Scientific American*, Axelrod and Wurtman proposed their "melatonin hypothesis," noting that, in response to environment light changes, the pineal gland would not only segregate its hormone, but it would also be able to modify reproductive functions in mammals. The great contributions of Axelrod (Fig. 1.16) to the knowledge of the role of monoamines in the CNS, primarily the discovery of presynaptic norepinephrine reuptake systems, earned him the Nobel Prize for Medicine and Physiology, which was received in 1970, together with Ulf S. von Euler (1905–1983) and Sir Bernard Katz (1911–2003).

Indeed, the development of chronobiology, especially since the publication in 1958 of the famous book entitled *Die physiologische Uhr* (*The Physiological Clock*) by Erwin Bünning (1906–1990), and the study of the biological rhythms have provided a powerful support to the scientific study of the pineal gland expansion. In fact, in the early 1970s, the existence of a circadian oscillator located in the suprachiasmatic nucleus of the hypothalamus was demonstrated,

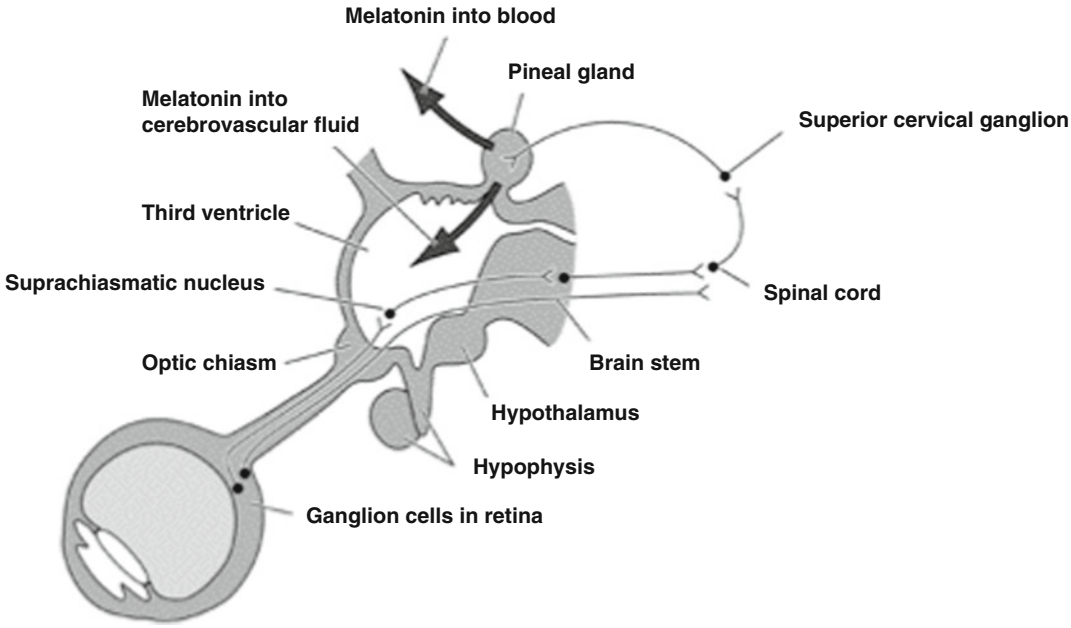


Fig. 1.15 Retina-pineal gland neuronal circuit, with corresponding hubs

Fig. 1.16 Julius Axelrod, director of the Pharmacology Section of the Laboratory of Clinical Sciences at the NIH (National Institutes of Health, Bethesda) between 1954 and 1984, in a photograph taken in 1973 working in his laboratory. Between 1960 and 1965, Axelrod was devoted to the study of the pineal gland and proposed his "melatonin hypothesis"

which would control the synthesis of melatonin in the pineal organ based on the activity of the enzyme serotonin-N-acetyltransferase [94–96]. Thus, in conditions of preserved integrity of the abovementioned neural circuit, melatonin exhibits a typical synthesis and secretion rhythmic pattern, so that during the day, plasma hormone concentrations are low (10–20 pg/ml), whereas at night they experience a significant increase (80–120 pg/ml), with a sharp peak between 24 and 03 h [97]. Therefore, the pineal gland would be part, as a gear of vital importance, of the "biological clock" responsible for the adjustment of biological rhythms to periodic changes in the environment [105]. Among the cyclical changes that normally occur in our bodies and which are regulated by this synchronizer system, the sleep-wake cycles, the secretion of various hormones, and body temperature are worth mentioning.

The identification of the biological targets on which melatonin exerts its physiological actions constituted one of the following research steps. Thus, in the late 1980s, the characterization of specific melatonin receptors and the definition of their pharmacological profiles were made possible by using the radioligand 2-[^{125}I]iodomelatonin, a high-affinity agonist thereof, by the group

of Margarita L. Dubocovich, of Northwestern University Medical School (Chicago) [99, 100]. To date, three melatonin receptor subtypes in mammals have been cloned, among which the MT_1 and the MT_2 (previously called Mel_{1a} or MTNR1A and Mel_{1b} or MTNR1B, respectively) are of high affinity, while MT_3 is of low affinity. These melatonin receptors belong to a distinct group of the superfamily of G protein-coupled (GPCR) metabotropic receptors [101, 102]. Initially another receptor, called Mel_{1c}, was identified in amphibians and birds, and not belonging to the family of GPCR [102]. The density and location of the melatoninergic receptors varies considerably among different mammalian species, and they have not only been found within the CNS but also in other peripheral organs, such as lymphocytes, platelets, prostate epithelium, preovulatory follicle granulosa cells, sperm, etc. [103–106]. This wide distribution can explain the role of melatonin on cardiovascular rhythms, gastrointestinal function, and the endocrine and immune functionalism.

From the academic perspective, the early years of the 1980s provided another series of scientific events leading up to the final maturity of the pineal research. In 1977, under the leadership of Professor Johannes Ariëns-Kappers (1910–2004) and the support of his disciple, Professor Paul Pévet, at Louis Pasteur University, Strasbourg, the *European Pineal Study Group* (EPSG) was founded, later called *European Pineal Society* (EPS), scientific society which held its first meeting in Amsterdam in 1978. The scientific results of this conference were published in the 52nd volume of the prestigious series *Progress in Brain Research* (1979). Two years later, under the direction of Professor Lutz Vollrath, Institute of Anatomy and Cell Biology, University of Mainz, the most complete and comprehensive book on the pineal gland was published: *The Pineal Gland* (1981). All the scientific knowledge available so far on this organ in all animal species was collected in this work. Probably the final step in the development of this process of consolidation of pinealogy as a scientific discipline was the launch, in 1984, of a periodical publication devoted specifically to this field, the *Journal of Pineal Researc*h, initially published by professors Russel J. Reiter, now in the Department of Cellular and Structural Biology, Texas University, authentic project inspirator and manager, and Wilbur B. Quay, Department of Zoology, University of California Berkeley, and Michal Karasek, Department of Neuroendocrinology, University of Lódz (Poland). This publication, even after its first 10 years, became the core of the scientific literature on the pineal research [107].

Conclusions

During the past five decades, scientific research on the pineal gland has experienced an enormous expansion in many different aspects related to this body and its functionalism, including physiological and pathological aspects. As a result, today we know that the mammal pineal gland is an organ that develops a very high biochemical activity, as evidenced by the presence in the body of abundant biogenic amines, not only melatonin, such as serotonin, norepinephrine or histamine, and multiple peptidergic substances (vasopressin, VIP, oxytocin, NPY, somatostatin, substance P, etc.) and other hormones (LH, FSH, TRH, ACTH, prolactin, etc.) [108, 109]. One can speak, therefore, of the pineal gland as a neuroendocrine organ capable of synthesizing and releasing active substances, which would exert their hormonal effect on a number of target organs and tissues [110], among which find themselves the hypothalamus, the pituitary, the gonads, the thyroid, etc. [111, 112]. In this sense, the epiphysis could be catalogued today, without risk of error, as an internal secretion gland and an important component of the photoneuroendocrine systems [113].

However, throughout history, this body has become a true anatomical enigma, and metaphysical and spiritual interpretations of its functional role have prevailed, perhaps excessively. In fact, always when the study of the *conarium* is approached from a historical perspective, the figure of René Descartes arises, one of the most read and studied philosophers in history, and considered one of the cornerstones of the seventeenth century

scientific and intellectual revolution, who came to postulate the supremacy of this body over any other anatomic organ, as it would house the ultimate essence of the human being, that is, his soul. From today's view, the scientific basis at the reach of Descartes to describe the function of the pineal gland is, obviously, totally rudimentary and lacks the rigor that modern science and technique require to positively evaluate a theory. However, it keeps surprising, almost four centuries away, the intuitive perspicacity of the French philosopher explaining his physiological model, as Jean Delay (1907–1987) noted, one of the great figures of the twentieth century psychopathology, "… even more curious is comparing the notion of the animal spirits… with the physiological notions of brain activation by hormones, of the chemical transmission of nerve impulses, of neurohormones … of the enzymes that release or destroy them, of the extraordinary preponderance of biogenic amines in the diencephalic region in relation to other brain regions" [114].

In any case, and leaving aside Descartes philosophical considerations in relation to the soul, the Cartesian description of sensory perception and the role played by the epiphysis are not so different, in essence, from what we know today, as Descartes presents the *conarium* to us as a signal "transducer," as a center of sensory integration and relation with the outside world. And this requires the harmonious cooperation of the "animal spirits," also by way of a sort of hormonal agents in the current physiological terminology. In this sense, today we know a "retina-epiphyseal" pathway, proposed by Moore [115], the axis on which the relationship between the pineal gland and the exterior world is based, and we know that this body is a "neuroendocrine transducer" [84] that converts external stimuli in hormonal responses (especially melatonin production). These hormones act on a variety of target organs, which, in turn, modify, through a feedback mechanism, the glandular response [112]. Thus, we can interpret the Cartesian hypotheses as a metaphorical progress (and even poetic) of the current scientific reality in relation to the pineal gland. That is why Descartes, together with the discovery by Lerner, occupies the two priority positions in the history of pinealogy, from which it is very difficult to evade.

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Bibliometric Study of Scientific Research on Melatonin During the Last 25 Years

2

Francisco López-Muñoz, Francisco J. Povedano, and Cecilio Álamo

2.1 Introduction

Throughout history, pineal gland has become an anatomical enigma, and metaphysical and spiritual interpretations of its functional role have prevailed. In fact, René Descartes, one of the most prestigious philosophers in history, postulated the supremacy of this body over any other anatomic organ and considered this organ as the seat of the human soul. But from this moment until the end of the 1950s of the twentieth century, the pineal gland was considered a vestigial organ without any defined physiological role, and it generated little interest in the scientific community. A series of breakthrough findings, mainly the discovery and isolation of melatonin (N-acetyl-5-methoxytryptamine) by the group led by Aaron B. Lerner in 1958, enabled the development of a new research field around the main hormone of the pineal gland (to deepen the historical development of the pineal gland, see [23, 24] and Chap. 1 of this book). During the last five decades, scientific research on melatonin and pineal gland has undergone an impressive expansion that has translated into an extraordinary increase in the scientific literature that covers all of its different aspects. The aim of this chapter is to analyze this literature through bibliometric tools.

Bibliometric studies, in spite of their methodological limitations, are useful tools for assessing the evolution of a given scientific activity, particularly discipline or subject, both from the historical and sociological viewpoints [2], since they permit analysis of the growth, size, and distribution of scientific literature on the topic in question during a given time period. Our group has studied, using a bibliometric approach, the evolution of scientific literature in psychiatry by specific research groups, on different psychiatric disorders, on aspects related to the discipline, and on specific therapeutic tools in the field of psychopharmacology [16, 18–22, 25–27], including the evolution of publications on the pineal gland and melatonin during the period 1966–1994, to detail its distribution and to interpret the changes that have affected its development [17]. The present bibliometric study extends this analysis until 2014.

F. López-Muñoz (✉)
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal
e-mail: francisco.lopez.munoz@gmail.com; flopez@ucjc.edu

F.J. Povedano
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

C. Álamo
Department of Biomedical Sciences (Pharmacology Area), Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain

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2.2 Method

2.2.1 Data Sources

In the present bibliometric study, we have used the databases MEDLINE (Index Medicus, US National Library of Medicine, Bethesda, MD, USA) and Excerpta Medica (EMBASE) (Elsevier Science Publishers, Amsterdam, Netherlands), considered to be the most exhaustive within the biomedical field. These databases are integrated in a single platform called OVID (Ovid Technologies Inc., New York, USA). For some specific sub-analysis, SCOPUS database (Elsevier BV, The Netherlands), which includes 55 million records, 21,915 titles, and 5,000 publishers (scientific journals, books, and conference proceedings), has also been used.

Using remote-download techniques, we selected documents that contained, in the TI (title) section, the descriptors *pineal**, *epiphys**, *melatonin**, or *agomelatin**, always confining the year of publication from the first document that appears in the database (pineal 1900, melatonin 1958, and agomelatine 2002) until the year 2014. Within this repertoire, we created several subgroups of documents referring to pineal gland, melatonin, and agomelatine. This study took into account all original articles, brief reports, reviews, editorials, letters to the editor, and so on; it was also made sure that the duplicated documents were eliminated. In this regard, the OVID platform used in the search has the useful feature of permitting the elimination of those documents that may be duplicated in the two databases (MEDLINE and EMBASE).

2.2.2 Bibliometric Indicators

Among the bibliometric indicators of production, we applied Price's law [29]. This law, undoubtedly the most widely used indicator for the analysis of productivity in a specific discipline or a particular country, takes into account an essential feature of scientific production, which is its exponential growth. In order to assess whether the growth of scientific production in this field follows Price's law of exponential growth, we carried out a linear adjustment of the data obtained and another adjustment to an exponential curve.

Other quantities related to growth are doubling time and annual growth rate. The first is the amount of time required for the subject matter to double its production; the annual growth rate represents how the magnitude has grown over the previous year, expressed as a percentage. The equation that calculates the doubling time (D) is represented by the following expression: $D = \mathrm{Ln}2/b$. Here, b represents the constant that relates the rate of growth to the size of the science already acquired. To calculate the annual growth rate, we used the following equation: $R = 100\,(e^b - 1)$.

As an indicator of the publications' repercussion, we used the impact factor (IF). This indicator, developed by the Institute for Scientific Information (Philadelphia, PA, USA), is published annually in the Journal Citation Reports (JCR) section of the Science Citation Index (SCI). The IF of a journal is calculated on the basis of the number of times the journal is cited in the source journals of the SCI during the two previous years and the total number of articles published by that journal in those 2 years. The JCR lists scientific journals by specific areas, ascribing to each of them their corresponding IF and establishing a ranking of "prestige" [8]. In this study, we used the IF data published in the JCR of 2014.

Another indicator included in the present analysis was the national participation index (PaI) in overall scientific production on this field. The PaI reflects the quotient between the number of documents generated by a given country and the total number of documents obtained in the repertoire.

Regarding investigators' productivity, Lotka's law [28] intents to calculate the number of expected authors for a given number of papers. This law is expressed as $\mathrm{An} = Kn^{-b}, n = 1, 2, 3, \ldots$, where An represents the probability that an author produces n publications on a subject, while K and b are parameters to estimate depending on the data. According to this law, if the studied period is long enough, and the bibliographic search is as complete as possible, "the number of authors that publish n papers is inversely proportional to n^2."

As a bibliometric indicator of the dispersion of the scientific information, Bradford's law [3] has been applied. According to this law, in the scientific production of a given area, there is a core of selected journals that are more widely used by the investigators and several groups or zones (Bradford's zones) which include the same number of journals as the core. Thus, the number of journals in the different Bradford's zones would be *1*, *n*, n^2,…. This model allows one to determine which journals are more widely used by a scientific community working in a specific field. The most common way to represent this law is through a semilogarithmic plot, which represents the calculated number of articles, $R(r)$, versus the cumulative number of journals, r. In this semilogarithmic diagram, the logarithm of the cumulative number of journals is used as the abscissa and the cumulative number of articles is used as the ordinate. In this way, once the data are graphed, the sorting of articles is distributed into approximately three equal parts. One is the nucleus or core, and the other two are the peripheral zones (linear zone). In the core, the number of articles increases slowly, giving rise to a curve, defined as the Groos droop [9]. This model allows the identification of the journals most widely used or with greatest weight in a given field of scientific output.

Other indicators that have been included are participation index (PaI) of the diverse countries in the global production, number of authors/paper index, and author's productivity index (PI). PI allows the establishment of three levels of productivity: PI = 0 (transience index, authors with a single paper), 0 < PI < 1 (those authors that published between two and nine papers), and PI ≥ 1 (very productive authors, with ten or more papers).

2.2.3 Document Allocation

For a proper interpretation of the results, all the selected documents were divided into five main groups: the first group, named TOTAL, included all the papers which included the descriptors *pineal**, *epiphys**, *melatonin**, or *agomelatin** in their title (period 1900–2014); the second group (PINEAL) included the papers with the descriptors *pineal** or *epiphys** in TI section (period 199–2014); the third group, named MELATONIN, contained all the documents which included in their title the descriptor *melatonin** (period 1958–2014); the fourth group PINEAL + MELATONIN included the papers with descriptors *pineal**, *epiphys**, or *melatonin** in TI (period 1900–2014); and the fifth group included only the documents on *agomelatin**, named AGOMELATINE (2002–2014).

2.3 Results

In our repertoire, 30,617 documents about diverse aspects of the pineal gland, its main secretory product (melatonin), or therapeutic melatonergic agents (agomelatine) have been included. The number of documents assigned to the subset PINEAL was 17,046, while 14,967 papers corresponded to the subset MELATONIN and 365 to the subset AGOMELATINE.

A notable increase in the number of publications on the pineal gland was observed (Fig. 2.1). The mathematical adjustment to an exponential curve ($y = 6E\text{-}58e^{0.0693x}$), as shown in Fig. 2.1, clearly confirms that this dataset follows Price's law, with a correlation coefficient $r = 0.9528$, which indicates that only a 9.2 % of variability remains unexplained by this adjustment, and with an average of annual increase of 24.93 %. In contrast, the linear adjustment of the values $y = 8.712x - 16{,}786$ produces a $r = 0.9139$ and, thus, a percentage of unexplained variability of 16.47 %. In subsets PINEAL and AGOMELATINE, but not in subset MELATONIN, also the fulfillment of the Price's law is observed; $y = 1E\text{-}49e^{0.0595x}$ ($r = 0.9142$) versus $y = 3.885x - 7455.2$ ($r = 0.8628$) in PINEAL subset, with an average of annual increase of 22.65 % (Fig. 2.2), and $y = 1E\text{-}83e^{0.0984x}$ ($r = 0.9349$) versus $y = 13.837x - 27{,}219$ ($r = 0.9552$) in MELATONIN subset, with an average of annual increase of 25.57 % (Fig. 2.3). In AGOMELATINE subset the data are $y = 8E\text{-}247e^{0.2836x}$ ($r = 0.9634$) versus $y = 6.0659x - 12{,}152$ ($r = 0.9289$), with an average of annual increase of 46.77 % (Fig. 2.4).

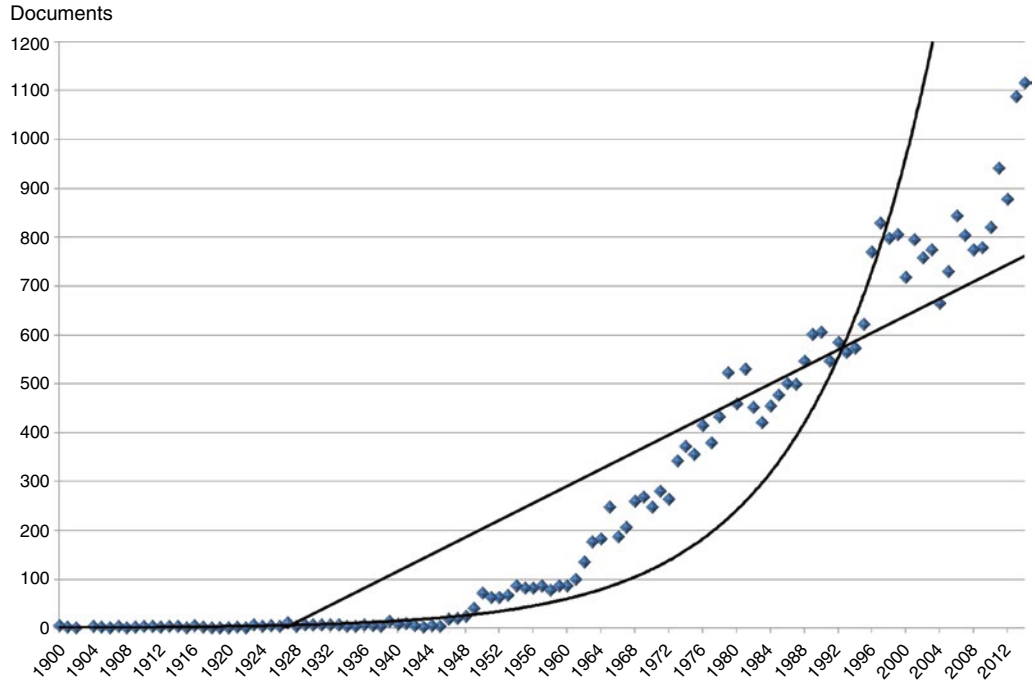

Fig. 2.1 Evolution in the number of international scientific publications on the pineal gland, melatonin, and agomelatine (TOTAL group). A linear adjustment of data and another adjustment to an exponential curve have been performed, to verify whether the analyzed production fits Price's law

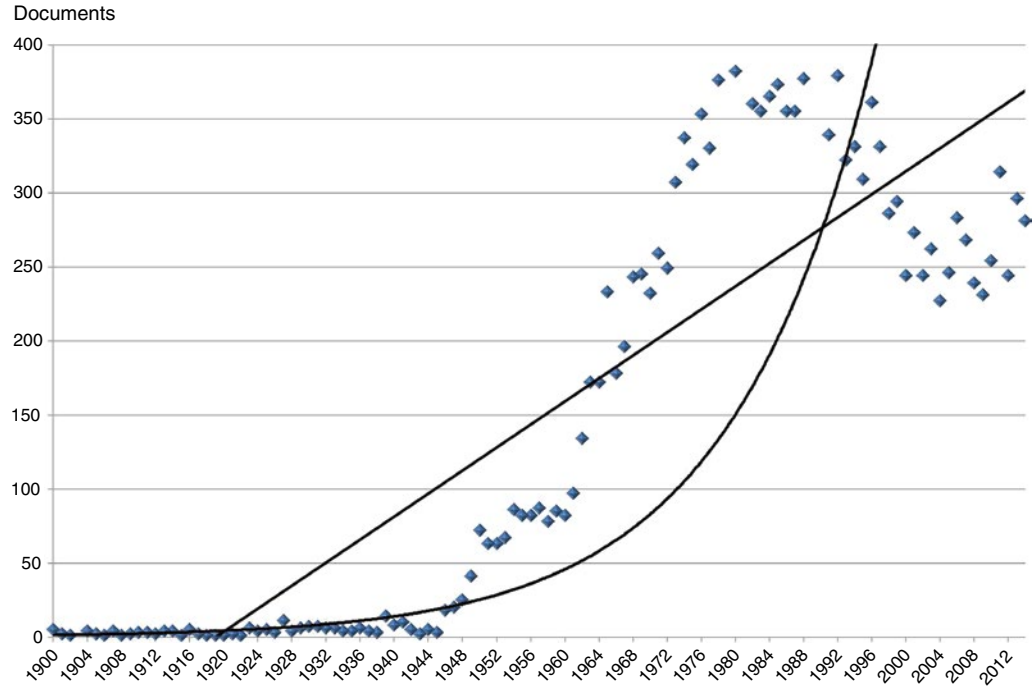

Fig. 2.2 Evolution in the number of documents for the PINEAL group (period 1990–2014). A linear adjustment of data and another adjustment to an exponential curve have been performed, to verify whether the analyzed production fits Price's law

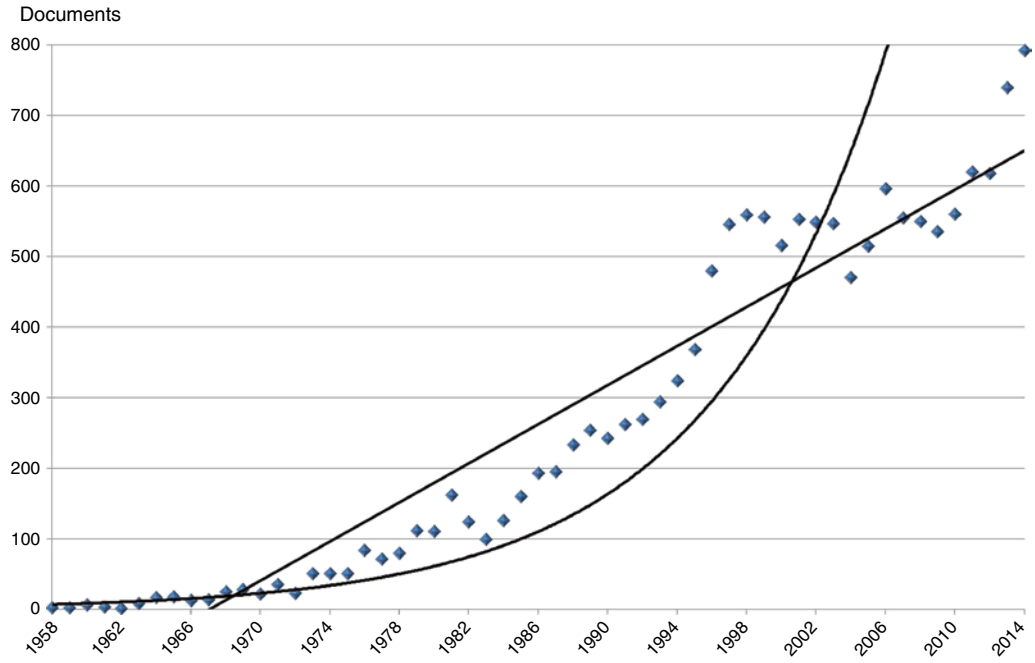

Fig. 2.3 Evolution in the number of documents for the MELATONIN group (period 1958–2014). A linear adjustment of data and another adjustment to an exponential curve have been performed, to verify whether the analyzed production fits Price's law

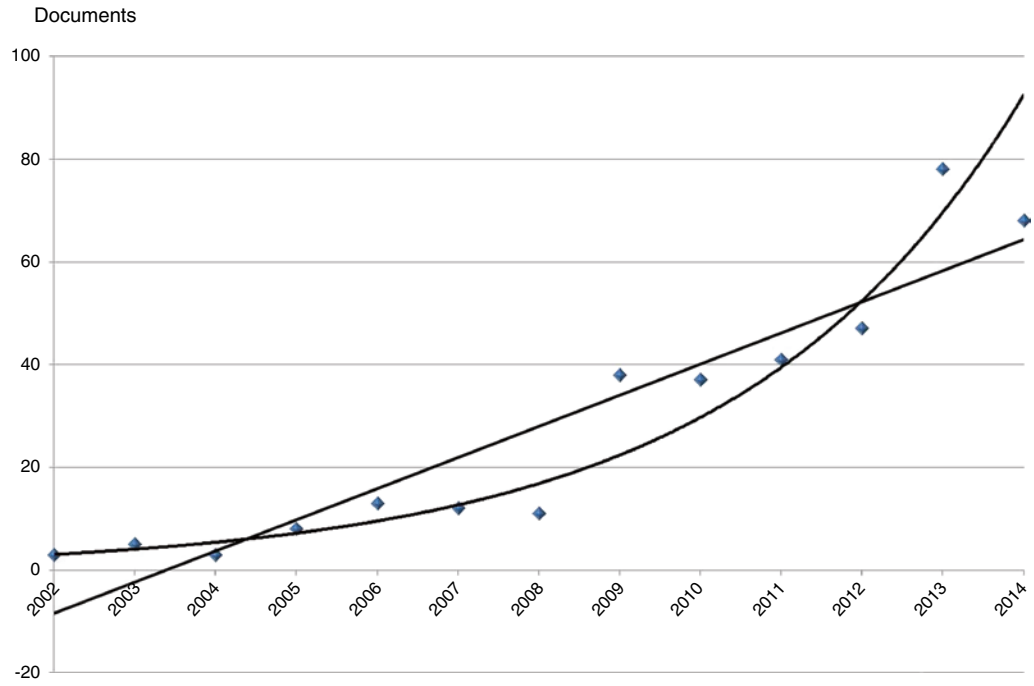

Fig. 2.4 Evolution in the number of documents for the AGOMELATINE group (period 2002–2014). A linear adjustment of data and another adjustment to an exponential curve have been performed, to verify whether the analyzed production fits Price's law

As shown in Fig. 2.3, the increase in the number of papers on melatonin is impressive, above all from the beginning of the 1980s, overcoming as of 1991; the number of publications stabilizes in the late 1990s and grows from year 2010. In contrast, the increment in the subset that we named PINEAL is stabilized in the 1980s, and a manifest decline is observed since the early 1990s (Fig. 2.2). The scarce path of the investigation in relation to agomelatine makes it impossible to properly analyze these aspects. Figure 2.5 further illustrates this issue. There is an important accumulative increase in the scientific production in each decade over the preceding one in the subset MELATONIN, mainly in the 1990s (133.35 %), while, in contrast, in the group PINEAL since 1980, a decrease is observed in scientific production (−20.93 % in the decade 2001/2010). In the TOTAL group we see rates of increment in the last three decades ranging between 14 and 33 % (Fig. 2.6).

To calculate the doubling time (D) ($y = 10.048e^{0.0764x}$; $r = 0.9634$), the temporary production of publications is depicted in the scatter plot (Fig. 2.7): D = Ln2/b = 0.68904/0.0764 = 9 years. The doubling time (D) in PINEAL subset is 9.5 years and 5.1 years and 1.82 years in the subsets MELATONIN and AGOMELATINE, respectively.

Table 2.1 indicates the journals more widely used for the diffusion of scientific literature on the pineal gland, both as a total, and divided according to the three subsets we analyzed, together with their respective impact factor (IF) according to the JCR of 2014. A total of 30,617 journals have been included (17,046 in the subset PINEAL, 14,967 in the subset MELATONIN, and 181 in the subset AGOMELATINE). The journal most used is *Journal of Pineal Research* (the core or first Bradford's zone), with 2,168 papers, followed, at a great distance, by *Brain Research* (369), *Neuroscience Letters* (335), or *Endocrinology* (332). For example, we have applied the models of Bradford and Gross to the subset AGOMELATINE, since this is a topic of

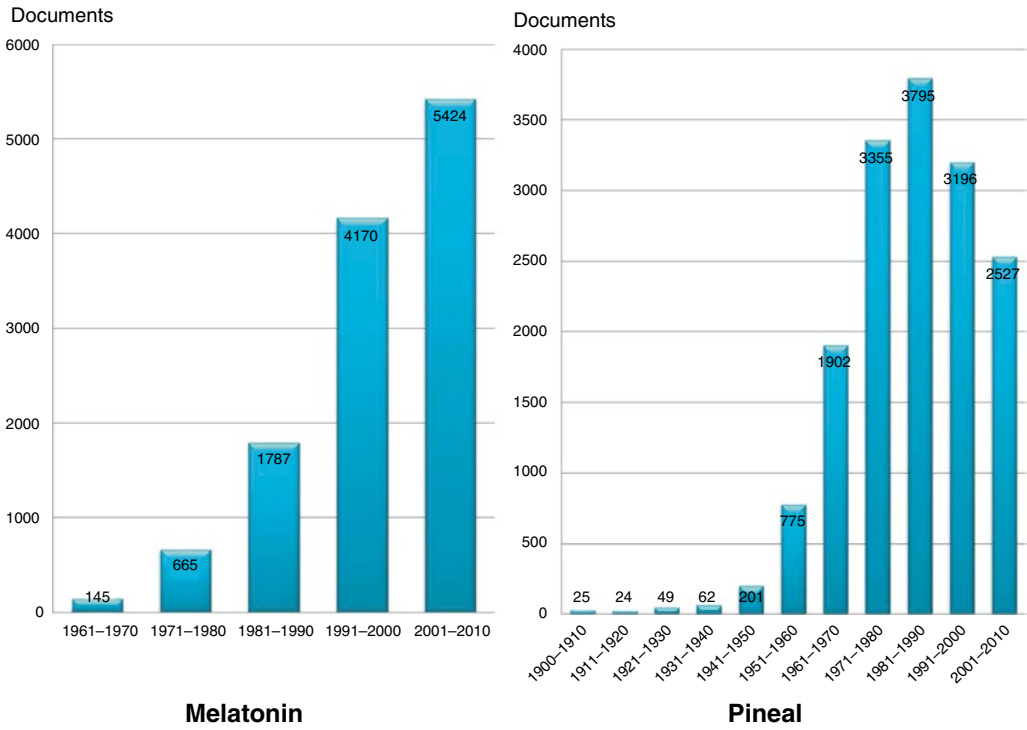

Fig. 2.5 Scientific production by decades for the MELATONIN and PINEAL groups

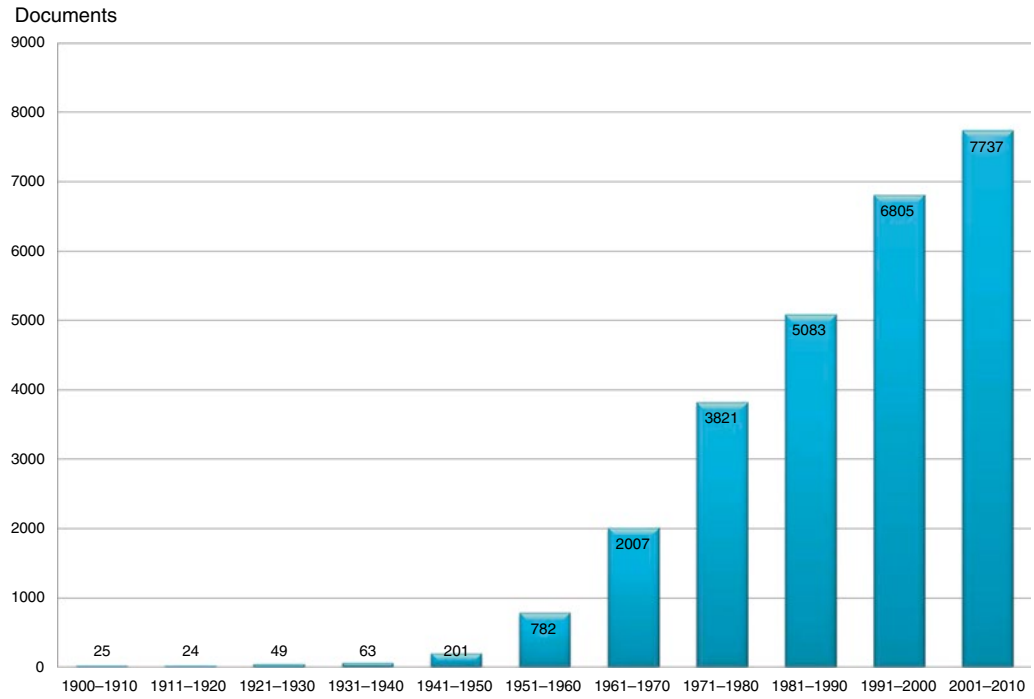

Fig. 2.6 Scientific production by decades of our repertoire (pineal gland, melatonin, and agomelatine)

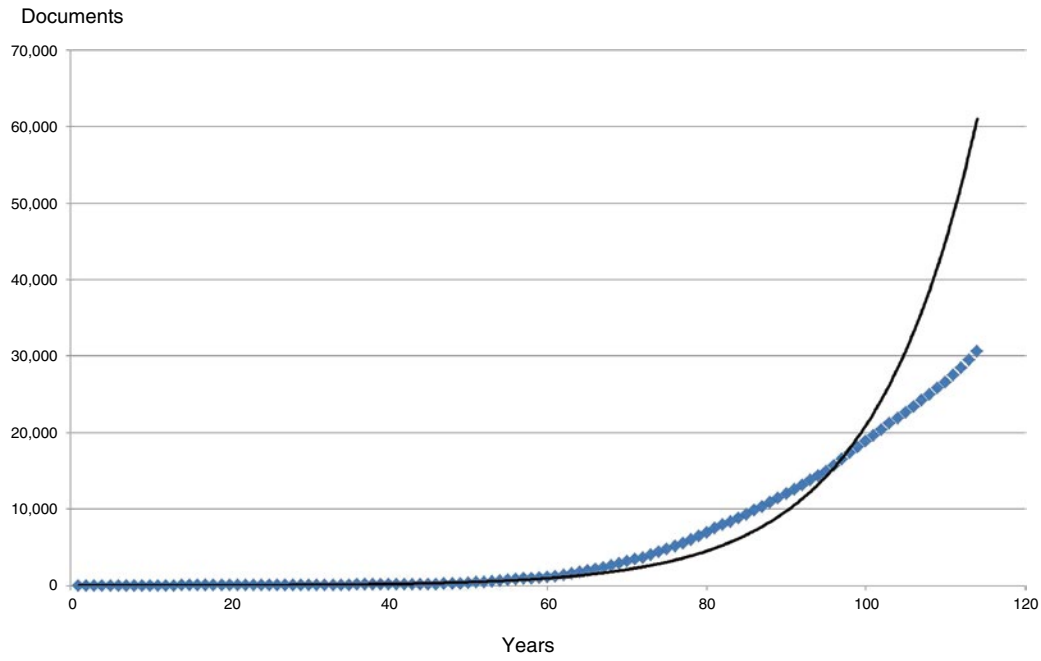

Fig. 2.7 Temporal evolution of scientific literature output in pineal gland, melatonin, and agomelatine

Table 2.1 Main journals used for diffusion of research papers on pineal gland

	Total			Pineal			Melatonin			Agomelatine		
	Journal	N° art.	IF	Journal	N° art.	IF	Journal	N° art.	IF	Journal	N° art.	IF
1	*J Pineal Res*	2,168	9.600	*J Pineal Res*	509	9.600	*J Pineal Res*	1,827	9.600	*Eur Neuropsychopharmacol*	16	4.369
2	*Brain Res*	369	2.843	*J Pediatr Orthopaed*	327	1.474	*Neurosci Lett*	237	2.030	*Psychopharmakotherapie*	14	–
3	*Neurosci Lett*	335	2.030	*Cell Tissue Res*	260	3.565	*Brain Res*	210	2.843	*Psychopharmacology*	14	3.875
4	*Endocrinology*	332	4.503	*Clin Orthopaed Relat Res*	241	2.765	*Neuroendocrinol Lett*	204	0.799	*J Clin Psychopharmacol*	12	3.243
5	*J Pediatr Orthopaed*	330	1.474	*Brain Res*	217	2.843	*Life Sci*	203	2.702	*Int J Neuropsychopharmacol*	10	2.456
6	*Life Sci*	320	2.702	*Endocrinology*	201	4.503	*Gen Comp Endocrinol*	176	2.470	*Int Clin Psychopharmacol*	10	4.009
7	*Neuroendocrinol Lett*	289	0.799	*J Bone Joint Surg Am*	191	5.280	*Endocrinology*	174	4.503	*Neuropsychiatr Dis Treat*	9	1.741
8	*Cell Tissue Res*	272	3.565	*J Neurochem*	191	4.281	*Chronobiol Int*	151	3.343	*J Clin Psychiatry*	7	5.498
9	*Neuroendocrinology*	264	4.373	*J Neural Transm*	168	2.402	*Neuroendocrinology*	143	4.373	*CNS Drugs*	6	5.113
10	*Gen Comp Endocrinol*	260	2.470	*Neuroendocrinology*	168	4.373	*J Clin Endocrinol Metab*	129	6.209	*Clin Neuropharmacoly*	6	2.009

IF impact factor (Journal Citation Report, Thompson Reuters, 2014)

Table 2.2 Distribution of the journals of AGOMELATINE subset in Bradford's zones

	N° of journals	% of journals	N° of articles	% of articles	Bradford's factor
Nuclear zone	16	8.84 %	128	35.07 %	
1° Zone	47	25.97 %	119	32.60 %	2.94
2° Zone	118	65.19 %	118	32.33 %	2.51
Total	181	100.00 %	365	100.00 %	2.73

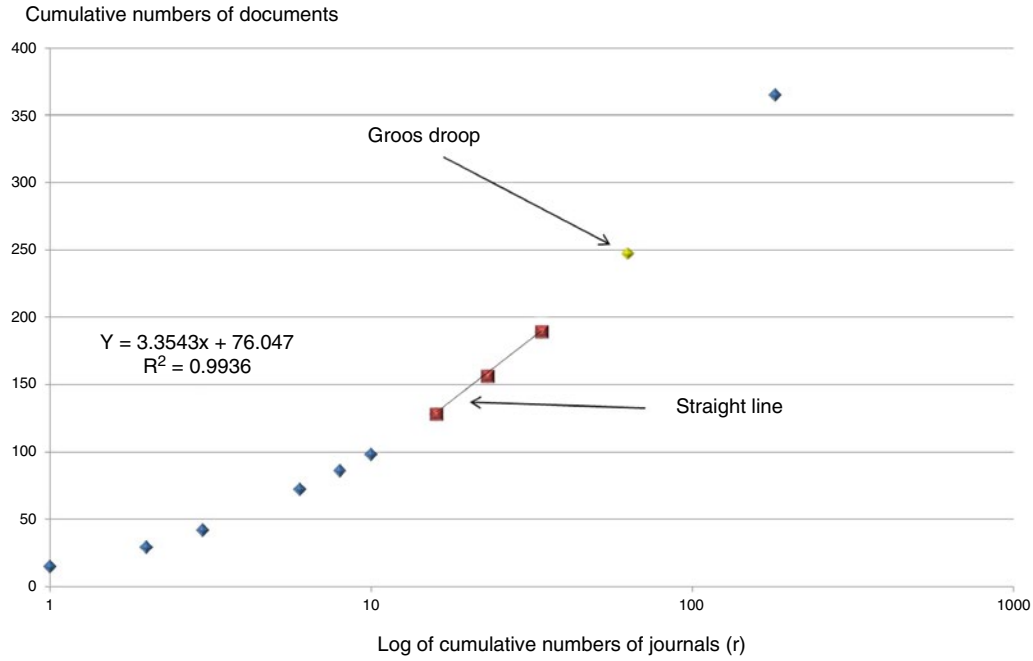

Fig. 2.8 Bradford's distribution of global data

great relevance and scientific growth in the last decade (Table 2.2). The first zone or core is composed of 16 journals, accounting for 35.07 % of all papers published. Table 2.5 shows the division by Bradford zones, the number of journals and articles, and the multiplication factor. The graphical distribution of Bradford's zones for the entire set of journals is represented in Fig. 2.8. It should be taken into account that it is a semilogarithmic diagram that represents the cumulative number of articles against the cumulative number of journals (r). The straight zone was fitted to the following equation, $y = 3.3543ln(x) + 76.047$, with a high correlation coefficient ($r = 0.9967$).

Another widely accepted bibliometric indicator is the one formulated in Lotka's law on the productivity of investigators. In summary, this law would be observed when less than one tenth of the authors are responsible of one third of the papers. The productivity index (PI; logarithm of the n values for each author) led to the establishment of three accepted levels of productivity. Continuing with the example of AGOMELATINE subset, there are four authors that have a PI ≥ 1 and that can be considered as large producers, i.e., they have published ten or more papers in this field. The elite of the authors, according to Price, is the square root of the total thereof (1,057), i.e., 32.5, which corresponds to the authors with five or more articles. In clear contrast, 838 authors (79.28 % of the total) have produced only one paper (PI = 0). In Table 2.3, the 20 most active authors in this dataset are listed.

Regarding the geographical distribution of the research production in this area (Table 2.4), the USA is the clear leader (PaI = 24.11), followed

Table 2.3 Most productive authors

	Total		Pineal gland		Melatonin		Agomelatine	
	Author	N° articles	Author	N° articles	Author	N° articles	Author	N° articles
1	Reiter RJ	1,086	Reiter RJ	423	Reiter RJ	822	Mocaer E	37
2	Klein DC	260	Klein DC	217	Tan DX	213	Gabriel C	19
3	Cardinali DP	256	Pevet P	154	Cardinali DP	181	di Giannantonio M	11
4	Pevet P	246	Vollrath L	140	Arendt J	167	Martinotti G	11
5	Tan DX	216	Cardinali DP	106	Pevet P	147	de Berardis D	9
6	Arendt J	176	Moller M	87	Delagrange P	130	Kennedy SH	9
7	Vollrath L	148	Karasek M	86	Brown GM	118	Guilleminault C	8
8	Guerrero JM	144	Ho AK	85	Pang SF	117	de Bodinat C	7
9	Delagrange P	135	Korf HW	84	Acuña-Castroviejo D	115	Delagrange P	7
10	Karasek M	133	Quay WB	83	Guerrero JM	114	Hale A	7
11	Lissoni P	131	Collin JP	81	Lissoni P	114	Kasper S	7
12	Brown GM	131	Arushanian EB	77	Zisapel N	95	Serroni N	7
13	Vaughan MK	124	Vaughan MK	74	Manchester LC	92	de Berardis D	6
14	Pang SF	122	Lissoni P	73	Fraschini F	91	Emsley R	6
15	Acuña-Castroviejo D	118	Chik CL	73	Kennaway DJ	84	Fornaro M	6
16	Arushanyan EB	118	Ebels I	63	Hardeland R	84	Millan MJ	6
17	Korf HW	113	Khavinson VK	61	Escames G	83	Moschetta FS	6
18	Sugden D	104	Damian E	61	Wetterberg L	82	Racagni G	6
19	Fraschini F	104	Weller JL	59	Claustrat B	80	Fornaro M	6
20	Wurtman RJ	101	McNulty JA	57	Dobocovich ML	78	Kasper S	6

Table 2.4 Geographical distribution of research production

	Total			Pineal			Melatonin			Agomelatine		
	Country	N° art.	PaI	Country	N° art.	PaI	Country	N° art.	PaI	Country	N° art.	PaI
1	USA	6,784	24.11	USA	3,745	27.98	USA	3,480	21.66	France	84	18.88
2	Germany	1,887	6.71	Germany	1,207	9.02	Spain	1,075	6.69	Italy	47	10.56
3	Japan	1,626	5.78	Japan	1,015	7.58	France	876	5.45	USA	40	8.99
4	France	1,623	5.77	France	824	6.16	UK	831	5.17	Germany	35	7.87
5	Spain	1,499	5.33	UK	645	4.82	Italy	803	5.00	UK	31	6,97
6	UK	1,448	5.15	Spain	491	3.67	Germany	773	4.81	Canada	23	5.17
7	Italy	1,237	4.40	Italy	465	3.47	Turkey	734	4.57	Spain	21	4.72
8	Canada	887	3.15	Canada	420	3.14	Japan	712	4.43	India	17	3.82
9	Turkey	886	3.15	India	339	2.53	China	686	4.27	Turkey	12	2.70
10	China	860	3.06	Poland	335	2.50	Poland	546	3.40	Switzerland	11	2.47
11	India	847	3.01	Netherlands	308	2.30	India	537	3.34	Russia	10	2.25
12	Poland	820	2.91	Switzerland	267	1.99	Canada	502	3.12	China	9	2.02
13	Australia	482	1.71	Sweden	218	1.63	Australia	333	2.07	Netherlands	9	2.02
14	Switzerland	481	1.71	Argentina	200	1.49	Argentina	288	1.79	Austria	8	1.80
15	Netherlands	479	1.70	China	191	1.43	Brazil	283	1.76	South Africa	8	1.80
16	Argentina	452	1.61	Turkey	189	1.41	Israel	246	1.53	Australia	7	1.57
17	Brazil	429	1.52	Australia	182	1.36	Switzerland	235	1.46	Argentina	7	1.57
18	Sweden	386	1.37	Brazil	167	1.25	South Korea	226	1.41	Poland	7	1.57
19	Israel	377	1.34	Denmark	164	1.23	Russia	219	1.36	Brazil	6	1.35
20	Russia	323	1.15	Hungary	157	1.17	Netherlands	191	1.19	Finland	6	1.35
Total		30,617			17,046			14,967			365	

PaI participation index

Table 2.5 Most productive institutions (TOTAL group)

	Institution	Documents	PaI
1	University of Texas at San Antonio	1,002	3.27
2	Uniwersytet Medyczny w Lodzi	311	1.02
3	Universidade de Sao Paulo – USP	217	0.71
4	National Institute of Child Health and Human Development	213	0.69
5	Universidad Complutense de Madrid	210	0.68
6	Université de Strasbourg	202	0.66
7	University of Surrey	195	0.64
8	Johannes Gutenberg Universitat Mainz	187	0.61
9	Facultad de Medicina de la Universidad de Buenos Aires	169	0.55
10	National Institute of Mental Health	165	0.54
11	CNRS Centre national de la Recherche Scientifique	159	0.52
12	Servier	153	0.50
13	Universita degli Studi di Milano	149	0.49
14	Universidad de Sevilla	138	0.45
15	The University of Hong Kong	136	0.44
16	Banaras Hindu University	134	0.44
17	King's College London	133	0.43
18	INSERM	131	0.43
19	VA Medical Center	129	0.42
20	Netherlands Institute for Neuroscience NIN-KNAW	123	0.40
Total		30,617	

PaI participation index

by Germany (PaI = 6.71), Japan (PaI = 5.78), France (PaI = 5.77), and Spain (PaI = 5.33). However, if the whole sample is split in the two study subsets, it is observed that in the MELATONIN group, Spain (ranked 2nd, PaI = 6.69) and the UK (ranked 4th, PaI = 5.17) stand out, while in the PINEAL group, the UK ranked 5th (PaI = 4.82). In the AGOMELATINE subset, France tops the ranking (PaI = 18.88), followed by Italy (PaI = 10.56). The most productive institutions in this field are shown in Table 2.5. This distribution of institutions is kept in PINEAL and MELATONIN groups, but not in the AGOMELATINE group, whose ranking is headed by Servier, the laboratory that developed the molecule (PaI = 14.79).

The most common language of the publications is English (82.66 %), followed by German (3.80 %), French (2.84 %), and Russian (2.49 %) (Fig. 2.9). Meanwhile, Fig. 2.10 shows the constitution of the repertoire depending on the type of document. Original articles accounted for 82.24 % of the same and reviews 5.66 %. Finally, analysis of subject areas shows that 40.21 % of the documents fits in "Medicine" area (17,581 documents); 27.87 % in "Biochemistry, Genetics, and Molecular Biology" (12,188 documents); 11.08 % in "Neuroscience" (4,846 documents); and 6.19 % in "Pharmacology, Toxicology, and Pharmaceutics" (2,708 documents) (Fig. 2.11). In subset MELATONIN the first two positions are reversed (34.16 % of documents in "Biochemistry, Genetics, and Molecular Biology" and 27.48 % in "Medicine"), and in subset AGOMELATINE, the "Pharmacology, Toxicology, and Pharmaceutics" area is second in the ranking (23.19 % of documents).

2.4 Discussion

Bibliometric studies constitute useful tools for assessing the social and scientific importance of a given discipline over a specific time period. The term "bibliometrics" was introduced by Pritchard in 1969, to define the application of mathematical

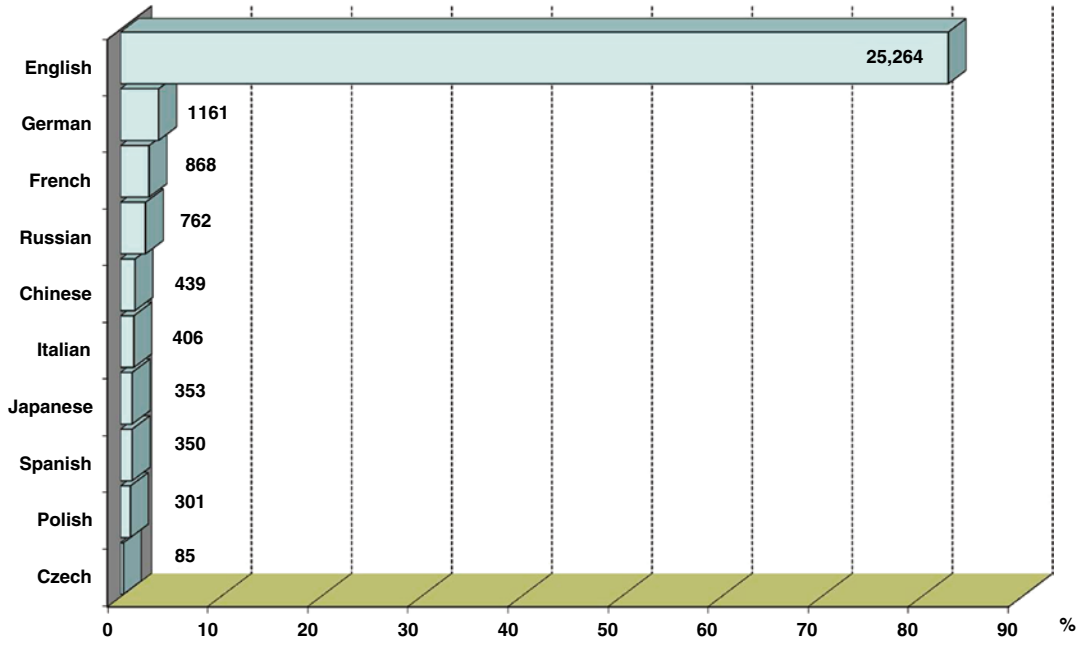

Fig. 2.9 Language of the publications

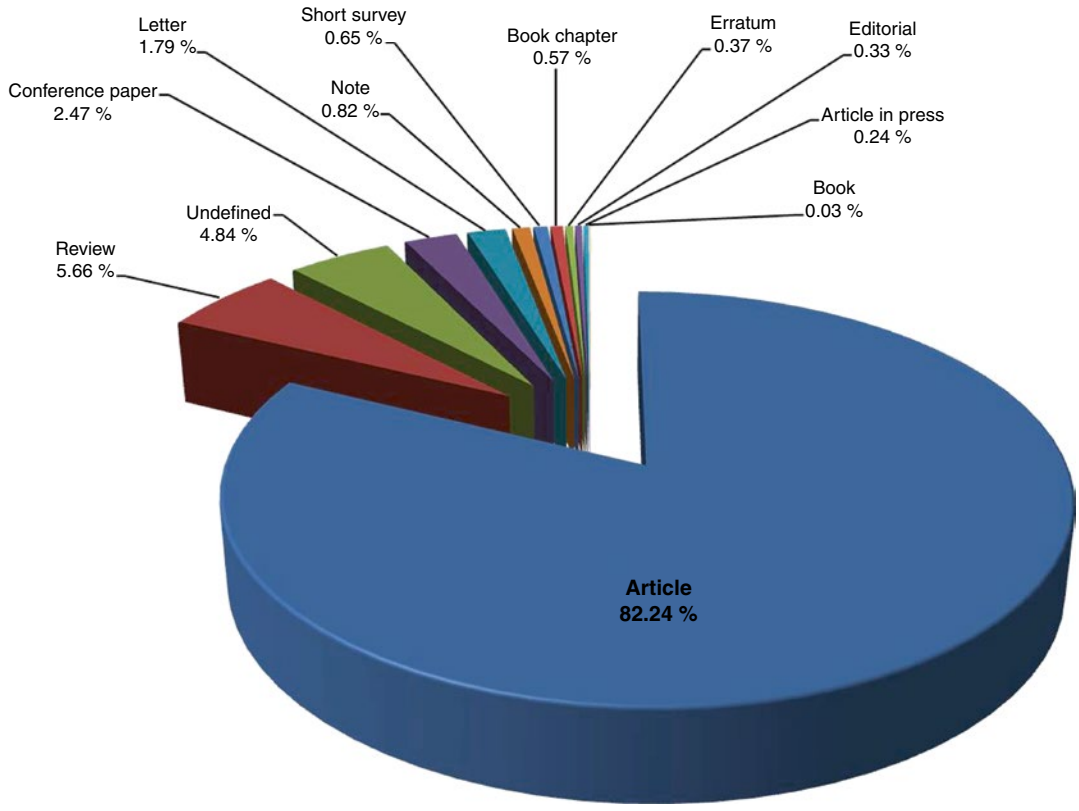

Fig. 2.10 Document types

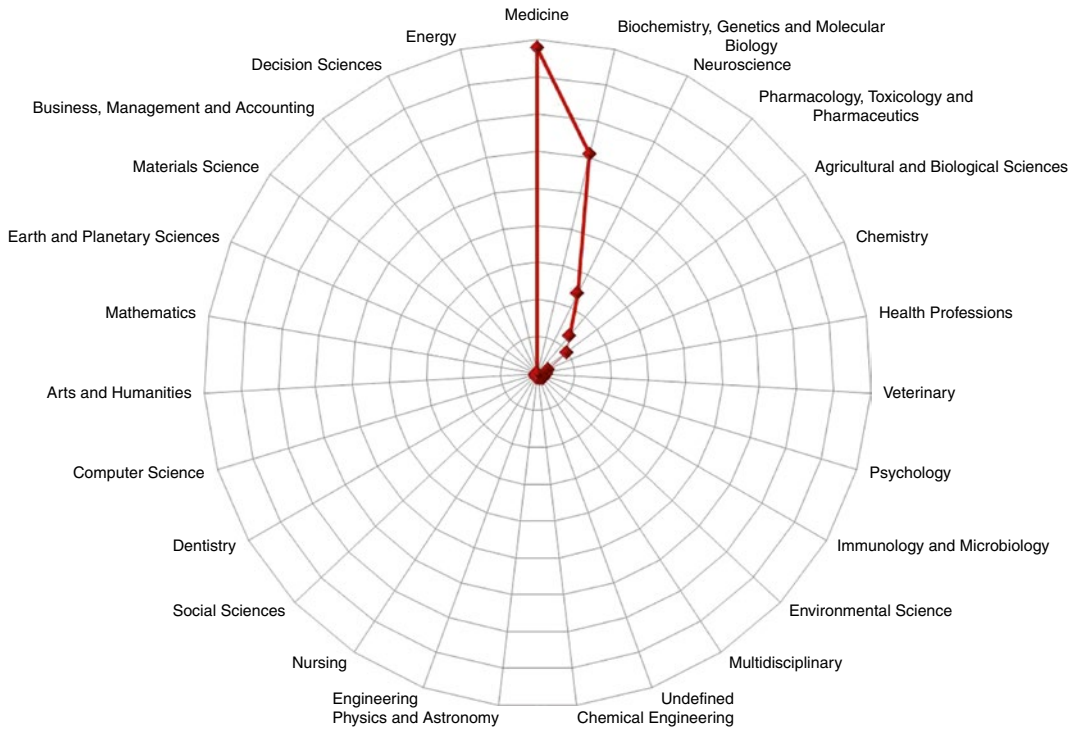

Fig. 2.11 Subject areas of total repertoire

and statistical methods to the process of dissemination of written communication in the area of scientific disciplines, using quantitative analysis of the different aspects of this type of communication [30]. These analyses give an overview of the growth, size, and distribution of the scientific literature related to a particular discipline and the study of the evolution of not only the biomedical specialty, field of specialization, or issue in question but also the scientific production of an institution, country, author, or research group [2]. The design of the present analysis permitted a global assessment on the growth of scientific publications on melatonin and pineal gland since 1900.

There were five main scientific events that made possible the impressive advance in this research field from the mid-1960s: the publication in 1954 of the book entitled *The Pineal Gland* by Kitay and Altschule [14], the isolation of melatonin in 1958 by Lerner and collaborators [15], the finding that this endocrine organ is directly controlled by external environmental factors [7, 11, 31, 36], the onset of clinical trials with melatonin in different diseases since the beginning of the 1980s and the characterization of melatonin receptors and the definition of their pharmacological profiles [6], and finally the development of new therapeutic tools of melatonergic action during the first decade of the twenty-first century, as agomelatine and ramelteon (see different chapters of this book). The appearance of the first three breakthroughs in a short period of time (1954–1965) initiated the basis of the current scientific knowledge of the pineal gland endocrinology that changed the old concept of the pineal as a vestigial organ without physiological importance to one that considered it to be a "neuroendocrine transducer" [35].

In a task that required several years, Julian Kitay and Mark D. Altschule reviewed all the controversial scientific literature published on the pineal gland until 1953 (a total of 1,762 original works), and they published in 1954 the book entitled *The Pineal Gland* [14]. From their critical review of the state of the art at that time, these authors concluded three probable functions of the

pineal organ: (1) control of the gonadal function, (2) participation in the chromatic response of the skin of lower vertebrates to light changes, and (3) a potential link with behavior disorders. About that time, Lerner began his studies to identify the factor responsible for blanching the skin in amphibians, which concluded in 1958 with the isolation, from 250,000 bovine pineal glands, of an indolamine extract that he named "melatonin" [15]. This discovery marks one of the milestones in the history of pineal research because finally, a chemical substance, synthesized and released by the pineal gland, was identified which demonstrated an endocrine function. During the following years, several authors [1, 7, 32, 36–38] showed that melatonin synthesis in mammals is under control of environmental light through the sympathetic innervation originating in the superior cervical ganglia [13]. In 1965, two papers were published where the neuroendocrine nature of the pineal gland was definitively confirmed. Hoffman and Reiter [11] showed that the gonadal changes in rodents exposed to dark conditions totally disappeared after pinealectomy. Wurtman and Axelrod [35] introduced the term "neuroendocrine transducer" to refer to the pineal gland. With this concept, these authors tried to explain the principle of the pineal physiology, i.e., the transformation of external luminic information that reach the gland from the retina and through a nervous pathway to an endocrine response consisting in the synthesis and release of the hormone melatonin.

By this time, a new discipline stimulated by the publication in 1958 of the book entitled *Die physiologische Uhr* (*The Physiologic Clock*) by Erwin Bünning [4] was also developing. This discipline called "chronobiology" rapidly consolidated during the 1960s, became firmly linked with pineal physiology. The development of chronobiology has been a powerful support to the scientific expansion of the study of the pineal gland.

Once these initial advances occurred, pinealogy came to age. In Fig. 2.1, it can be observed that after the mathematical adjustment, the number of publications on the pineal gland and melatonin shows an exponential growth after 1966; this curve does not show the saturation phenomenon described by Price [29] in his theory on the expansion of the scientific literature. This phenomenon assumes a faster growth rate for science that for any other human activity and that its size would double every 10–15 years.

As it has been mentioned previously, there seems to be two well-defined periods in the growth of the scientific production with the junction around the year 1980. From this year, the increase in the number of publications is more pronounced. The first years of the decade of the 1980s provided the main facts that led to the maturity of pineal and melatonin research. In 1977, under the leadership of Professor Johannes Ariëns Kappers and Dr. Paul Pévet, the *European Pineal Study Group*, EPSG, was founded (today known as *European Pineal Society*, EPS), and its first meeting was held in Amsterdam in 1978. The scientific results of this conference are published in the issue number 52 of the prestigious series *Progress in Brain Research* (1979). Two years later, the most complete and comprehensive book on the pineal gland was published under the authorship of Professor Lutz Vollrath: *The Pineal Gland* [33]. In this work, all the scientific knowledge on this organ in all the animal species studied up to that time was reviewed. Probably, the final step in this development process was the launching in 1984 of a specific journal devoted to this field: *Journal of Pineal Research*, initially edited by Professors Russel J. Reiter, Wilbur B. Quay, and Michal Karasek. This journal constitutes the nucleus or first Bradford's zone in the analysis that we carried out on the dispersion of the scientific literature on pineal research.

The general growing tendency of pineal research, together with the absence of a "saturation point" in the growth of the scientific production on this matter, leads to encouraging horizons for this discipline. However, as it has been already pointed out, and after interpreting Figs. 2.2, 2.3, and 2.5, there are two clearly different periods in this process. Until 1980, the increment is mainly due to the papers included in the group PINEAL; in contrast, in the last 35 years, the increment in the number of publications is the result of the

large number of papers contained in the group MELATONIN. Thus, it seems that melatonin research (and its therapeutic implications) is currently the major prime mover in pineal research, overcoming all research on other aspects of the pineal gland. This fact confirms the natural evolution of biomedical research, where basic approaches to diverse disciplines are progressively substituted by more clinically oriented applications. Endocrinology initially and psychiatry and pharmacology later have taken over morphological and physiological disciplines that dominated pineal research during the 1960s and the 1970s.

Although the application of techniques of cellular and molecular biology including immunohistochemistry, tissue culture, in situ hybridization, or microinjection techniques [34] accounted an active research area in terms of in morphology and physiology until the late twentieth century (mainly the interrelation between the central nervous system and the gland), the present seems to be more oriented to the study of the systemic effects of the pineal secretory products and their derivatives and their clinical implications. Thus, new research areas in this field seem to be the relationship of the gland to reproductive function disorders; psychiatric disorders including depression, manic-depressive disease, and schizophrenia; sleep disorders; and alterations of the biological rhythms ("jet lag") or even cancer and degenerative diseases of aging are increasingly recognized (see different chapters of this book).

Another interesting quantitative aspect of this bibliometric analysis is the quality of the scientific production. We approached this problem with the evaluation of the repercussion index of the publications included in the dataset. The impact factor (IF) is a repercussion index that, in spite of its limitations and biases, such as underestimation of original papers when compared with reviews, higher scores for English language-edited journals, or the tendency to ignore certain areas of knowledge that only concern to a small number of investigators [8], is the most frequently used tool by the scientific community to evaluate the quality of research papers. It is notable the high number of journals with an IF > 2.5 included in the list of the most used ten journals (*Journal of Pineal Research*, *Brain Research*, *Endocrinology*, *Life Sciences*, *Cell and Tissue Research*, *Neuroendocrinology*) (Table 2.1).

Pineal research is a multidisciplinary field where very different areas of knowledge, both basic (morphology, pharmacology, biochemistry, physiology, etc.) and clinically oriented (endocrinology, neurology, neurosurgery, psychiatry, radiology, pharmacology, etc.) converge. As a result, the most widely used journals for publication by pineal investigators are those with multidisciplinary content (*Journal of Pineal Research*, *Brain Research*, *Life Sciences*, *Neuroscience Letters*), together with those related to endocrinology (*Neuroendocrinology*, *Endocrinology*, *Neuroendocrinology Letters*, *General and Comparative Endocrinology*, *Journal of Clinical Endocrinology and Metabolism*) and morphology (*Cell and Tissue Research*, *Journal of Neural Transmission*). As expected, in MELATONIN subset predominate the papers included in endocrine-related journals and in AGOMELATINE subset the psychopharmacology-related journals (see Table 2.1).

Team-working research and author collaborations are reflected in our repertoire. It is clear that a tendency exists toward the increment in the number of authors on scientific papers and that this effect is mainly due to a higher degree of collaboration between different investigators. This increase in scientific collaboration is not only the result of interdepartment support, which is essential given the high complexity and diversity of the current technology and methodology, but a notable increase in the collaboration of pineal research groups located in different countries is apparent.

Previous bibliometric studies have addressed limitations' characteristic of this sociometric approach [12]. Regarding this particular chapter, there are some main limitations. First, we might have excluded some papers on melatonin and pineal gland if the authors did not put our study inclusion descriptors in the titles or as key words. Second, it is clear that the papers that have been included in this work constitute a partial sample of

the global production in this discipline and that the scientific literature on melatonin and the pineal gland is considerably larger, but the boundaries that the bibliographic databases introduce determine the subsequent analysis. Third, the use of the SCI impact factor to determine the merit or quality of scientific contributions is debatable. The citation count applied in calculating the impact factor may not directly reflect the importance or quality of one study; on the contrary, it may only represent the topic of a given study being "more fashionable" or even "not yet mature" and/or "in need of more studies" [5, 10]. However, the well-known reputation of the journals included in the databases used and its wide coverage make for a representative sample of the international research on the pineal gland and melatonin. Despite the inherent limitations of bibliometric studies, we feel that, given the applied design, we have offered a comprehensive overview of the evolution of the international scientific production and research impact assessment on melatonin and pineal gland.

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Neuroimaging of the Pineal Gland: Focus on Primary Insomnia

3

J. M. Bumb and Ingo S. Nölte

Abbreviations

AA-NAT	Arylalkylamine N-acetyltransferase
aMT6s	6-Sulphatoxymelatonin
ASMT	Acetylserotonin methyltransferase
CT	Computed tomography
FLAIR	Fluid-attenuated inversion recovery
MPRAGE	Magnetization-prepared rapid gradient echo
MRI	Magnetic resonance imaging
PGV	Pineal gland volume
TrueFISP	True fast imaging with steady-state precession

3.1 Background

Anatomically, the pineal gland is part of the epithalamus, which again belongs to the diencephalon. The gland is localized at the dorsal wall of the third ventricle, vis-à-vis the superior colliculi of the quadrigeminal plate and between the cerebral hemispheres. The habenulae, denoted as the pedunculus of the gland, connect it to the thalamus.

Not only is it found in almost all mammals but also in a variety of other species. In some of these species as in amphibians, the pineal gland is connected with the parietal eye ("third eye," "pineal eye," photoreceptor) and regulates circadian rhythmicity and thermoregulation. Interestingly, it has been suggested that pineal gland volume (PGV) depends on (i) the degree of latitude (the more distant from the equator the larger) and (ii) on nocturnality and diurnality (smaller volume in owls and bigger volume in horse) [1].

In humans, the pineal gland arises out of the third ventricle, and it was proposed that the gland is fully grown after the first years of life [2, 3], instead of developing and adapting during adolescence and even later, as it is known for many other brain structures. Further studies have demonstrated that the PGV is correlated positively with age-related parameters (e.g., body height, body weight, and head circumference) [4, 5]. A recent study in a large clinical sample [6] showed a negative correlation of the PGV and age. In insomnia patients, age and PGV are correlated negatively as well [7], whereas in healthy individuals the influence of age on PGV seems negligible [8]. Volumetric analyses of PGV have been carried out in the past predominantly by postmortem measurements [8, 9]. In vivo measurements

J.M. Bumb (✉)
Department of Psychiatry and Psychotherapy,
Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University,
Mannheim J5, 68159, Germany
e-mail: jan.bumb@zi-mannheim.de

I.S. Nölte
Department of Neuroradiology, University Hospital Mannheim, Mannheim 68167, Germany
e-mail: ingo.noelte@medma.uni-heidelberg.de

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with CT or MRI on PGV are rare. In a healthy Chinese population, mean PGV was 94.2 ± 40.7 mm^3 [5]. In clinical samples (inpatients suffering from various medical diagnoses) comprising infants and adults, PGV ranged between 94.3 ± 159.1 mm^3 in infants [4] and 78.33 ± 89.0 mm^3 in adults [6].

The gland's major task is the synthesis and release of melatonin, a very versatile hormone regulating many physiological body functions involving chronobiotic properties and sleep processes [10]. Melatonin is synthesized following a circadian (approximately 24 h) rhythm. Hormone levels are high at night and low during the day [11, 12]. The rate-limiting enzymes in the synthesis of melatonin are called arylalkylamine N-acetyltransferase (AA-NAT) and acetylserotonin methyltransferase (ASMT), which synchronize melatonin's acrophase during the daytime and at the beginning of the night (AA-NAT) and the melatonin peak at night (ASMT) [13]. Human melatonin levels increase continuously until the age of 3 years, establishing the 24 h circadian rhythm [2, 12, 14, 15] and decrease with age, which might be causal for sleep disorders in the elderly. Nevertheless, only a few studies have investigated pineal gland morphology, pineal gland volume, and possible links to mental and/or somatic illnesses, such as sleep disorders (e.g., insomnia).

Insomnia is characterized by difficulties in falling asleep, maintaining sleep, or non-restorative sleep accompanied by significantly impaired daytime functioning in the absence of a specific physical, mental, or substance-related cause [7, 16]. Additionally, insomnia patients suffer from a "hyperarousal" on an autonomous and central nervous level, which is indicated by highly elevated numbers of micro-arousals and awakenings during REM sleep in comparison with healthy good sleepers [7, 17].

Recently, several magnetic resonance imaging (MRI) and computed tomography (CT) studies determined the prevalence of pineal calcification [18–20] and pineal cysts [4, 19], speculating about their potential role in the etiopathology of various disorders (e.g., sleep disorders). In a pilot study, researchers demonstrated that the degree of pineal calcification, obtained by CT, might cause alterations of the sleep-wake cycle (e.g., chronic daytime tiredness and sleep disturbances) [21]. Using specific MRI techniques, the direct quantification of pineal volume is possible, even the separate assessment of cystic and solid compartments. In fact, melatonin levels in human plasma have indeed been shown to correlate positively with solid PGV [18].

Intriguingly, interrelations of PGV and insomnia have been addressed lately, while it has been known for quite some time that PGV is reduced in male schizophrenic patients compared to healthy controls [22]. Nevertheless, PGV does not differ significantly when comparing bipolar patients and healthy controls [23].

Picking up on these issues, we hypothesize that reduced pineal gland volume may be related to altered melatonin secretion and that the latter may be correlated with parameters of disturbed sleep and primary insomnia [7]. To elucidate mutual interferences of these factors, the use of comparative volumetric analyses of the pineal gland in patients and healthy controls and a link with polysomnographic parameters, sleep quality scores, as well as melatonin levels might represent suitable approaches.

3.2 Neuroimaging of the Pineal Gland

3.2.1 Pineal Gland Volume

For a long time, morphological analyses of the pineal gland were mainly based on anatomical autopsy studies [8, 9, 24], which are characterized by some limitations, such as postmortem changes due to fixation techniques and relatively small sample size. Additionally, the mean age of the investigated groups is very high and the groups are often comprised of patients and not of healthy subjects. In anatomical autopsy studies, PGV is estimated on the basis of a geometric model (e.g., globe) by multiplication with a distinct factor (depending on the geometric model used) x length x height x width. Based on this model, PGV ranged between 20–270 mm^3 [9]

and 109–131 mm^3 [8]. The introduction of new techniques involving MRI has improved the precision and the richness of detail of volumetric analyses.

In MRI, the pineal gland usually appears isointense to gray matter in T1-weighted and T2-weighted imaging. For characterization of the pineal gland, the adjacent quadrigeminal plate, the medial borders of the thalami, and the habenulae have to be distinguished. Since the pineal gland is surrounded by cerebrospinal fluid (except for the attachment to the habenulae), it is readily identifiable [7]. Special care has to be taken to discriminate between the pineal gland and the adjacent vessels (e.g., the thalamostriate veins).

The morphology of the pineal gland was analyzed in conventional MRI sequences [2, 4, 6, 25–27], but only a few provide reference data on PGV. In a Chinese population, the mean PGV of healthy volunteers was 94.20 ± 40.65 mm^3 [5]. In clinical samples (inpatients suffering from various medical diagnoses) comprising infants and adults, PGV ranged between 94.3 ± 159.1 mm^3 in infants [4] and 78.33 ± 89.0 mm^3 in adults [6]. The results vary in dependence with the study design, e.g., MRI sequences (such as MPRAGE or TrueFISP) and slice thicknesses (between 800 µm and 10 mm [25, 28]). It has to be emphasized that the evaluation of small anatomical structures, like the pineal gland, measuring approximately 10 mm at most, should be performed with small slice thicknesses (≤1 mm) and, on the basis of strongly T2-weighted high-resolution sequences (as TrueFISP), to overcome the partial volume effect and to facilitate the separation of parenchyma, cysts, and other structural abnormalities [27]. Up to now, the volume assessment of the pineal gland is based on the manual delineation of its borders (Fig. 3.1); automated segmentation as for other brain regions has not been performed yet.

3.2.2 Pineal Calcifications

Pineal calcifications are very frequent in humans. They have a diameter of a few millimeters and are mostly round or berry-shaped [29]. They are localized extracellular, in the pineal lobules or in the interlobular trabeculae [30]. Pineal calcifications are radiopaque and were used as landmarks for skull X-rays [31, 32]. Analysis and measurement of the calcifications are best achieved by using CT [30, 33], mainly because of its high spatial and contrast resolution. In CT, calcifications are hyperdense to gray matter, whereas they appear hypointense to gray matter in most MRI sequences [30, 32].

Despite the fact that pineal calcifications are very frequent in humans (prevalence ranges between 27 % [18] and 71 % [9] in brain imaging of adults), their etiology and potential associations to various disorders are still under debate.

Some authors have suggested that pineal calcifications develop during lifetime and that their prevalence increases with age [32, 34–36]. Helmke et al. [37] analyzed 1.044 consecutive lateral skull X-rays of children. The incidence of calcifications was 3 % in very young children (<1 year) rising to 7.1 % in older children and adolescents (≤18 years). The researchers suggested that pineal calcifications already exist in children and young adults and increase with age.

Other researchers have investigated whether pineal and habenular calcification is associated with the onset of schizophrenia [38, 39]. The same group [40] has hypothesized that the degree of pineal calcification may be correlated to non-responsiveness to electroconvulsive therapy in bipolar patients. However, appropriate evidence has not been put forward.

3.2.3 Pineal Cysts

For the development of pineal cysts, mainly three theories exist. Firstly, Tapp et al. [24] suggested that pineal cysts might arise if the pineal parenchyma did not develop correctly during embryogenesis ("theory of incomplete fusion"). According to this hypothesis, pineal cysts would be of primary origin. Alternatively, cysts could be the result of a secondary pathophysiological process after a complete development. This process may be ischemic ("theory of ischemia") or

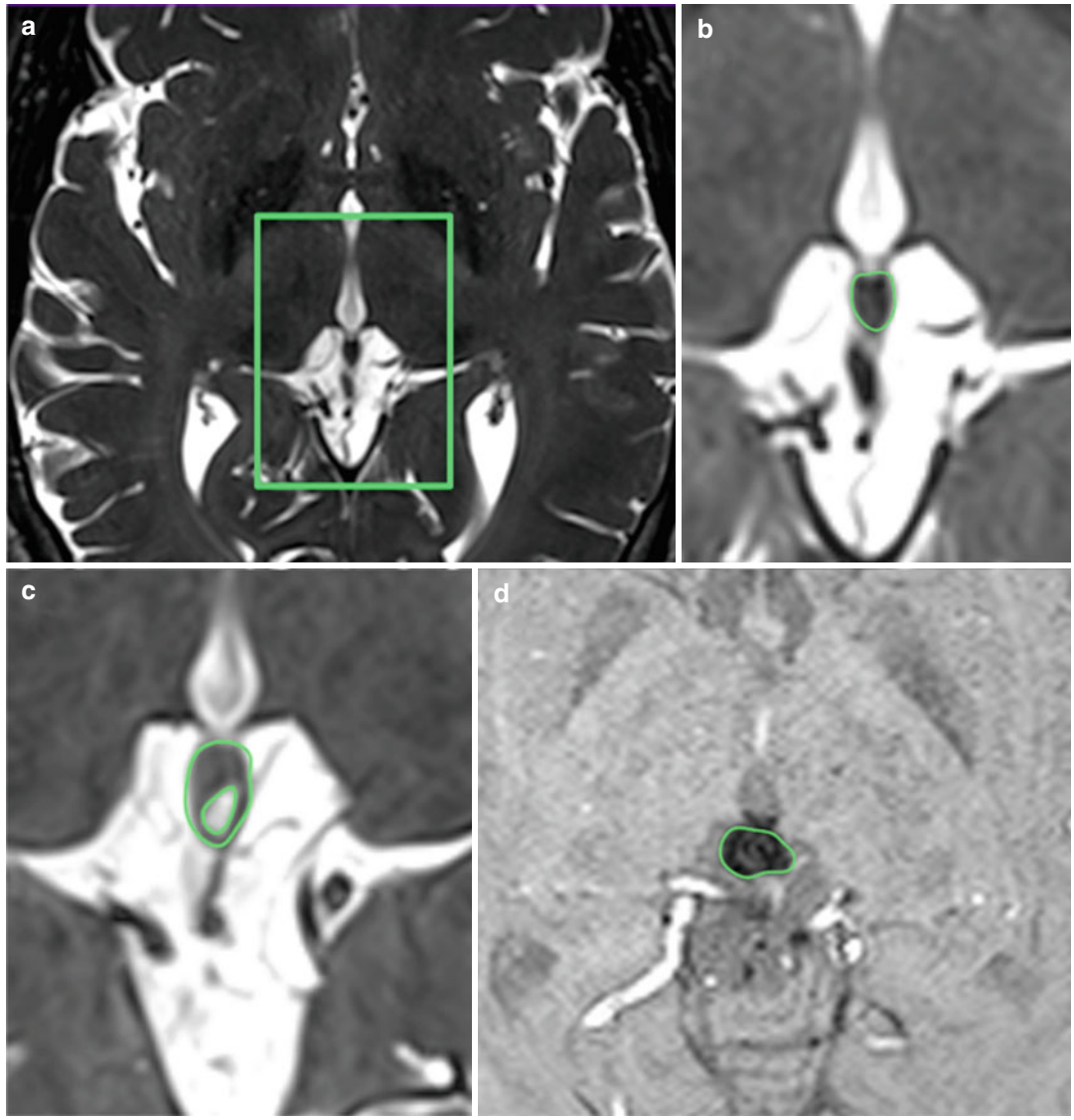


Fig. 3.1 Manual volume assessment in solid, cystic, and calcified pineal glands [19]. The pineal outline (as highlighted by *green circles*) is depicted in T2-TSE images reconstructed axially to the pineal gland axis (**a**, **b**). The measurement of cystic areas is shown in (**c**). Intraparenchymal hypointensity in susceptibility-weighted imaging (SWI, **d**) is used to estimate calcification

primary necrosis ("theory of necrosis"). The theory of ischemia states that pineal cysts might be left over after an ischemic process leading to degeneration of cells in the center of a glial plaque. Similarly, according to the theory of necrosis, necrosis and invagination of pinealocytes might be the pathophysiological process leading to cyst formation [41, 42].

Autopsy studies have revealed a cyst prevalence of 25–40 % [9, 43]. Two recent MRI studies using TrueFISP sequences reported prevalence of 33 % [18] and between 29 and 54 % depending on age and gender [27].

In T1-weighted sequences and in FLAIR, cysts are isointense to hyperintense (in dependency of their protein content) when compared to cerebrospinal fluid. In T2-weighted sequences, cysts are isointense to cerebrospinal fluid [4, 27, 44].

In most cases, only big cysts may cause severe and clinically relevant symptoms [45–47].

Follow-up studies of pineal cysts did not reveal a substantial upgrowth over time [26, 45, 48]. Therefore, follow-up of asymptomatic simple- and small cysts seems negligible [26].

3.3 Melatonin, Pineal Gland Volume, and Insomnia

Human melatonin levels increase continuously until the age of 3 years [2, 14, 15, 49]. It is well known that levels finally decrease with age [50–52]. In this regard, decreasing melatonin levels are likely to have an influence on sleep disorders frequently observed in the elderly [53–55].

Structural abnormalities as calcifications [18–20] and cysts [4, 19] might influence the total amount of melatonin production by reducing the functional parenchyma.

Already in 1990, Bojkowski and Arendt [56] measured 6-sulphatoxymelatonin (aMT6s), a major metabolite of melatonin, in urinary samples and tried to correlate their findings to pineal calcification as determined by lateral skull X-rays. These analyses revealed no significant correlations between pineal calcifications and aMT6s. The authors suggested that the limited contrast resolution of skull X-rays might explain the missing correlation. Some years later, Schmid et al. [57] found in a post-mortem study (i) that pineal calcifications are positively correlated to aging and (ii) that in night samples (pineal glands of subjects that died at night), pineal calcium supernatant levels and melatonin were correlated negatively. The sample was composed of 33 subjects and calcium deposits were detected by atomic absorption spectrometry. In 1998, Kunz et al. [21] published a hypothesis-driven pilot study to elucidate whether the degree of pineal calcification, as determined by CT, may be correlated to subjective sleep parameters. They found that pineal calcification is associated with the occurrence of daytime tiredness and sleep disturbances. The group hypothesized that this effect may be due to lower melatonin levels, caused by reduced pineal parenchyma. Of course this result should be considered preliminary, because of the small sample size ($n = 36$). In a subsequent study, this group found that uncalcified pineal tissue (measured by CT), but not pineal calcifications, is correlated with aMT6s levels in urinary samples [58]. In 2009, Mahlberg et al. [20] showed that uncalcified pineal tissue (measured by CT) is positively correlated to aMT6s levels in the urine of primary insomnia patients. Moreover, the degree of pineal calcification was negatively associated with REM sleep percentage, total sleep time, and sleep efficiency. Therefore, Schmitz et al. [33] suggested that the determination of uncalcified pineal tissue might serve as a suitable diagnostic tool to draw conclusions on melatonin deficits due to reduced solid pineal tissue. In addition, it was recently demonstrated that pineal cysts might have a substantial influence on melatonin levels as well [18, 19]. In these studies, the solid and uncalcified pineal volume correlated positively to melatonin maximum (Figs. 3.2 and 3.3), and intriguingly, solid (excluding pineal cysts and pineal calcification) and uncalcified pineal volume correlated negatively with the sleep rhythm disturbances subscore (Table 3.1). In summary, it is reasonable that the amount of uncalcified and noncystic pineal tissue is likely to have an influence on blood melatonin levels.

At the behavioral level, insomnia patients suffer from difficulties in falling asleep, maintaining sleep, and/or non-restorative sleep. These factors are accompanied by subsequent significantly impaired daytime functioning. Specific physical, mental, or substance-related features potentially disturbing sleep processes have to be ruled out before diagnosis [7, 16]. At the biological level, insomnia patients suffer from a "hyperarousal" on an autonomous and central nervous level, indicated by highly significantly elevated numbers of micro-arousals and awakenings during REM sleep in comparison with healthy good sleepers [7, 17].

Differences of PGV in insomnia patients and healthy controls have so far only been addressed by one study [7]. In this study, pineal gland volume was significantly smaller in patients with primary insomnia than in controls (Fig. 3.4). Moreover, PGV and age were negatively correlated in insomnia patients. In a large clinical

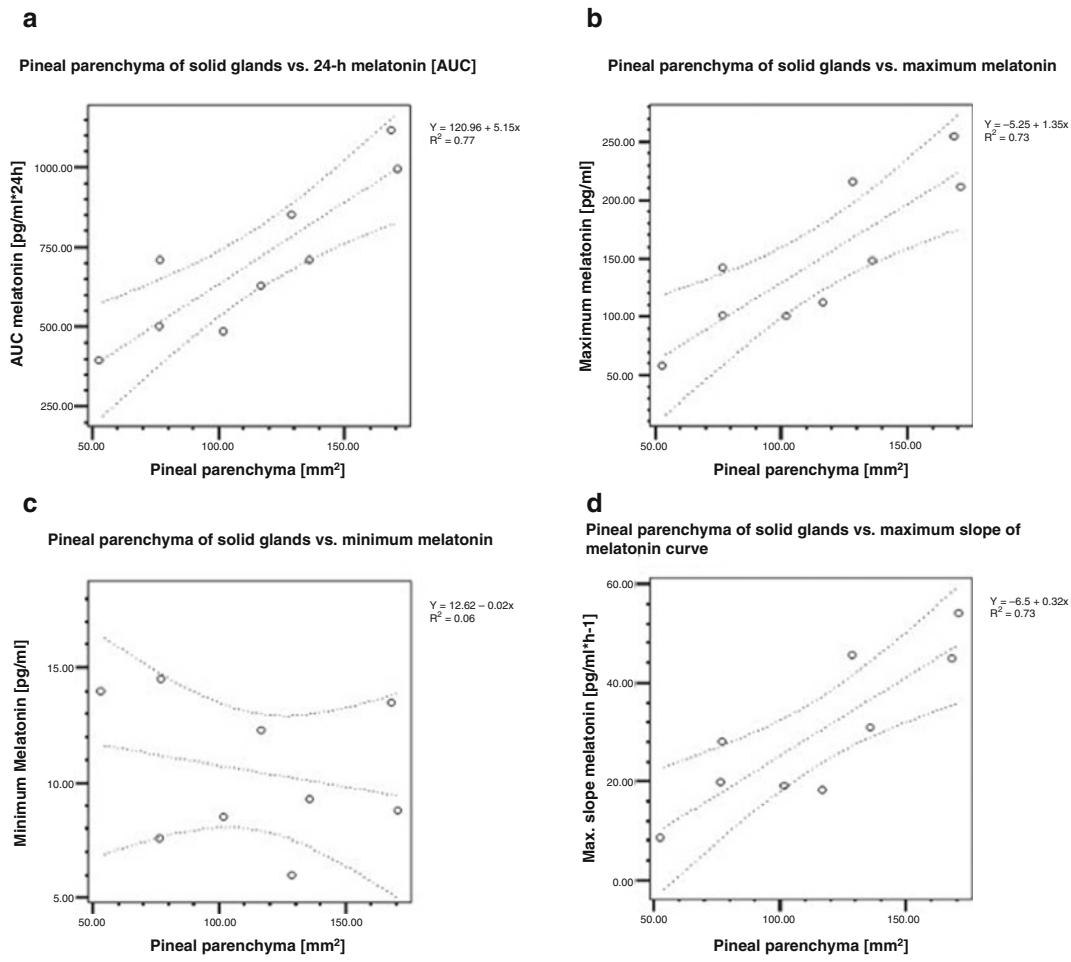
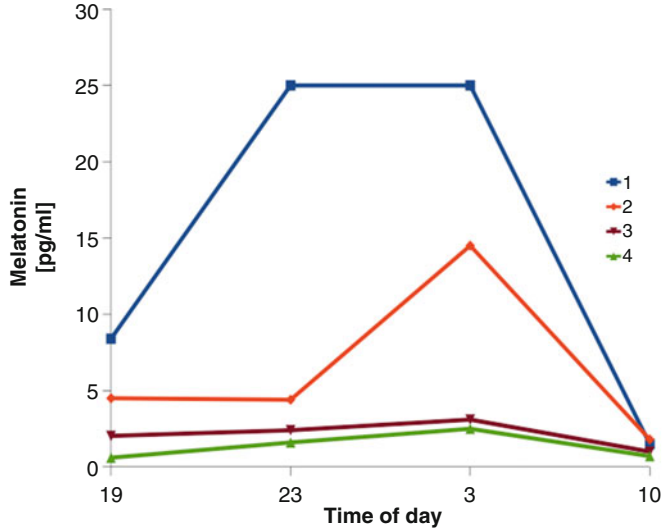


Fig. 3.2 Regression diagrams of pineal parenchyma of solid pineal glands vs. determinants of the 24-h melatonin curve [18]. The regression diagrams (*dotted line*: linear regression and 95 % mean prediction interval) show good correlation of pineal parenchyma to 24-h melatonin (**a**), maximum melatonin (**b**), and maximum slope of 24-h melatonin curve (**d**). Correlation to minimum melatonin levels reveals no linear relationship between pineal parenchyma and minimum melatonin levels (**c**)

sample [6], PGV and age were also correlated negatively, whereas the influence of age on PGV is likely to be negligible in healthy individuals [8]. It is well known that sleep processes are substantially linked to aging. Older humans often suffer from poorer sleep, and shorter sleep duration as well as decreased sleep efficiency [59]. Based on the study by Bumb et al. [7], it might be suggested that this effect is even more pronounced in insomnia patients, and prerequisites for insomnia might be prerequisites for an age-related degenerative process resulting in smaller PGV. Moreover, Bumb et al. [7] showed a positive correlation between PGV and sleep quality (measured by the SF-B questionnaire) encouraging the hypothesis that bigger pineal glands might produce more melatonin, which in turn may promote sleep processes adequately. This finding is in line with another recent study [19] showing a trend of a negative correlation between solid pineal parenchyma and the sleep quality disturbance subscore of the Landecker Inventar für Schlafstörungen in a homogeneous population without sleep impairments. Furthermore, Kunz et al. [60] stated that REM sleep is impaired in the context of many sleep disorders. Actually,



N°	Selection criteria	SPV [mm³]	CCPV [mm³]	AUC [pg/ml/15h]	Calcification [%]
1	95 % percentile of CCPV	221.6	214.8	171.2	3.1
2	95 % percentile of solid pineal glands	61.6	61.6	156.1	0
3	5 % percentile of solid pineal glands	29.2	29.2	34.4	0
4	Most calcified gland	92.7	32.4	23.8	66.8

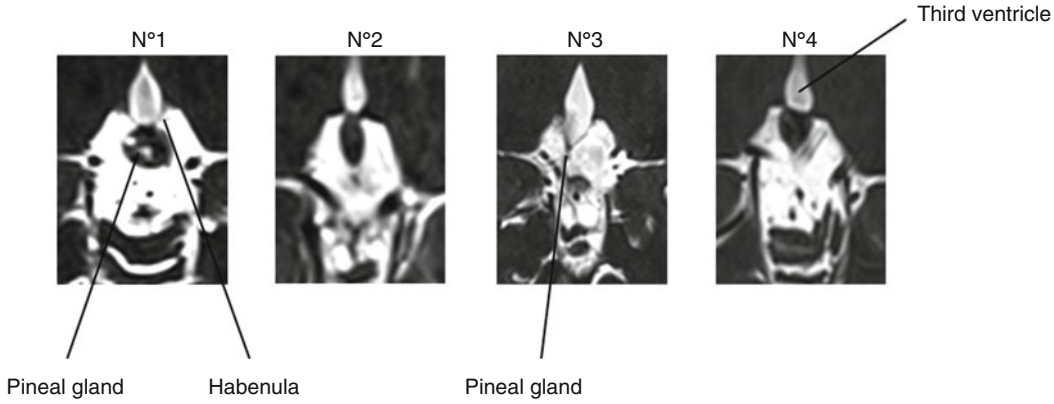


Fig. 3.3 Morphometric and hormonal characterization of four selected pineal glands [19]. Pineal gland No 1 is one of the glands with the most hormonal active pineal parenchyma (upper 5 % of the parenchyma corrected for cysts and calcification [CCPV]). Small cysts can be seen in the TrueFISP sequence within the pineal gland (*lower row*), the saliva melatonin levels show a high nocturnal increase (*upper diagram*). No 2 is one of the largest solid glands (upper 5 %) without signs of calcification. Again, the saliva melatonin levels display a clear nocturnal increase. One of the smallest solid and the most calcified pineal glands (No 3 and No 4) both have a small amount of hormonal active pineal tissue (CCPV) and a correspondingly low level of nocturnal saliva melatonin with a very small peak (*upper diagram*)

Table 3.1 Correlation of pineal volume, sleep parameters, and melatonin curve (p-values in parentheses, significant values in bold. All tests were two-tailed except for the LISST) [19]

		Correlation coefficient to pineal parenchyma		
	Mean ± STD	PV (mm^3)	SPV (mm^3)	CCPV (mm^3)
MLT_min [pg/ml] ($n=90$)	0.67 ± 0.74	0.12 (0.253)	0.19 (0.069)	0.18 (0.097)
MLT_max [pg/ml] ($n=90$)	12.33 ± 8.01	0.14 (0.188)	0.26 (0.013)	0.28 (0.008)
$AUC_{19:00–10:00}$ [pg/ml*15] ($n=90$)	90.15 ± 53.44	0.16 (0.141)	0.27 (0.011)	0.29 (0.006)
ESS ($n=103$)	7.17 ± 2.99	0.07 (0.464)	−0.02 (0.877)	0.01 (0.937)
PSQI ($n=103$)	3.74 ± 2.51	0.14 (0.165)	−0.12 (0.235)	0.10 (0.672)
LISST (rhythm disorder)	17.55 ± 6.26	−0.11 (0.259)	−0.17 (0.0431)	−0.17 (0.0421)
LISST (sleep disturbances)	15.79 ± 5.48	−0.15 (0.130)	−0.17 (0.089)	−0.14 (0.168)

In this study, 24-h melatonin saliva profiles were analyzed in 90 healthy volunteers (*MLT_min* minimum melatonin, *MLT_max* maximum melatonin, *AUC* area under the curve/24-h melatonin) and magnetic resonance imaging-based pineal volumetry was used to quantify pineal volume (*PV*), solid pineal volume (cysts excluded, *SPV*), and calcification, and cyst-corrected pineal volume (*CCPV*). Sleep quality and daytime sleepiness were assessed using the Pittsburgh Sleep Quality Index (*PSQI*), the Epworth Sleepiness Scale (*ESS*), and the Landecker Inventar für Schlafstörungen (*LISST*). The better the identification of productive pineal parenchyma (CCPV > SPV > PV), the higher the correlation to Mlt_max and AUC. Despite a very homogenous population without evident sleep disturbances, SPV and CCPV correlated to subscores of the LISST showing that the amount of productive parenchyma is related to sleep quality

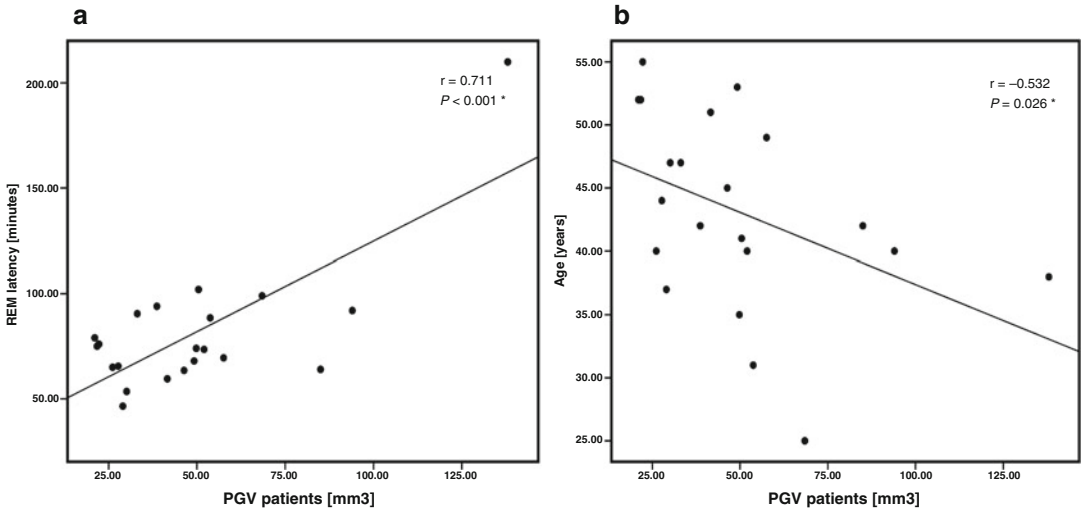


Fig. 3.4 Spearman correlation analyses of (**a**) pineal gland volume and REM latency in insomnia patients adjusting for the effect of age and of (**b**) pineal gland volume and age in insomnia patients (adjusted statistical significance is denoted by an asterisk "*") [7]

Bumb et al. [7] showed that mean REM latency was shorter in insomnia patients, reflecting substantial disturbances of normal sleep processes, and an explorative analysis (adjusting for the effect of age) revealed a significant correlation between PGV and REM latency in insomnia patients. Already in 1999, Monti et al. [61] showed that REM sleep latency and REM sleep time are reduced in insomnia patients if compared to healthy controls. Melatonin, administered exogenously, increased REM sleep latency as measured by polysomnography. In addition, it has been stated that exogenous melatonin increases sleep propensity in healthy volunteers while decreasing both sleep onset latency and REM latency [62].

It has to be emphasized that Bumb et al. [7] performed MRI volumetric studies without

correction for cysts or pineal calcification. Using only MRI, it cannot be clarified to which degree pineal calcifications and cysts might have influenced the results of the PGV measurements and the correlation analyses between PGV, polysomnographic parameters, and sleep quality. Therefore, it was not possible to analyze functional parenchyma instead of considering the total pineal volume. Additional CT scans would represent a helpful tool to further validate the results. Moreover, it should be kept in mind that the findings might be influenced by a selection bias with respect to the analyzed patients. These subjects decided, or were recommended by their physician, to seek help in a sleep clinic. Insomnia patients experiencing more than one of the following risk factors involving small pineal gland, low melatonin levels, as well as poor sleep/insomnia and resulting psychological strain may be more likely than others to seek contact with a sleep clinic.

Further issues concerning the relation of PGV and insomnia comprise genetic, epigenetic, and exogenous prenatal and postnatal influences. At this point, however, it is not clear whether smaller glands reflect the cause or the consequence of insomnia. On the one hand, there was no correlation between PGV and the duration of illness [7], which does not support the idea of small PGV being a consequence of insomnia. On the other hand, it has been hypothesized that PGV in humans underlies a strong genetic influence, and an interesting question in this context is why sleep problems occur at such a late stage if the pineal gland is fully grown in early childhood [2, 3]. Moreover, aside from genetic determination, no one has ever investigated whether epigenetic factors, i.e., environmental conditions during pregnancy, such as light exposure at night, interindividual differences in maternal rhythmicity (e.g., "morning"-ness versus "evening"-ness), sleep disorders, as well as medication, have a relevant impact on the growth of the fetal PGV in humans. All these effects might result in smaller pineal glands in the newborn, which again might cause sleep disturbances in the years to come. Intriguingly, several animal studies, for instance, in primates [63] and in rodents [64] have shown that certain conditions during pregnancy might influence the development of the newborn circadian system and its components (reviewed in [65]). Besides genetic and early-life environmental factors, an increase of dysfunctional tissue (i.e., calcification and/or pineal cysts) may contribute to "low" PGV as a predisposing factor of primary insomnia. However, the validation of these hypotheses is still pending.

Conclusion

Remarkable interrelations between morphometric analyses and both endocrinological function and sleep have been found.

It is very likely that primary insomnia is related to reduced pineal gland volume and that the latter is correlated to parameters of disturbed sleep. Recently, comparative volumetric analyses revealed that PGV is significantly smaller in primary insomnia patients than in healthy controls. Additionally, it has been hypothesized that PGV and polysomnographic parameters as well as sleep quality scores are correlated significantly. The exact pathophysiological relationship, however, needs further investigation.

Future imaging aspects include the amelioration of the volumetric analysis of productive pineal tissue, such as cyst and calcification quantification and new approaches as perfusion analysis or molecular imaging.

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Conflict of Interest The authors declare no conflict of interest.

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Melatonin: Basic and Clinical Aspects

4

Agata Carpentieri, Vanessa Areco,
Gabriela Díaz de Barboza, María Angélica Rivoira,
Solange Guizzardi, and Nori Tolosa de Talamoni

4.1 Introduction

Melatonin (N-acetyl-5-methoxytryptamine, MEL) is an endogenous indolamine that has several important physiological functions [1]. It is synthesized and secreted mainly by the pineal gland in a circadian fashion and by other extrapineal tissues. For many years, it was exclusively considered as a chronobiotic molecule, but lately many other functions have been shown beyond its classical role. Hardeland and Poeggeler [2] indicated that the ubiquity, pleiotropy, and complexity are the three terms that summarize the properties and actions of MEL. In fact, this compound is present in bacteria and eukaryotes. The different distribution of MEL receptors and the diversity of metabolism and actions of metabolites explain, at least in part, the pleiotropic functions. The different binding sites and the signal transduction pathways as well as the influence on other hormones and neuronal subsystems contribute to increasing the complexity of this molecule.

MEL, also called the "hormone of darkness," is secreted at night by the pineal gland and transmits the signal darkness to other organs, particularly to the suprachiasmatic nucleus, and to the eminentia mediana and pituitary in seasonal breeders. As a hormone, it circulates in very low concentration, but its synthesis and quantity in extrapineal tissues exceed those in pineal and blood plasma [3]. MEL participates in the circadian rhythms, the modulation of season changes, in reproduction, as well as an antioxidant, antiapoptotic, anti-inflammatory, oncostatic, and anticonvulsant drug [4, 5].

Exogenous MEL is employed in a number of physiopathological conditions, mainly for the treatment of sleep disorders and jet lag [6]. MEL has been shown to have other sedative and

A. Carpentieri
"Dr. Cañas" Laboratory, Biochemistry and Molecular Biology, Faculty of Medical Sciences, Universidad Nacional de Córdoba, INICSA (CONICET-UNC), Córdoba, Argentina

Biological Chemistry, Faculty of Dentistry, Universidad Nacional de Córdoba, Córdoba, Argentina

V. Areco • G.D. de Barboza • M.A. Rivoira
S. Guizzardi
"Dr. Cañas" Laboratory, Biochemistry and Molecular Biology, Faculty of Medical Sciences, Universidad Nacional de Córdoba, INICSA (CONICET-UNC), Córdoba, Argentina

N. Tolosa de Talamoni (✉)
"Dr. Cañas" Laboratory, Biochemistry and Molecular Biology, Faculty of Medical Sciences, Universidad Nacional de Córdoba, INICSA (CONICET-UNC), Córdoba, Argentina
e-mail: ntolosa@biomed.fcm.unc.edu.ar; ntolosatalamoni@yahoo.com.ar

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anti-excitatory effects that go beyond the sleep induction, because these effects were also observed in nocturnally active animals [2]. It has also been used in the treatment of chronic pain in humans [7, 8]. Because of its hypnotic, antinociceptive, and anticonvulsant properties, MEL has a potential use to modulate the anesthetic procedures in both adults and children [1]. The spectrum of the uses of MEL seems to be wide, although more investigation is needed in order to know better the molecular mechanisms and the possible side effects [6].

4.2 Melatonin Biosynthesis in Pineal Gland with Regulation of Secretion

Tryptophan and serotonin are MEL precursors. Two well-characterized enzymes participate in its synthesis: N-acetyltransferase (AANAT or NAT), the first-rate limiting enzyme in MEL production, which converts serotonin to N-acetylserotonin (NAS), and hydroxyindole-O-methyltransferase (HIOMT), which converts NAS to MEL. In the pineal gland, MEL biosynthesis is regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus and is synchronized to the environmental light-dark cycle [9]. The SCN is connected with the pineal gland through paraventricular nuclei and preganglionic sympathetic neurons. Norepinephrine released from postganglionic sympathetic fibers at pinealocyte membrane stimulates its adrenoceptors leading to cAMP formation as well as other second messengers, which stimulate the expression and activity of AANAT [10].

Both the synthesis and secretion of MEL at night are stimulated, reaching a peak value (80–150 pg/mL) between midnight and 3 a.m., while its concentration during the day is low (10–20 pg/mL) [11]. Exposure to bright light during the dark hours of the night suppresses MEL production by degradation of NAT enzyme [12]. Once synthesized in the pineal gland, MEL is not stored and is immediately secreted into the blood and cerebrospinal fluid and reaches other body fluids. Small amounts of unmetabolized MEL are excreted in the urine.

At the beginning, it was thought that MEL catabolism was almost exclusively done by the P450 monooxygenases in the liver, followed by a conjugation of the resulting 6-hydroxy-MEL to produce the main urinary metabolite 6-sulfatoxy-MEL. This might occur with the circulating hormone. However, in the central nervous system (CNS), the oxidative pyrrole-ring cleavage predominates and no 6-hydroxy-MEL was detected after MEL injection into the cisterna magna, which may be important because much more MEL is released via the pineal recess into the cerebrospinal fluid than into the circulation. The primary cleavage product is N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK). Several different reactions lead to the same product, AFMK, which is converted into N^1-acetyl-5-methoxykynuramine (AMK). AFMK and AMK form metabolites by interaction with reactive oxygen and nitrogen species. Some other metabolites have been also detected, but in minor quantities [13].

4.3 Melatonin Synthesis in Other Tissues and Organs

MEL is not only synthesized in the pineal gland of vertebrates. It has been demonstrated that other tissues and organs are able to synthesize MEL such as the retina [14], lens [15], brain [16], placenta [17], skin [18], testis [19], oocytes [20], etc.

The gastrointestinal tract is one of the main sources of extrapineal MEL [21]. In mammals, MEL shows differences in regional distribution, with the lowest levels in the jejunum and ileum and the highest levels in the rectum and colon. Similar distribution has been confirmed in rabbit, mouse, and humans [22]. MEL was also detected in luminal fluids of the gut. The origin of this MEL can be from food, mucosa, or the microflora. Large amounts of MEL have been also detected in the gastrointestinal tract of embryos either from birds or mammals. In the bile, MEL is also present, but its sources remain to be elucidated.

MEL is synthesized in the enterochromaffin (EC) cells, and its distribution is comparable to the density of EC cells in the gut. L-tryptophan is a crucial precursor of MEL synthesis in the gut. Oral administration of L-tryptophan causes greater MEL synthesis in the intestine than the intraperitoneal administration. Although the MEL synthesis in the gut is independent from that of the pineal gland [23], some MEL of pineal origin is accumulated in the digestive tract, mainly in the lower tract [24].

MEL concentration in the intestine is 10–100 times higher than in serum and is 400× more concentrated than in the pineal gland, at any time of the day or night. The levels of MEL in the gut depend on age, food, and luminal content and maybe on the movement of digesta. Gut MEL concentration peaks at birth in rats and then diminished to stable level at 21 days [25].

Fisher et al. [26] have shown that cultured keratinocytes contain high levels of intracellular MEL. They calculated that a single keratinocyte has about 34 pg MEL (146 pmol/1,000 cells), and based on a cell volume of keratinocytes of 1.43×10^{-6} μL, they concluded that the MEL concentration should be around 102 mM. Considering the size of the skin, the amount of MEL in this organ would be extraordinarily high. The data should be confirmed by other researchers.

It is very interesting to quote that the extrapineal MEL appears not to contribute significantly to the MEL circadian rhythm in the circulation. Pinealectomy or chemical pinealectomy (constant light exposure) decrease markedly the circulating MEL levels. Therefore, despite the large quantity of extrapineal MEL, it does not constitute a chemical signal of light/dark. Tan et al. [27] think that locally generated MEL is consumed by the tissues mainly as a protective mechanism of oxidative stress. This is reasonable since the gut and skin, the two major extrapineal MEL generators, are exposed to hostile outside environment factors such as bacteria, parasites, toxins, irradiations, etc.

Another interesting issue is the presence of MEL-binding proteins in several rat tissues [28]. These proteins would avoid the free movement of MEL inhibiting the hormone transport to blood and facilitating the local MEL storage. If this is the case, the reason of MEL storage remains to be investigated.

4.4 Melatonin Receptors

Most of the MEL's actions are mediated by membrane and nuclear receptors. However, some other MEL effects seem to be independent of the involvement of receptors [29]. Two membrane-bound MEL receptors have been identified and characterized, MT1 and MT2. Both belong to the family of G protein-coupled seven-transmembrane receptors. MT1 receptor is composed of 350 amino acids and is coupled to G_i and $G_{q/11}$. MT1 receptor is present in the brain, cardiovascular system, immune system, testes, ovary, skin, liver, kidney, adrenal cortex, placenta, breast, retina, pancreas, and spleen. In the brain, the presence of MT1 receptor is predominant in the hypothalamus, cerebellum, substantia nigra, and ventral tegmental area [30]. MT2 receptor consists of 363 amino acids and has 60 % homology to the MT1 receptor. The distribution of MT2 receptors is quite similar to that of the MT1, and it is also related to a G protein and coupled to the activation of G_i. The MEL-related receptor (MRR) or GPR50 is an orphan receptor, which exhibits 45 % amino acid homology to MEL receptors. MEL does not bind to GPR50, but this receptor may heterodimerize with the MT1 receptor inhibiting its activity [31]. The binding sites of both MT1 and MT2 receptors are located in the immediate vicinity of the five-transmembrane domain, precisely in the extracellular half of the transmembrane domain. The C-terminal tails of MT1 and MT2 contain a single cysteine residue that seems to be essential for adenylate cyclase activity inhibition and phosphorylation sites for PKA and PKC [32]. It has been proposed that homo- and heterodimerization of MT1 and MT2 receptors constitute a regulatory mechanism with possible signaling and pharmacological effects. Although coexpression of both MT1 and MT2 has been shown in several regions of CNS and other tissues, the interaction remains to be identified in tissues.

In the market, all the melatoninergic drugs act on both types of receptors without significant selectivity [33].

A third receptor, MT3, has been found in hamsters and rabbits [34], but not yet in humans. It has a nanomolar affinity for MEL, is not coupled to G proteins, and is not sensitive to cations such as Na^+, Ca^{2+}, or Mg^{2+}. It has been reported that MT3 is equivalent to quinone reductase II (NQO2) and is modulated by MEL, resveratrol, and compound S29434 [35].

MEL is also bound to nuclear receptors named retinoid orphan receptors (ROR) and retinoid Z receptor (RZR). MEL is able to bind to RZRα, RORα, RORα2, and RZRβ. These nuclear receptors consist of an N-terminal domain, a DNA-binding domain with a zinc double finger, a hinge region, and a ligand-binding domain in the C-terminal. RZRβ has been found in the nervous tissue, and RZRα in the skin, testes, liver, cartilage, and adipose tissue [36]. The binding of MEL to ROR is questioned by some investigators. The field of MEL nuclear receptors is unclear and needs further investigation.

Other interactions with proteins such as calmodulin, calreticulin, and microtubular proteins, which could act as MEL sensors, have been also reported [30, 37].

4.5 Signal Transduction Pathways

MEL is involved in many physiological processes. This can be understood knowing its modes of action through the cellular and molecular mechanisms. Some of MEL effects occur through receptor-dependent pathways, meanwhile others are receptor independent or related to Ca^{2+}-binding proteins (See Fig. 4.1).

4.5.1 Receptor-Dependent Mechanisms

AC/cAMP/PKA/CREB Cascade When MEL binds to either MT1 or MT2 receptor, this pathway is inhibited through a pertussis toxin-sensitive G_i protein. The outcome of this activation is the phosphorylation of CREB. The classic acute effects of MEL on a small cluster of genes in the pars tuberalis of the pituitary gland are mediated by this pathway [38].

Phospholipase C (PLC)-β and PLC-η Cascade In some cells, MT1 or MT2 receptors couple to $G_{q/11}$ causing activation of PLC-β and perhaps PLC-η, which increases the levels of inositol triphosphate and 1,2-diacylglycerol (DAG), provoking an activation of Ca^{2+} signaling by calmodulin kinases (CaMK) and PKC, respectively [38].

Rafs/MEK1/2/ERK1/2 Cascade MEL triggering of this pathway might occur through the activation of the PLC-β/DAG/PKC or PLC-β/IP_3/Ca^{2+}/CaMK via the dissociation of βγ subunit. In addition, MEL could cause transactivation of SOS/Ras/Raf signaling promoted by G proteins or via matrix metalloproteinase activation. This pathway is a major component of MEL signaling during oxidative stress [39] together with the stress-activated protein kinase JNKs [40].

Other Pathways The activation of MT1 receptor causes an inhibition of GC/cGMP/PKG pathway with the possible contribution of low NOS activity [41]. Other pathways that also seem to be involved in the MEL signaling are the PI3K/Akt(PKB) and the Kir (GIRK) inward rectifier potassium channels [32].

4.5.2 Receptor-Independent Mechanisms

MEL has been proposed to interact with low-affinity intracellular enzymes, transporters, nuclear receptors, and cytoskeletal proteins generating independent signals or secondary interactions with molecules involved in the receptor-dependent cascades. The classic example is the enzyme quinone reductase 2, which was first described as the MT3 receptor presumably involved in the regulation of the redox status [42].

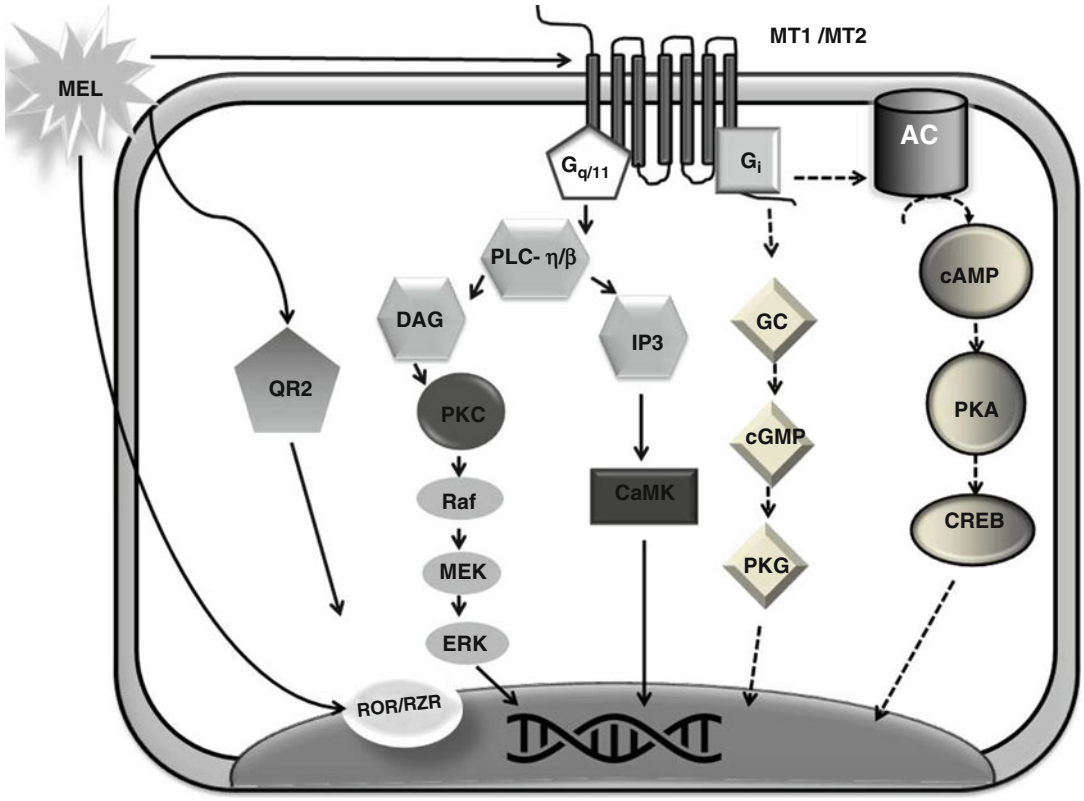

Fig. 4.1 Scheme of multiple signal transduction pathways involved in MEL effects. MT1 and MT2 membrane receptors can form homo- and heterodimers. They are coupled to the G protein subunits G_i and $G_{q/11}$. The G_i-coupled MT1 receptor lowers cAMP levels by inhibition of adenylyl cyclase (AC). The MT1 receptor can also be coupled to $G_{q/11}$ and activates PLC-C, which in turn can increase DAG and IP3, triggering the Raf/MEK/ERK cascade or CaMK cascade, respectively. MT1 receptor causes inhibition of GC/cGMP/PKG pathway. Membrane MEL receptors also promote the activity of c-Jun-N-terminal kinase (JNK) (not shown). RORα/RZR1 are MEL nuclear receptors. QR2, quinone reductase II, or MT3 receptor is a cytosolic enzyme only present in some species

Ca^{2+}-CaM complex plays a role in both receptor-dependent and receptor-independent actions of MEL. At nanomolar concentration, MEL interacts with the complex reversing the inhibitory effect of this complex on microtubule polymerization and the rearrangements of the cytoskeleton [43]. When MEL concentration is high (10^{-5} M), it binds directly to tubulin causing inhibition of microtubule formation. These MEL effects probably control the main events involved in motility, division, organelle function, and trafficking and signaling function of scaffold proteins [44]. At micromolar concentration, MEL also triggers a transient ROS increase in human leukocytes by a calmodulin-dependent mechanism, which is a receptor-independent effect not compatible with oxidative stress and associated to low apoptosis [45].

Calreticulin is a nuclear protein that binds MEL with high affinity. It is quite possible that calreticulin contributes to signaling and genomic effects in the nucleus [46].

The anticancer activity of MEL has been reported in C6 glioma cells and in other cancer cells to be mediated by changing the redox status of the cells. This redox-dependent antiproliferative action of MEL was not demonstrated in noncancerous cells, which indicates that cancerous cells have a peculiar ROS signaling that represents a condition that MEL requires in order to produce an anticancer effect [47].

4.6 Physiological Actions with Its Therapeutic Potentials

The pineal gland becomes mature after birth. Therefore, the embryo and fetus are dependent on maternal MEL. The hormone crosses all physiological barriers, including the placental one, and has been involved in placental function in humans and animals [48]. MEL concentration increases in maternal blood during pregnancy, reaching a maximum at term. The presence of MEL has also been shown in amniotic fluid [49]. MEL is secreted in a circadian manner, showing the highest levels of secretion at night. The activity of the NAT is increased from 30- to 70-fold at night, and the duration of darkness determines the period of MEL secretion. The onset of a circadian rhythm of MEL biosynthesis appears between 6 and 8 weeks of age in humans [50]. A peak of MEL secretion occurs between 4 and 7 years [51] then declines to adult levels around 15–18 years. The levels remain stable in the adulthood until 70 years approximately. At this age, a marked declination is produced, maybe as a result of a weakness of the circadian system, calcification of the pineal gland, and insufficient environmental illumination [52].

Most of the MEL's clinical trials are devoted to treat sleep disorders in various populations. Diverse doses and formulations have been proved to be effective in the treatment of primary insomnia for subjects at different ages. In 6–12-year-old children with chronic sleep-onset insomnia, MEL advanced sleep onset in 1 h and decreased sleep-onset latency approximately by half an hour [53]. MEL was able to advance sleeping time, reduce sleepiness, and increase school alertness in adolescent students with sleep-onset insomnia when it was administered in the afternoon [54]. Positive results were also obtained among the elderly population, mainly by using the prolonged-release MEL (PRM), and there was no rebound insomnia or withdrawal symptoms reported [55–57]. Furthermore, a preservation or improvement of the daytime psychomotor performance was observed [58, 59]. Apparently, MEL could be a safe sleep inducer in old people with cardiac risk and chronic respiratory conditions [60, 61]. MEL treatment has been also shown to improve the sleep quality in patients with Parkinson disease [62].

The obstacle to use MEL in primary chronic insomnia is that the half-life of circulation is short, 20–30 min and even less. This problem has been partially solved by drugs that prolong MEL release and the melatoninergic agonists with longer half-life synthetic melatoninergic agonists [13]. In a clinical study, 40 healthy subjects slept exposed to simulated intensive care unit (ICU) noise and light (NL). These subjects were randomly assigned to four groups: NL, NL plus placebo (NLP), NL plus the use of earplugs and eye masks (NLEE), and NL plus 1 mg of oral MEL. Compared with earplugs and eye masks, MEL improved sleep quality, and serum MEL levels better in healthy subjects exposed to simulated ICU noise and light [63].

Recently, Gandhi et al. [64] have shown in zebra fish lacking MEL due to a mutation of AANAT2 that MEL is required for circadian regulation of sleep. The authors have found that MEL promotes sleep downstream of the circadian clock as it is not necessary to initiate or maintain circadian rhythms.

MEL's cognitive benefits have been observed in Alzheimer disease (AD) patients and AD mice. MEL treatment of AD mice decreased the levels of mRNA of SOD-1, GPx, and catalase [65].

Exogenous MEL has been used in the management of pain in pathological conditions such as fibromyalgia, irritable bowel syndrome, and migraine and cluster headache. Srinivasan et al. [66] have reviewed the available evidence indicating that MEL, acting through MT1/MT2 receptors, plays an important role in the pathophysiological mechanism of pain. On the other hand, some data indicate that nocturnal production of MEL is not reduced in children with migraine [67]. This area merits further investigation.

There is evidence that MEL has antioxidant properties. At low concentrations, MEL seems to act through an upregulation of glutathione peroxidase gene expression and antioxidant activity, whereas at high MEL levels, the antioxidant

effects are attributed to direct radical scavenging actions [68]. It has also been demonstrated that MEL scavenges nitrogen-based reactive molecules and increases glutathione (GSH) synthesis and mRNA levels and the activity of superoxide dismutase (SOD) [69]. The antioxidant properties have been detected in several organs such as the brain [70], eye [71], liver [72], intestine [5], vascular endothelium [73], etc. The antioxidant properties of MEL have been proven to be beneficial to patients with rheumatoid arthritis [74], females with infertility [75], elderly patients with primary essential hypertension [76], and multiple sclerosis patients [77]. Although there are many data related to a potential hepatoprotective role of MEL, based on its antioxidant properties, there are also negative findings, so the usefulness of MEL under conditions of liver injury or liver transplantation is not quite clear [78].

Because of the lipophilic nature of MEL, this molecule crosses cell membranes to easily reach subcellular compartments including mitochondria. MEL enhances the activity of citrate synthase, the expression and activity of complex IV, and the activity of complex I of the electron transport chain, thus improving mitochondrial respiration and ATP synthesis [79]. Other benefits of MEL to the mitochondria include a decrease in membrane lipid peroxidation, cytochrome c release, and mitochondrial permeability transition and in mtDNA oxidation, as well as an increment in GSH synthesis [6].

MEL is apparently involved in preventing tumor initiation, promotion, and progression. The antiproliferative effects of MEL have been mainly detected in cancer breast and colon cells. In human breast cancer, the effect of MEL is mediated by the MT1 receptor [80], whereas the oncostatic effect of MEL on cancer colon cells was demonstrated to be mediated through MT2 receptors and RZR/ROR receptors [30]. In a mouse model of colitis-associated colon carcinogenesis (CACC), it was found that MEL treatment attenuates the progression of CACC by modulating autophagy and Nrf2 signaling pathways [81]. Apoptosis is another mechanism that seems to be involved in the anticancer action of MEL [82]. Although at physiological concentrations, MEL has an antioxidant activity; at pharmacological levels, MEL exhibits prooxidant actions enhancing ROS-mediated DNA fragmentation leading to apoptosis and reducing the viability of cells [83].

4.7 Concluding Remarks

A great effort has been made to advance on the knowledge of molecular aspects of MEL, its receptors and metabolism, as well as on the signal transduction pathways involved in its physiological responses. It can be considered a first-line drug for the treatment of some sleep disorders, but it potentially has beneficial properties to treat or prevent a wide spectrum of diseases such as cancer, cardiovascular and gastrointestinal diseases, and other pathologies. It is necessary to do more research work in order to know better the molecular mechanisms triggered by MEL under those conditions and its possible side effects.

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Melatonin and Melatonin Receptors in Neuroprotection

5

Omur Gulsum Deniz, Aysın Pınar Turkmen, Mehmet Emin Onger, Berrin Zuhal Altunkaynak, and Suleyman Kaplan

5.1 Introduction

Melatonin (Mel) is a neurohormone which is synthesized from tryptophan with the activation of beta-adrenergic receptors of the pineal gland [2, 11, 36]. It is chemically known as N-acetyl-5-methoxytryptamine [1]. The rhythm of the body and protecting the biological clock of the body is mainly adjusted by the Mel and many of physiological and biological processes in the body is also regulated by this hormone [11, 34, 58]. At this point, apart from the protection of the tissues by preventing the lipid peroxidation [8, 33], Mel is directly a free radical scavenger, and it is stronger than all known antioxidants by this effect [1, 55] as having a neuroprotective effect mechanism [2, 69]. Also, Mel shows tissue protective effect by increasing antioxidant enzyme levels or inhibiting pro-oxidative enzymes through receptors [49]. With this perspective, this chapter of the book reviews the therapeutic effects of Mel and Mel receptors in neuroprotection.

O.G. Deniz • A.P. Turkmen • M.E. Onger
B.Z. Altunkaynak • S. Kaplan (✉)
Department of Histology and Embryology, Medical School, Ondokuz Mayıs University, Samsun, Turkey
e-mail: skaplan@omu.edu.tr

5.2 What Do We Know About the Melatonin?

The chemical formula of Mel is expressed as N-acetyl-5-methoxytryptamine. It is secreted largely from the pineal gland, also in small amounts from the harderian gland, retina, hypothalamus, certain blood cells, and gastrointestinal cells, and its molecular weight is 232 g/mol [21, 39]. This hormone was defined by American dermatologists Lerner et al. [31] as a color pigment in the melanocytes of frogs and fish, but later it was found to be a hormone that was present in all mammals and regulated the biological rhythm of organisms [31]. After 1996, its presence was proven in the structure of many living things including invertebrates and protozoa. Also, it was shown that Mel is produced by plants and it is located in plant roots, leaves, and seeds. Although this hormone is present in plants and unicellular organisms, the Mel production in these organisms is independent from dark and light signals [39]. This hormone is defined as a molecule which has important physiological effects in animals and humans. It has an important role as a strong biological modulator in the regulation of cardiac rhythm, immunoregulation mechanisms, free radical scavenging, antioxidant functions, oncostatic effects, control of reproductive functions, emotion regulation, circadian

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rhythms, neuroprotection, sleep, and retinal physiology [21, 70].

5.3 Biosynthesis and Metabolism of Melatonin

Mel is synthesized from tryptophan as a result of a series of enzymatic reactions within the pinealocyte cytoplasm [1, 45]. The biosynthesis of this hormone is regulated by photoperiodic environment [48]. In this way, the knowledge of light and darkness reaches the pineal gland and, thus, Mel synthesis cycle is determined. Postganglionic sympathetic nerves release the noradrenaline with the darkness. Noradrenaline dominantly binds to beta-1 adrenergic receptors (a small amount binds to alpha 1), and this reveals N-acetyltransferase (NAT) and serotonin which is stored in the cell. Mel is synthesized from serotonin by the acetylation reactions which are catalyzed respectively by the NAT and 5-hydroxyindole-O-methyltransferase (5-HIOM) enzymes. The activity of NAT is controlled by the specific cAMP-dependent transcription factors which regulate the synthesis of Mel (Figs. 5.1 and 5.2) [3, 48]. This hormone is not stored after the synthesis, and due to its high lipophilic feature, it easily passes the cell membrane by free diffusion, and it is transported in the blood by binding to albumin with a rate of 60–70 % [4, 42]. After it is metabolized in the kidneys and liver (90 %), this hormone is excreted in urine and feces [42].

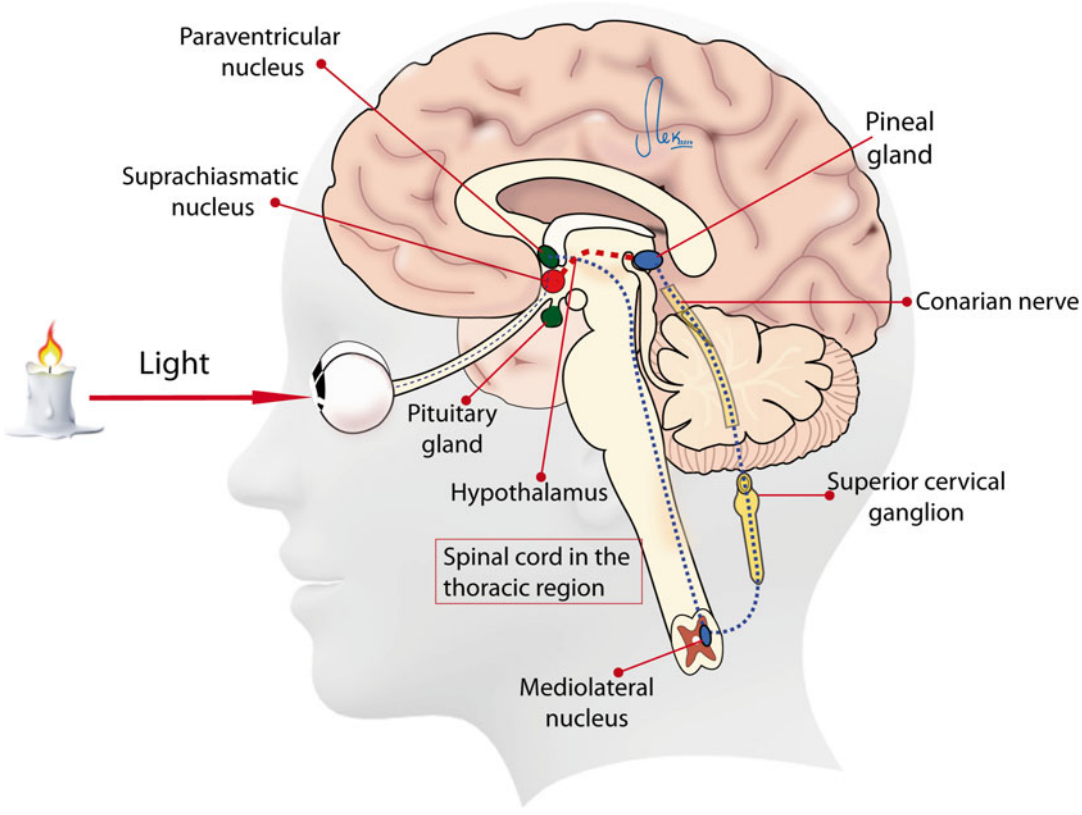

Fig. 5.1 Mel synthesis pathway (Redrawn from Özçelik et al. [38])

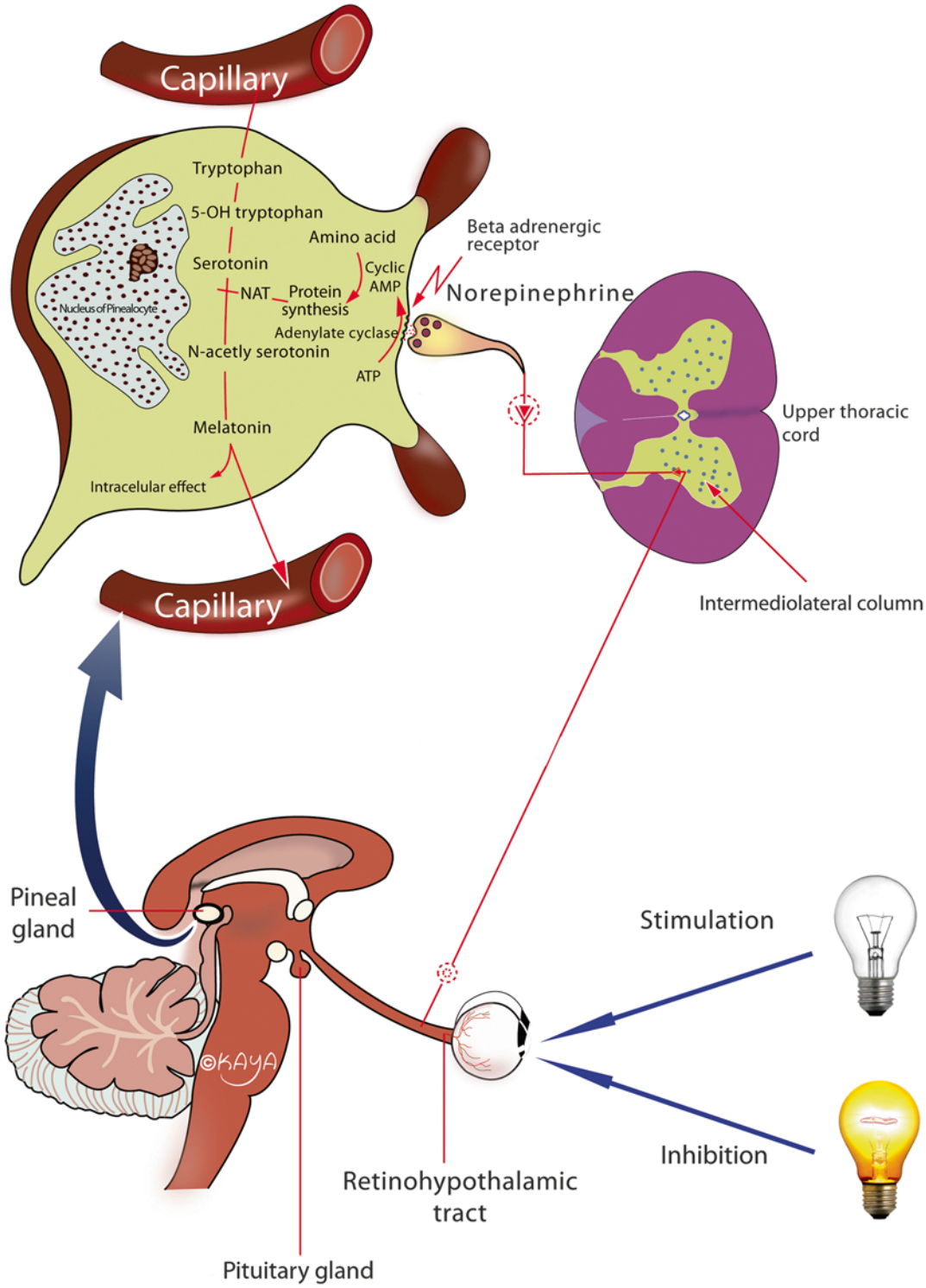

Fig. 5.2 Synthesis and control of Mel in the pineal gland (Redrawn from Reiter [44] and Brzezinski [4]). *NAT* N-acetyltransferase, *ATP* adenosine triphosphate, *AMP* adenosine monophosphate

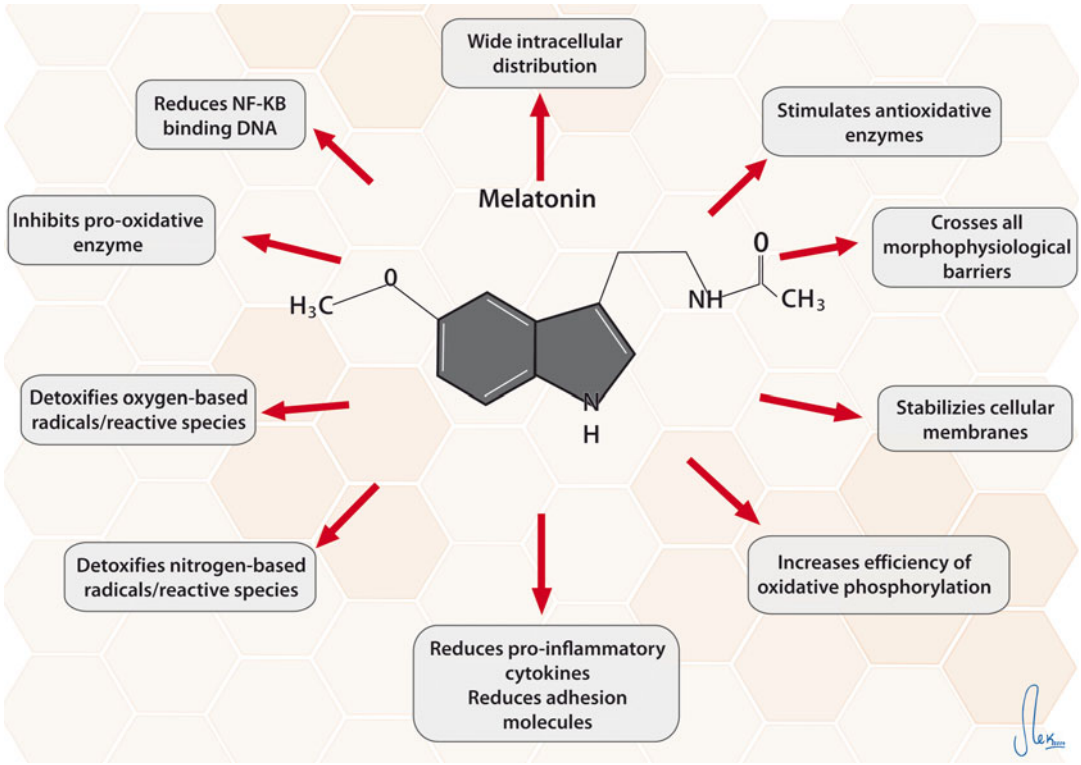

Fig. 5.3 Antioxidant properties of Mel (Redrawn from Reiter [48])

5.4 Melatonin as an Antioxidant Agent

Mel is physiologically the most powerful free radical scavenger [37]. During aerobic respiration, the 5 % part of oxygen is carried as hydroxyl radical (OH), hydrogen radical (H_2O_2), superoxide anion (O_2), or oxygen radicals (O_2^-) in the organism [41, 58]. As is known, these oxygen radicals are toxic products for the cell membrane. These radicals directly affect the cell membrane and react with phospholipids to break the integrity of the membrane. At this point, Mel is one of the antioxidant agents that protect cells against oxygen radicals (Fig. 5.3) [41]. Also, this hormone shows indirect antioxidant effects by activating GSH-Px enzyme which metabolizes hydroperoxides, increasing the activity of superoxide dismutase (SOD) which catalysis O_2 radicals to H_2O_2, preventing the decrease of catalase activity during the oxidative stress, and inhibiting the nitric oxide synthase (NOS) which is responsible for the formation of NO [47, 50, 51]. Mel is not the only material which is effective as an antioxidant and which shows an extensive intracellular distribution; however, it is more effective as a scavenger of free radicals than the other endogenous antioxidants which have high toxicity [60].

5.5 The Neuroprotective Effects of Melatonin

In several studies it has been shown that Mel has a neuroprotective effect and improves nerve regeneration [2, 24, 36]. The Mel primarily shows strong antioxidant effect by free radical scavenging activity and enhancing the activity of antioxidant enzymes in both central and peripheral nervous system. Glutathione peroxidase enzyme which is a crucial element of antioxidant defense system in the neural tissue is stimulated by this hormone [22]. In a study carried out by Reiter et al. [47], it has been shown

that Mel significantly increased glutathione peroxidase in the neural tissue, and glutathione peroxidase prevented the hydroxyl radical formation by metabolizing water to hydrogen peroxide. The tissues of the central nervous system are the most affected tissues from oxidative damage that may occur in the organism [28–30]. In spite of having a small percentage (2 %) of the body weight, the brain consumes a large portion of inspired oxygen (2 %). The by-products of oxygen are extremely toxic, and it is known that the nerve tissue is more affected by this toxicity [17, 58].

The neural tissues of the central nervous system contain relatively low antioxidant enzyme levels than the other tissues. Also, the brain contains quite high concentrations of polyunsaturated fatty acids that can easily start and continue oxidative processes [30]. Therefore, the only way to protect against free radical-mediated diseases and premature aging is the ability of resisting the molecular degradation resulting from oxidative damage process. Due to special feature which can easily pass the blood-brain barrier, Mel shows antioxidant properties in the central nervous system. Therefore, a potential barrier in the secretion of this hormone may result in the start of an oxidative process in the nervous tissue [6]. In the current studies, oxidative damage and cell loss in the layer of the hippocampus of the brain have been reported in case of the absence of Mel synthesis. Externally applied, this hormone has been reported to ameliorate the tissue damage due to pinealectomy [5, 7].

Mel also plays a role in the physiological regulation of neural function by suppressing nitric oxide synthase (NOS) activity in the cerebellum at physiological concentrations. The suppression of this hormone and NOS activity reduces the oxidative damage by decreasing the production of nitric oxide (NO) [20, 50]. In the light of the knowledge that Mel is effective in the reduction of oxidative damage with NO, a study by Chang et al. [10] investigated the neuroprotective activity of this hormone after disconnecting the peripheral branches of the hypoglossal nerve. According to the study, it has been shown that Mel effectively reduces the expression of NADPH-d and neuronal NOS which are induced by the damage of the hypoglossal motor neurons. At this point, researchers concluded that the Mel is effective in reducing oxidative stress, and it can create a positive effect on regeneration after peripheral nerve injury.

Mel reduces electrophysiological degeneration, which can be seen in peripheral nerves. The effect of this hormone administration on the peripheral nerve function of ovariectomized rats was investigated in a study, and the average distal latency was shorter in the Mel-treated group when compared with the control group, and the speed of neural transmission was significantly higher in the Mel-treated group when compared with the control group [15]. At this point, based on these data, researchers advocate that Mel has a place in the treatment of postmenopausal peripheral nerve degeneration. In another study, it has been shown that after the suture repair of rat sciatic nerve in the nerve repair area, giving exogenous Mel reduces neuroma formation and deposition of collagens and improves nerve regeneration [65, 66].

Another study which investigated the neuroprotective effect of Mel with low (10 mg/kg) and high (50 mg/kg) doses on the lipid peroxidation and nerve fiber damage after sciatic nerve damage showed that although low doses of Mel was beneficial, substantially almost all of the ultrastructural changes were neutralized with high doses of this hormone [57]. In a study by Reiter [46], it has been shown that Mel decreases the apoptotic neuronal cell death after peripheral axotomy in both central nervous system and peripheral nervous system. Retinal ganglion cell (RGH) axotomy model is often used to examine the effects of neuroprotective agents and degeneration mechanism of neurons in the central nervous system in experimental studies. Kilic et al. [26] investigated the protective effect of intraperitoneal Mel at the degeneration of retrograde right parahippocampal gyrus (RHG) on the rats that they performed optic nerve transection after pinealectomy. In this context, researchers concluded that endogenous Mel inhibits late degeneration of neurons in the central nervous system, and the exogenous Mel may have neuroprotective effect on neurons in case of lack of this

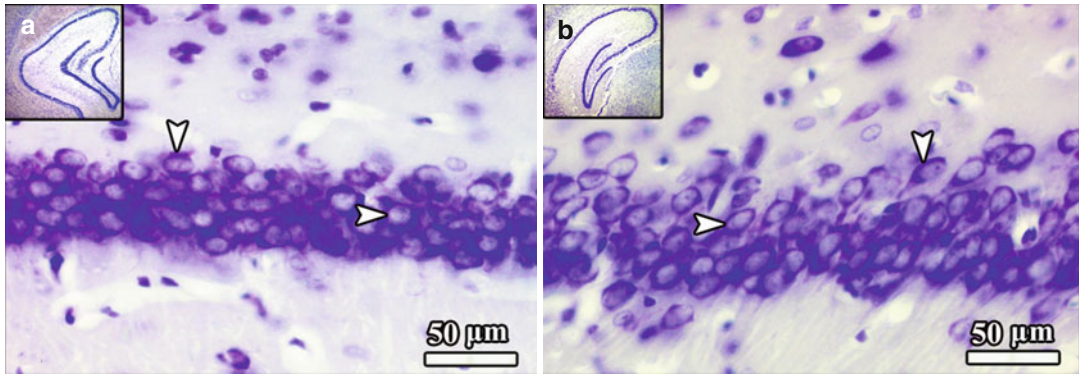

Fig. 5.4 Photomicrographs of the hippocampus belonging to the control (**a**) and Mel (**b**) groups. Mel treatment has a positive effect on the pyramidal cell regeneration in comparison of the control group. *Arrowheads* show the pyramidal cells in the hippocampus

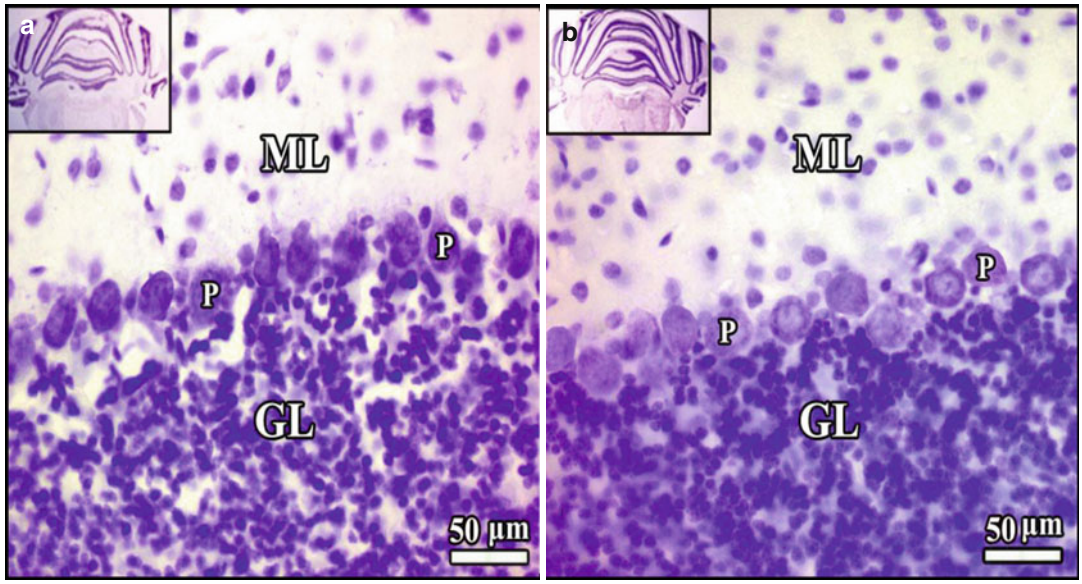

Fig. 5.5 The photomicrographs related to the cerebellum in the control (**a**) and Mel (**b**) groups. Mel treatment has a positive effect on the Purkinje cell regeneration in comparison to the control group. *ML* molecular layer, *GL* granular layer, *P* Purkinje cell

hormone. Also Fujimato et al. [19] carried out a study, and they examined the Mel effects on lipid peroxidation in spinal cord injury, and the protective effect was demonstrated by them. In another study, the antioxidant effects of Mel were shown on the spinal cord injury model, and this hormone has been found to reduce intracytoplasmic edema in neurons [25]. Therapeutic effect of Mel on nerve regeneration is shown in Figs. 5.4, 5.5, and 5.6.

5.5.1 Effects of Melatonin on the Neurodegenerative Events in the Central Nervous System

The decline in the nervous system with aging is associated with neurodegenerative diseases and physiological aging, so the best indicators are loss of memory and cognitive functions.

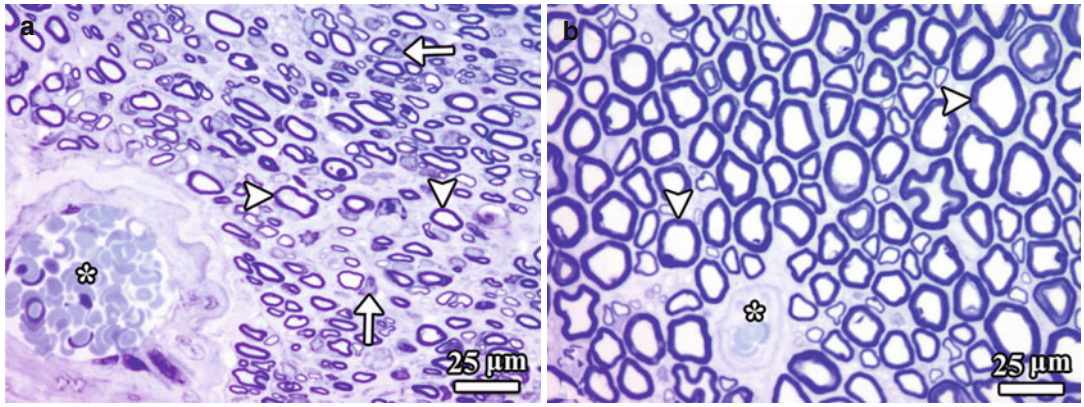

Fig. 5.6 Photomicrographs of peripheral nerve belonging to the control (**a**) and Mel (**b**) groups. Given the Mel group, increased myelinated axon number and remarkable myelination can be seen in comparison with the control group (**a**). *Arrowheads* myelinated axons, *stars* blood vessels, *arrows* Schwann cells

The relationship between aging and free radicals is one of the most topical issues of today. Brain tissues being very sensitive to oxidative damage, the development of inflammation, and the accumulation of oxidative damage in the macromolecules with aging have brought to mind that Mel treatment can be protective against age-related changes in the functions of the central nervous system. At this point, it was found that the Mel enhances neurogenesis in aged animals [56, 63]. Neurodegenerative diseases cannot be diagnosed without the formation of extensive damage in the brain. For example, when Parkinson's disease has been diagnosed in patients, 75 % of dopaminergic neurons have already been lost in the substantia nigra. Since Mel secretion declines with age, neuronal apoptosis is likely to increase with aging. Because of the in aging is seeing, taking into account all of these, starting from the Middle Ages, it would be a positive approach for Mel or other antioxidants to reduce neuronal loss rate. On the other hand, the fact that Mel lipophilic structure can easily pass through the blood-brain barrier and also the fact that the clinical applications of pharmacological doses in animals or in humans in long periods have been shown not to cause any toxicity support that this indole can be safely used [67].

5.6 The Affect Mechanisms of Melatonin and Its Receptors

Mel can easily enter cells due to its high lipid solubility when compared with water. Therefore, its effects are not just for the membrane. Its partial dissolve in the aqueous media contributes to the formation of the intracellular effects. Recent studies have shown that Mel exists in the nucleus with high concentration and Mel has specific binding points. According to these results, it has been suggested that the effects of this hormone can be associated with the molecular events in the nucleus, similar to the thyroid and steroid hormone [40]. At this point, while Mel performs molecular effect by binding to the target cells receptors, there are also cases when it does not need receptors. This hormone shows its receptor independent effects by blocking the binding of calcium to calmodulin, and it is modified by the intracellular events which are associated with the Ca^{+2} [16]. Studies have shown that Mel receptors belong to the family of G protein-coupled receptor and that they have pharmacologically different binding sites on the target structure membranes. Also it was observed that the mentioned receptors function with a circadian rhythm like a Mel receptor. Moreover, it has been suggested that in addition to Mel receptors in the cell

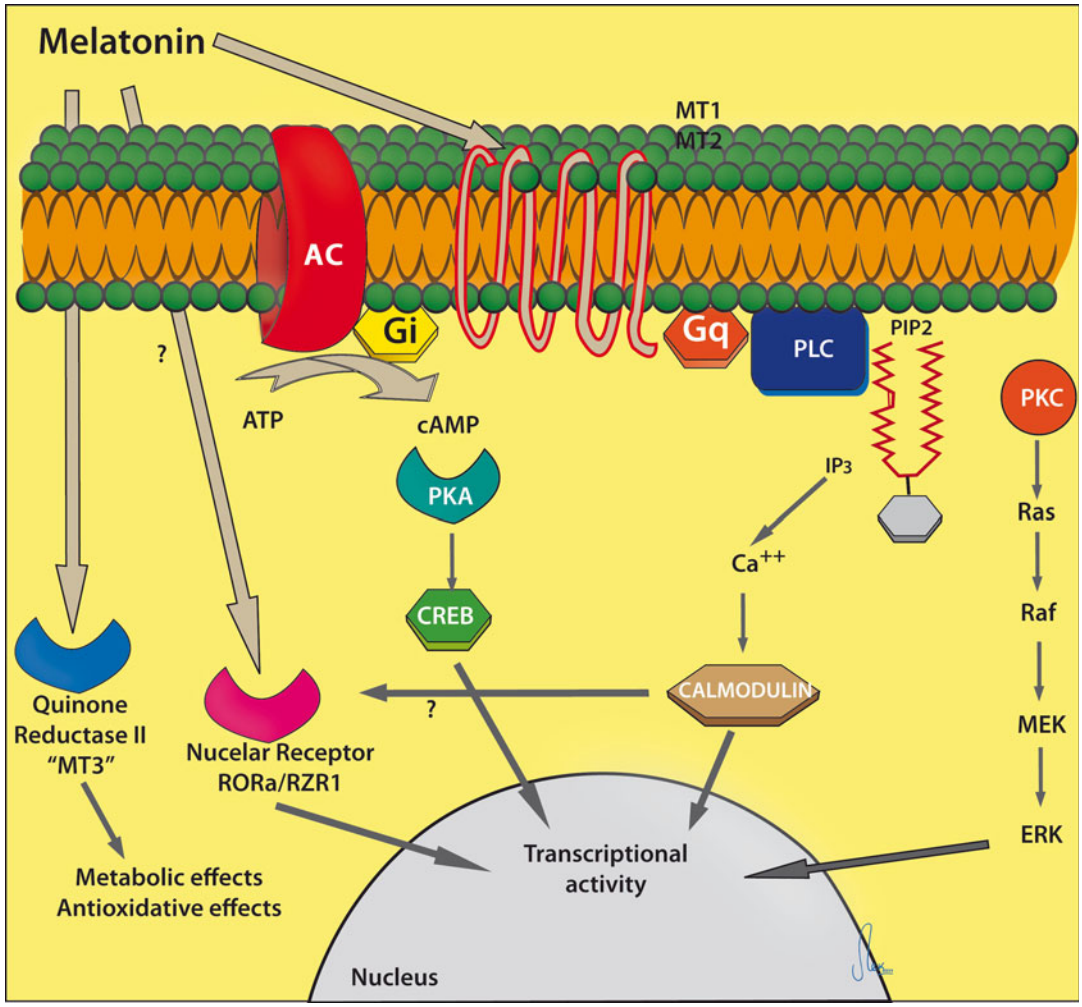

Fig. 5.7 Signaling schematic of Mel receptor subtypes (Redrawn from Slominski et al. [59])

membrane, there are similar receptors in the nucleus and Mel creates an effect on the DNA Reppert et al. (1994) [59]. A schematic diagram of Mel receptor signaling is shown in Fig. 5.7.

5.7 Melatonin Receptors: A Perspective

It has been known that Mel has receptors in many tissues, although the prevalence depends on factors such as environment, hormone, and age. Receptors of this hormone are very common especially in the central nervous system. Mel has a variety of receptors, so with this aspect, it is different from other molecules. Two types of membrane-dependent Mel receptors known as ML1 and ML2 have been identified which are members of different pharmacological families [16].

It was determined that ML1 receptors are high-affinity (Kd ~ 75 pm) receptors in the chicken and rabbit retina; however, ML2 receptors showed low affinity to the same Mel agonist which is called "2-[125I]iodomelatonin" [23, 43]. ML1 receptors include three subtypes which are Mel1a (MT1 receptors), Mel1b (MT2 receptors), and Mel1c (MT3 receptors) [12, 32]. MT1 receptor gene is encoded in localization of 4q35.1 on human chromosome. Inhibition of adenylate cyclase on the target cells can be seen after acti-

vation of these receptors. While Mel has important roles in thermoregulation, arterial vasoconstriction, proliferation of cancer cells, reproduction and metabolic functions, detection of circadian and reproductive effects, and neuroprotection via MT1 receptor, it has an important function for neuronal thermoregulation in the suprachiasmatic nucleus (SCN), inhibition of retinal dopamine secretion, induction of vasodilation, prevention of the formation of arterial bed leukocytes' rolling formation, and improvement of the immune response via MT2 receptor [64]. MT1 receptor expression is limited with the suprachiasmatic nucleus and the pars tuberalis. MT2 receptor which is encoded in 11q21-22 area on human chromosome is expressed in the brain and retina. The MT3 which is a low-affinity receptor is found in mammals, but it has not been found in humans yet [14].

ML1 receptors show neuronal settlement in the cerebellum and hippocampus. Also ML1 receptors presence have been demonstrated in the SCN, hypothalamus, thalamus, preoptic area, and plexiform layer of the retina and in many regions of the cerebral cortex [54, 61]. ML1 receptors which are nonneuronal take place in the cerebral and caudal arteries, hypophyseal pars tuberalis, ovary, kidney, and small intestine [18, 54]. MT2 receptor takes place in the immune system, brain (hypothalamus, suprachiasmatic core), retina, testes, kidney, digestive tract, mammary glands, adipose tissue, and skin [13, 53, 68]. Due to the diversity of receptors, Mel attracts attention as a multifaceted molecule in different tissues with different functions.

Conclusion

The Mel which is released from the pineal gland and which is primarily dependent on the darkness for synthesis is being investigated all over the world with a growing interest in recent years. The rhythm of the body and protecting the biological clock of the body is mainly adjusted by the Mel and many of physiological and biological processes in the body is also regulated by this hormone. Also, it has a role in many biological and physiological processes in the body. Cell regeneration, immune system strengthening, antioxidant functions, thermoregulation, anticancer agent, aging, neuroprotective effects, and sleep rhythm regulation are among the most important tasks of this hormone [4, 27, 34, 35, 49]. Also, Mel is the most powerful antioxidant because of its lipophilic feature. With this feature, it reaches all areas of the body and can easily pass the blood-brain barrier [67].

It seems that Mel which is secreted in the dark will enlighten many questions under the dark. Today, researches related to neuronal protection are one of the most comprehensive areas of neuroscience. However, although extensive studies have been done, the neuroprotective effects of this hormone are still not fully known. Therefore, the revision of existing information will guide scientists and will help for the future studies of Mel, and new trends will be uncovered.

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Melatonin Supplementation in Neurodegenerative Diseases: Current Status

6

Giovanni Polimeni, Claudio Guarneri, and Salvatore Cuzzocrea

6.1 Introduction

Neurodegenerative diseases are chronic and progressive disorders characterized by selective destruction of neurons in motor, sensory, and cognitive systems. Despite their different origins, the majority of central and peripheral nervous system degenerative diseases (including Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), and amyotrophic lateral sclerosis) share the reduced capacity to maintain the balance between free radical formation and antioxidative mechanisms as a common critical factor [1].

In recent years, research on melatonin revealed a potent activity of this hormone against oxidative and nitrosative stress-induced damage within the nervous system. Besides its antioxidant activity, melatonin has also shown to reverse chronic and acute inflammatory processes, probably due to a direct interaction with specific binding sites located in lymphocytes and macrophages [2–7]. As a consequence, melatonin may improve the clinical course of illnesses which have an inflammatory etiology [7]. With specific reference to the brain, a large body of evidence supports the use of melatonin for the preventive treatment of major neurodegenerative disorders, given also the absence of any harmful side effects even at high doses. This review summarizes the literature on the neuroprotective effects of melatonin and its potential clinical implications in those neurodegenerative diseases where free radical-mediated insult is involved.

G. Polimeni, Pharm D
Sicilian Regional Centre of Pharmacovigilance, C/O Unit of Pharmacology, A.O.U. Policlinico "G. Martino", Torre Biologica, Via Consolare Valeria - Gazzi, Messina 98125, Italy

C. Guarneri, MD (✉)
Department of Clinical Experimental Medicine, Section of Dermatology, The University of Messina C/O A.O.U. Policlinico "G. Martino", Via Consolare Valeria - Gazzi, Messina 98125, Italy
e-mail: guarneri@unime.it

S. Cuzzocrea, Pharm D
Department of Biological and Environmental Sciences, The University of Messina, viale Ferdinando Stagno D'Alcontres, 31, Messina 98166, Italy

6.2 Oxidative Mechanisms Involved in Neuronal Degeneration

Central nervous system (CNS) susceptibility to oxidative and nitrosative stress damage depends on the inherent biochemical and physiological characteristics of the brain: high metabolic activity utilizing a disproportionately large amount (20 %) of the total inhaled oxygen [8], small quantity of endogenous scavengers, wide axonal and dendritic networks, and high content of polyunsaturated fatty acids representing valid

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substrates for the formation of reactive oxygen species (ROS). Also, the presence of high concentrations of metals like iron can contribute to free radical damage by catalyzing the formation of reactive hydroxyl radicals, by inducing secondary initiation of lipid peroxidation, and by promoting the oxidation of proteins [9, 10]. Abnormally high levels of iron have been demonstrated in a number of neurodegenerative disorders including Alzheimer's disease and those characterized by nigral degeneration such as Parkinson's disease, multiple system atrophy, and progressive supranuclear palsy [11]. Astroglial cell dysfunction seems to be particularly involved in neurodegenerative damage; in physiological conditions, astrocytes participate to several brain functions (like neuronal development, synaptic activity, and cellular repairing after brain injuries) and contribute to neuroprotection through inactivation of ROS [12]. Nevertheless, "activated" astrocytes may induce the inducible nitric oxide synthases (iNOS), leading to an excessive formation of NO (and its toxic metabolites), which inhibits the mitochondrial neuronal respiration and causes cellular energy deficiency and, eventually, neuronal death [13]. Glia activation seems to originate by a chronic inflammatory state causing an immune response, with cytokine production (interferons and interleukins) and macrophages, lymphocytes, and other immune system cell involvement. The consequent, sustained release of large amounts of NO has been related to several neurological diseases with inflammatory component, including HIV-1-associated dementia, Alzheimer's disease, multiple sclerosis, and stroke [14, 15].

6.3 Role of Melatonin in Neurodegenerative Diseases

In recent decades, many research groups have focused their attention on the potential role of melatonin in neuroprotection [16–18], basing on the observation that, differently from other free radical scavengers that are either hydrophilic or lipophilic, melatonin readily passes across all morphophysiological barriers (including the blood-brain barrier) because of the amphiphilicity of its chemical structure, thus limiting oxidative damage in both the lipid and aqueous phases of cells [19]. The majority of endogenous melatonin is directly released from the pineal gland to the cerebrospinal fluid (CSF) of the brain's third ventricle; from this location, melatonin readily diffuses into the surrounding neural tissue [20]. This ability to be taken up rapidly by neural structures has prompted an intense research activity on the potential clinical use of melatonin in defense of morphological and functional integrity of the CNS. Evidences from several studies suggested that melatonin neuroprotective activity may be related to its ability to accumulate at the mitochondrial level due to its lipophilicity [21, 22] and to protect brain mitochondrial membranes from free radical attack, thus maintaining mitochondrial homeostasis and inhibiting mitochondrial cell death pathways [23]. In particular, melatonin acts not only by lowering electron leakage but also inhibiting the opening of the mitochondrial permeability transition pore (mtPTP), thus maintaining the mitochondrial respiratory electron flux [24]. This aspect is particularly important since abnormalities in mitochondrial functions such as defects in the electron transport chain/oxidative phosphorylation system and ATP production have been suggested as the primary causative factors in the pathogenesis of neurodegenerative disorders [22].

Recent works have also shown that melatonin can modulate astrocyte reactivity or death through an upregulation of antioxidative astrocytic defenses: melatonin protection against NO-induced impairment is also associated with decreased upregulation of oxidative stress-responsive genes, such as Hsp70, whose expression is a valid indicator of astroglial stress [25].

Melatonin also prevents specifically the activation of the pro-inflammatory enzymes COX-2 and iNOS in glioma cells, thus indicating an anti-inflammatory action. Importantly, melatonin does not alter COX-1 protein level, one of the major disadvantages of nonspecific nonsteroidal anti-inflammatory drugs (NSAIDs). The mecha-

nism through melatonin limits the nitrite/nitrate production and reduces iNOS expression in glioma cells which involves NF-κB pathway [26].

The efficacy of melatonin and its metabolites in either reducing the severity or delaying the onset of neurodegenerative disorders has been estimated in various conditions such as Alzheimer's or Parkinson's disease [26–30], amyotrophic lateral sclerosis [31, 32], and neural trauma [33, 34].

Moreover, data from clinical tests showed that even at the higher concentrations (dosages up to 1 g melatonin daily for 30 days), melatonin is safe and well tolerated by humans [34–36], which could encourage its long-term administration for therapeutic uses.

6.3.1 Melatonin in Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by a progressive loss of neurons, especially cholinergic neurons of the basal forebrain. Clinical manifestations of the disease consist in an irreversible memory impairment, cognitive decline, and behavioral changes. AD can occur in any decade of adulthood, but it is the most common cause of dementia in people older than 70 [37]. As a consequence, given the increasing longevity of population in developed countries (and in absence of effective treatments), the incidence of AD is expected to dramatically grow in the future.

Although its etiology is largely unknown, there is increasing evidence suggesting that neuroinflammation, immune activation, and nitrosative and oxidative stress play a critical role in the pathogenesis of AD [38]. The postmortem histopathological analysis confirmed an elevated lipid peroxidation in the brains of AD patients [39, 40], also showing effects of the disease on DNA oxidation and protein reorganization in the brain cortex, with typical abnormalities in cytoskeletal architecture [41, 42].

The inflammatory component in AD significantly contributes to cell stress by promoting microglial activation with the resultant generation of inflammatory cytokines and neurotoxic free radicals [43]; however, inflammatory manifestations in AD typically lack in some features such as neutrophil infiltration and edema, whereas other characteristics including acute-phase proteins and cytokine accumulation can be identified.

The neurotoxic effect has been ascribed to the intracellular formation of (i) neurofibrillary tangles (NFT), that are histopathological lesions consisting of hyperphosphorylated microtubule-associated protein tau at the cytoskeletal level, and (ii) senile plaques, derived from the extracellular accumulation of soluble beta-amyloid peptides (A-beta) in arterial walls of cerebral blood vessels [44].

Tau protein promotes microtubule assembly and stabilization; it also takes part in the formation and maintenance of the axonal structure [45]. Hyperphosphorylated tau protein reduces the ability to stabilize microtubules, leading to disruption of the cytoskeletal arrangement and neuronal transport [46]. In AD, the cytoskeleton is abnormally assembled into NFT, and impairment of neurotransmission occurs. It is widely accepted that hyperphosphorylation of the tau protein is due to an imbalance between the activities of protein kinases and protein phosphatases, suggesting that these proteins could serve as therapeutic targets for AD.

Amyloid-beta1-42 peptide (A-beta1-42), which is a fragment derived from proteinase-induced cleavage of amyloid precursor protein (APP), is believed to play a major role in promoting neuronal degeneration. Actually, although the mechanism underlying A-beta neurotoxicity remains to be fully elucidated, there is increasing evidence that A-beta induces mitochondrial dysfunction, triggers apoptosis, and increases the intracellular levels of calcium and ROS in the AD brain, thus leading to a series of events which destroy adjacent neurons [47, 48].

The involvement of ROS in neuronal death associated to AD led to investigate the effects of antioxidants in preventing or delaying the sequence of events causing neuronal destruction. Among these, melatonin has been deeply evaluated, due not only to its potent activity as

free radical scavenger but also to the finding that lower levels of endogenous melatonin were observed both in serum and in CSF from AD patients compared with that in age-matched control subjects [49]. Moreover, it has been observed that the concentration of melatonin in CSF decreases with the progression of AD neuropathology [50] and that melatonin levels both in CSF and in postmortem human pineal gland are already reduced in AD subjects manifesting only the earliest signs of AD neuropathology, in absence of any sign of cognitive impairment [51–53]. The decrease in melatonin levels correlates with the severity of dementia and appears to be a consequence rather than a cause of the disease [51]. As a consequence, the determination of CSF concentration of melatonin has been proposed as an early marker for the detection of AD [54].

Restoration of melatonin levels might induce a benefit in AD patients whenever melatonin receptors are functional [55]. The antioxidative protection against A-beta of melatonin has been confirmed by Pappolla et al. which showed that co-incubation of both murine neuroblastoma (N2a) and pheochromocytoma (PC12) cells with A-beta peptides and melatonin greatly reduced the degree of A-beta-induced lipid peroxidation, thus greatly increasing the survival of the cells [56]. In vitro studies demonstrated that melatonin could efficiently protect from alterations in neurofilament hyperphosphorylation and accumulation induced by calyculin A (CA), through not only its antioxidant effect but also its direct regulatory effect on the activities of protein kinases and protein phosphatases [57]. A recent in vivo study by Yang et al. [29] confirmed that administration of melatonin intraperitoneally for nine consecutive days before injection of CA in 78 male Sprague-Dawley rats could prevent CA-induced synaptophysin loss, memory retention deficits, as well as hyperphosphorylation of tau and neurofilaments.

Furthermore, supplementation with melatonin by prior injection and reinforcement during haloperidol administration significantly improved memory retention deficits, arrested tau hyperphosphorylation and oxidative stress, and restored protein phosphatase 2A activity [57].

Melatonin decreased not only oxidative stress and tau hyperphosphorylation but also reversed glycogen synthase kinase 3 (GSK-3) activation, thereby showing that melatonin's actions exceeded its antioxidant effects and also interfered with the phosphorylation system, especially stress kinases [56]. Tyrosine kinase (trk) receptors, representing an additional important element of the phosphorylation system and neurotrophins, were also shown to be affected by oxidotoxins, including A-beta. In neuroblastoma cells, melatonin was capable of normalizing trk and neurotrophin expression [58].

Melatonin has shown to reduce the generation of A-beta peptide [59, 60] and also thereby secondarily reduces neuronal death more efficiently than other antioxidants [61]. This effect is secondary to the inhibition of the proteolytic processing of soluble derivatives of amyloid precursor protein (sAPP), as shown by in vitro experiments [62, 63].

Counteraction by melatonin against A-beta-induced apoptosis has been repeatedly demonstrated in a number of cellular models of AD including mouse microglial BV2 cells, rat astroglioma C6 cells, and PC12 cells [64–66]. Studies in transgenic AD mice and cultured cells have suggested that administration of melatonin prevented the A-beta-induced upregulation of apoptosis-related factors such as Bax and suppressed caspase-3 activity [64, 67–69]. Experiments in mouse microglial BV2 cells in vitro showed that melatonin also decreased caspase-3 activity, inhibited NF-κB activation, and reduced the generation of A-beta-induced intracellular ROS [70]. In addition, in vivo observations showed that melatonin-treated animals had diminished expression of NF-κB compared to untreated animals [71]. Moreover, melatonin inhibited the phosphorylation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase via a PI3K/Akt-dependent signaling pathway in microglia exposed to A-beta1-42 [72]. Taken together, the abovementioned evidences suggest that melatonin may provide an effective means of treatment for AD through its antiapoptotic activities.

Melatonin displays its antifibrillogenic activity even in the presence of the profibrillogenic

apolipoprotein E4 (apoE4) and antagonizes the neurotoxic, synergistic potentiation between A-beta protein and apoE4 or apoE3 [73]. ApoE4, which aggravates A-beta effects, is also produced by astrocytes.

The anti-amyloidogenic properties of melatonin in AD have been also observed in transgenic mice: melatonin not only inhibited amyloid plaque deposition but also improved learning and memory deficits in an APP695 transgenic mouse model of AD [67]. However, the antifibrillogenic effect was not observed when the treatment was started in old transgenic mice [74], confirming that, after numerous amyloid plaques have been formed and neuronal damage has progressed, melatonin is no longer capable of efficiently antagonizing amyloid deposition and amyloid-dependent damage.

In a recent study melatonin administration and physical exercise were tested on 3xTg-AD mice with moderate to advanced phases of AD. Both treatments protected against cognitive impairment, brain oxidative stress, and a decrease in mitochondrial DNA. Furthermore, results showed an additive effect of melatonin plus physical exercise against a range of AD symptoms (sensorimotor reflexes, learning, etc.) [75].

Interestingly, the neuroprotective and anti-amyloidogenic properties of melatonin are not mediated by MT1 and MT2 melatonin membrane receptors: experimental studies with MT1 and MT2 receptor agonists without antioxidant properties showed no effects on neuroblastoma cells and primary hippocampal neurons [59]. Nevertheless, MT1 and MT2 expression in the human brain appears to be altered by pathological conditions such as AD, as observed in post-mortem brain from AD patients [76]. The role of MT2 receptors in hippocampal synaptic plasticity and in memory processes is also suggested by the fact that transgenic mice deficient in MT2 receptors demonstrated deficient hippocampal long-term potentiation.

With regard to the anti-inflammatory effects of melatonin, the most important feature is its inhibition of mitochondrial iNOS expression [77, 78]. Antioxidant and anti-inflammatory properties of melatonin are relevant in mitochondrial physiology, and they may play a neuroprotective role in neurodegenerative disorders [79]. Melatonin supplementation in patients with AD significantly slows down the progression of cognitive impairment and decreases brain atrophy [80]. In a study of 14 patients at various stages of AD, melatonin supplementation for 22–35 months improved sleep and significantly reduced the incidence of "sundowning." Furthermore, patients experienced no cognitive or behavioral deterioration during the study period [81.

Administration of melatonin to AD patients has been found to improve significantly sleep and circadian abnormality and generally to slow down the progression of the disease [82, 83]. Jean-Louis et al. reported the effects of melatonin administration in two patients with AD [84]. Melatonin enhanced and stabilized the circadian rest-activity rhythm in one of the patients along with some reduction of daytime sleepiness and mood improvement. The results of this and many other clinical studies are not statistically significant because of small sample size. Therefore, the supplementary use of melatonin in AD patients cannot be recommended, but further evidences from clinical trials are needed.

6.3.2 Melatonin in Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease, occurring most commonly in the elderly. It is believed to affect approximately 1 % of the population over 55 years of age [85]. Pathologically, PD is characterized by loss of pigmented neurons and gliosis, most prominently in the substantia nigra pars compacta (SNpc) and locus coeruleus (LC), and by the presence of fibrillar cytoplasmic inclusions, known as Lewy bodies [86]. The Lewy bodies are concentric eosinophilic cytoplasmic intraneuronal inclusions with peripheral halos and dense cores, whose presence is essential for the pathological confirmation of PD. This depletion of neurons presents clinically with severe motor symptoms including uncontrollable resting tremor, bradykinesia, rigidity, and postural imbalance.

The exact etiology of PD remains to be fully elucidated, but the key theories propose either an environmental toxicants or a genetic origin or a combination of both [87–90]. Currently, the only therapies approved for the treatment of PD are agents that attenuate the symptoms of the disease.

Research data have clearly indicated that during PD, SN dopaminergic neurons are subject to oxidative and nitrosative stress, mitochondrial dysfunction, proteasome inhibition, and protein aggregation, leading eventually to cell death [91, 92]. Nevertheless, the precise relationship among these pathways has not been completely defined. Moreover, brains from PD patients show evidence of elevated oxidative damage to DNA [93], lipid peroxidation and oxidative modification of proteins [94], decreased levels of reduced glutathione (GSH), and increased monoamine oxidase (MAO) activity [95] that indicate reduced antioxidant defense mechanisms [91]. Dopamine oxidation by MAO leads to the formation of ROS [96] and, if not effectively detoxified by glutathione, hydrogen peroxide might potentially induce the generation of highly reactive hydroxyl radicals in the presence of excess iron via the Fenton reaction.

There are several neurotoxin-based models that have been important in studying the mechanisms of PD pathogenesis. These include the neuronal oxidotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) [97], which produced symptoms very similar to PD after injecting in mice, cats, and primates. After administration, MPTP crosses the blood-brain barrier and is metabolized into the 1-methyl-4-phenylpyridinium ion (MPP+), by MAO type B; MPP+ is then selectively taken up by dopaminergic neurons. MPP+ toxicity is believed to result from the mitochondrial inhibition of complex I, leading to oxidative stress [98], depletion of NAD and ATP, and apoptosis [99, 100]. Exposure to MPTP results in nigrostriatal dopaminergic degeneration with 50–93 % cell loss in the SNpc and more than 99 % loss of dopamine in the striatum [101].

Protection by melatonin was demonstrated in a variety of experimental PD models. MPTP-induced stress was antagonized by melatonin at the levels of mitochondrial radical accumulation, mitochondrial DNA damage, as well as breakdown of the proton potential [102]. Lewy bodies, which are considered cytopathologic markers of parkinsonism, comprise abnormal arrangements of tubulin and microtubule-associated proteins, MAP1 and MAP2. Melatonin effectively promotes cytoskeletal rearrangements and was assumed to have a potential therapeutic value in the treatment of parkinsonism and, perhaps, generally in dementias with Lewy bodies [103]. In unilaterally 6-OHDA-injected-hemi-parkinsonian rats, protective effects by melatonin were also attributed to normalizations of complex I activity [104]. Suppression of NO formation and scavenging of reactive nitrogen species by melatonin and its metabolite AMK should additionally support cell survival, along with other protective effects, such as upregulation of the antioxidant enzymes Cu, ZnSOD, MnSOD, and GPx, which has been demonstrated in cultured dopaminergic cells [105]. However, while complex I inhibition is a plausible cause of neurodegeneration in the toxicological animal models, it would be of particular importance to know whether mitochondrial dysfunction is relevant in the PD patient. In fact, recent investigations did not reveal any differences in complex I, II/III, and IV activities in mitochondria from platelets [106]. However, this does not entirely exclude striatal mitochondrial dysfunction in advanced stages of PD, because of an impairment by iron-mediated oxidative stress [22].

Melatonin secretion patterns have been studied in patients suffering from PD. An increase in the secretion of melatonin was noted in PD patients under dopaminergic treatment but not in untreated, as compared to control subjects [107]. This increased melatonin secretion in response to dopaminergic therapy may be related to recent findings linking dopamine to the regulation of the pineal gland. Animal models have identified the D4 dopamine receptor on the pineal gland [108]. Furthermore, the release of serotonin and melatonin from the pineal gland is reported to be controlled by circadian-related heterodimerization of adrenergic and dopamine D4 receptors [109].

The finding that dopaminergic treatment profoundly affects the secretion of melatonin and the regulation of circadian phase and sleep timing in PD patients [107] suggests that there may be some form of melatonin resistance among patients during the activation of their circadian systems and could account for the limited success of this therapy in PD patients with insomnia [27, 110].

Alternatively, the additional increase of daytime melatonin under medication with L-DOPA can be interpreted as an adaptive mechanism in response to the neurodegenerative process and possibly reflect a neuroprotective property of melatonin. Further studies are needed to identify the mechanism affecting these phenomena.

Studies undertaken in elderly insomniacs have demonstrated that melatonin can increase sleep efficiency and decrease nighttime activity [111, 112]. Administration of melatonin in 5 mg/day for 1 week reduced the nocturnal wake time for about 20 min in eight patients with PD [113]. In a recent double-blind, placebo-controlled study on 40 subjects conducted over 10 weeks, Dowling et al. [110] noted that administration of a higher dose of melatonin, 50 mg/day, increased actigraphically scored total nighttime sleep in PD patients, when compared with 5 mg or placebo-treated patients. Subjective reports of overall sleep disturbance improved significantly with 5 mg of melatonin compared to 50 mg or placebo [110]. This study may indicate that very high doses of melatonin can be tolerated in PD patients over a 10-week period as in healthy older adults.

6.3.3 Melatonin in Huntington Disease

Huntington disease (HD) is a neurodegenerative disorder with a progressive motor impairment, cognitive decline, and psychiatric disturbances [114, 115]. It is a genetically programmed neuronal degeneration inherited in an autosomal dominant manner and caused by an expanded CAG triplet in the gene encoding the protein huntingtin (HTT), a cytoplasmic protein whose functions are not fully understood [115]. Brain areas initially involved are the striatum and then the cortex.

Many evidences suggest the presence of an abnormal conformation of mutant HTT [116]. The clinical course, probably resulting by neuronal deterioration dysfunction and cell death [114], is characterized by a typical motor dysfunction such as involuntary, unwanted movements that primarily involve distal extremities and facial muscles and then all other muscles, with a distal to proximal progression. If movement disorders are distinguishing, also other signs fulfill the clinical picture: unintended weight loss, sleep and circadian rhythm disturbances, dysarthria, dysphagia, dystonia, tics, cerebellar signs, psychiatric symptoms (depression, anxiety, apathy, obsessions, compulsions, irritability, aggression, loss of interest, psychosis), autonomic disturbances, cognitive decline, muscle wasting, metabolic dysfunction, and endocrine disturbances [117], suggesting neurological and non-neurological-mediated mechanisms [118, 119]. Cell death heavily involves striatal area [120] with pronounced loss of GABAergic medium spiny projection neurons. Also described is atrophy of the cerebral cortex, subcortical white matter, thalamus, etc. Pathognomonic of HD are intranuclear inclusion bodies, which are large aggregates of abnormal HTT in neuronal nuclei, also described in cytoplasm, dendrites, and axon terminals [120, 121]. A possible molecular mechanisms in HD pathogenesis involved intracellular calcium overload causing mitochondrial blockade, NMDA receptor-mediated excitation, and oxidative stress [81]. Currently, evidence is lacking as to whether antioxidant, neuroprotective, and antiapoptotic activities of melatonin and its action on mitochondria have an impact in HD [22, 26].

6.3.4 Melatonin and Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease, affecting both the first and second motoneuron. The progression of ALS is characterized by a degeneration of motor neurons associated with a dramatic demyelination in the anterior horn of the spinal cord. The etiology is only partially understood.

Pathophysiologically, three major mechanisms are discussed in ALS: (a) mutations of the superoxide dismutase 1 (SOD1) gene, causing a toxic gain of function with enhanced reactivity toward abnormal substrates (tyrosine nitration), along with an impaired ability to bind zinc leading to a reduced antioxidant capacity; (b) mutations in neurofilament genes and oxidative modifications or hyperphosphorylation of cytoskeletal proteins leading to selective motor axon degeneration; and (c) excitotoxicity caused by increased cerebrospinal fluid glutamate levels together with a loss of excitatory amino acid transporters [122].

There is no promising treatment available to date. The only compound used for its effects in survival time is an anti-excitotoxin, riluzole. As the common basis of cellular and extracellular alterations in ALS seems to be oxidative stress mediated by reactive nitrogen/oxygen species, future attempts of treatment might focus on antioxidant strategies involving suppression of nitric oxide (NO) synthase.

Melatonin is a candidate compound for neuroprotection in ALS patients, who tolerated high doses of melatonin [32]. Melatonin has a unique broad spectrum of effects including scavenging of hydroxyl, carbonate, alkoxyl, peroxyl, and aryl cation radicals; stimulation of glutathione peroxidase; and other protective enzymes but also suppression of NO synthase. The interference with NO metabolism has multiple consequences: downregulation of NO formation counteracts damage by peroxynitrite-dependent radicals as well as Ca^{2+} -dependent excitotoxicity. This pleiotropy may explain, at least in part, why melatonin has been identified as a potent neuroprotectant, e.g., by attenuating oxidative damage after experimental neurotrauma [123, 124].

Furthermore, since melatonin appears to be free from side effects even upon long-term applications, melatonin can be used as a prophylaxis to treat those patients that are at risk of developing ALS. Such patients would be those that have been identified with the genetic marker associated with ALS, i.e., familial form of ALS, and those patients who exhibit early signs of motor neuron disease, such as impaired motor control.

Although melatonin seems to have a rapid turnover, the administration of slow-release preparations maintains high plasma levels for about 6 h. In SOD1(G93A)-transgenic mice, high-dose oral melatonin delayed disease progression and extended survival. In a clinical safety study, chronic high-dose (300 mg/day) rectal melatonin was well tolerated during an observation period of up to 2 years. Importantly, circulating serum protein carbonyls, which provide a surrogate marker for oxidative stress, were elevated in ALS patients but were normalized to control values by melatonin treatment. This combination of preclinical effectiveness and proven safety in humans suggests that high-dose melatonin is suitable for clinical trials aimed at neuroprotection through antioxidation in ALS [31]. The role of melatonin in this disease needs to be explored further conducting well-designed clinical trials.

6.4 Perspective

Besides its well-known regulatory role on circadian rhythm, the growing body of observations from experiments with endogenously produced and exogenously administered melatonin disclosed its involvement in a broad range of biological functions, ubiquitously occurring in the body. These activities, particularly the capacity of melatonin to reduce the degree of tissue damage and limit the progression of those diseases associated with oxidative stress, have been documented in a number of in vitro and in vivo studies and are being tested for their clinical implications.

With specific reference to the brain, as mentioned above, the fact that melatonin readily crosses the blood-brain-barrier, coupled with its optimal safety profile at the highest dosages, represents two major advantages compared to other available antioxidants. An indirect proof confirming the preventive effect of this hormone against free radical insult is the significant presence of melatonin in several plant species known for their neuroprotective properties, which are often consumed with the food or used in the context of Chinese traditional medicine [125].

The increased prevalence of neurodegenerative diseases in developed countries and absence of effective and/or well-tolerated treatments for many of them urge further investigation to elucidate the role of free radicals in such disorders and the potential clinical role of melatonin (as well as other antioxidants) in slowing down their progression. Confirmations from large clinical trials will also be essential to identify clinically relevant concentrations of melatonin required under different pathological conditions.

Regrettably, the number of randomized controlled trials testing the effectiveness of melatonin on neurodegenerative disorders is still very low, and their quality is often poor. The limitations of interventional trials in this field are that, because diseases have a long induction period, these types of studies may not be very feasible because of high costs and excessive time required. Furthermore, although epidemiological studies clearly show a correlation between the increased consumption of food rich in antioxidants and a decreased risk of oxidative stress-induced diseases, it is often very difficult to establish whether supplementation beyond dietary intake levels is of benefit, as well as to interpret the role of the nutraceutical supplementation in the progression of the disease. As a consequence, hard endpoints (such as mortality) are rarely used. To overcome this difficulty, the development of validated biomarkers as intermediate endpoints may help to understand the complexity of degenerative diseases at their different stages.

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7 Therapeutic Potential of Melatonin in Combination with Other Drugs Against Neurodegeneration

Eva Ramos, Paloma Patiño, José Luis Marco-Contelles, Ramón Cacabelos, and Alejandro Romero

7.1 Introduction

It is well known that melatonin, a pleiotropic molecule, originally discovered as a hormone synthesized mainly in the mammalian pineal gland during the dark phase, is also produced by immune system cells and in many peripheral tissues including the brain, airway epithelium, bone marrow, gut, ovary, testes, skin, and likely other tissues [1]. In mammals, three major melatoninergic membrane receptors have been identified. Among them, MT1 and MT2 represent the melatonin receptor system, their physiological functions and pharmacological properties being well documented [2]. Also MT3 receptor has been identified as quinone reductase 2 [3]. Nuclear binding sites/receptors also exist for this indolamine and have been identified as a further class of melatonin receptors [4]. The antioxidant abilities of this neurohormone could derive either from direct scavenging of free radicals or by increasing the activity and expression of antioxidant enzymes. Thus, deficiencies in melatonin production or melatonin receptor expression and decreases in melatonin levels can lead to numerous dysfunctions. The toxicity and tissue damage induced by a large number of drugs and substances are a consequence of free radical generation and related reactants [5]. Recent advances in melatonin knowledge have suggested the possibility of enhancing the therapeutic activity of different drugs and/or to reduce the possible side effects when they are administered [5]. Melatonin has been prescribed in both physiological and pharmacological amounts to humans and animals, and there is widespread agreement that it is a nontoxic molecule. In addition, this wide therapeutic range gives the possibility to adapt melatonin doses depending on the different effects expected.

Neurodegenerative diseases are characterized by a chronic and progressive deterioration of

E. Ramos • A. Romero, PhD (✉)
Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Complutense University of Madrid, Avda. Puerta de Hierro s/n, Madrid 28040, Spain
e-mail: manarome@ucm.es

P. Patiño
Paediatric Unit, La Paz University Hospital, Paseo de la Castellana 261, 28046-Madrid, Spain

J.L. Marco-Contelles
Medicinal Chemistry Laboratory, Institute of General Organic Chemistry (CSIC), Juan de la Cierva, 3, Madrid 28006, Spain

R. Cacabelos
Chair of Genomic Medicine, Camilo José Cela University, Madrid 28692, Spain

EuroEspes Biomedical Research Center, Institute of Medical Science and Genomic Medicine, Bergondo, Corunna 15165, Spain

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specific neuronal populations, and despite of the different origin, there are three frequently interrelated processes, such as glutamate excitotoxicity, free radical-mediated damage, and mitochondrial dysfunction, identified as common pathophysiological mechanisms leading to neuronal death [6].

Increasing evidence suggests that melatonin rhythmicity plays by itself a key role in several metabolic functions as an antioxidant, anti-inflammatory, and epigenetic regulator, through mechanisms which include nuclear receptors and histone acetylating and DNA methylating enzymes [7]. Therefore, the neuroprotective properties of melatonin against these three processes, its regulatory effects on circadian disturbances coupled to its remarkable tolerability and safety, point melatonin as a promising molecule in neurodegenerative disease treatment.

Currently, we can find many in vitro and in vivo studies in the literature showing the neuroprotective effect of melatonin in neurodegenerative diseases, mainly alone but also in combination with other drugs. In this chapter, we summarize the interesting perspectives given by the studies carried out with this multifunctional indolamine in combined therapy with other pharmaceutical agents used for the treatment of neurodegenerative diseases.

7.2 Alzheimer's Disease

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by progressive loss of cognitive function, loss of cholinergic neurons, impaired memory, extracellular senile plaques of aggregated β-amyloid (Aβ), intracellular neurofibrillary tangles (NFTs) mainly composed of abnormally hyperphosphorylated tau protein, and other neurological manifestations. Thus far, the etiology of AD remains unknown; genetic factors, chronic inflammation associated with cytokine release, oxidative stress, and metal ion neurotoxicity have been suggested as possible causes of the disease [8]. Therefore, Aβ, NFTs, inflammation, oxidative stress, and metal ions are important therapeutic targets in AD. It has been shown that melatonin reduces Aβ toxicity, reducing Aβ generation or deposition, and efficiently protects against Aβ oxidative damage in cells [9–12]. Tau hyperphosphorylation reduces its functions leading to cytoskeletal disruption [13]; thus, it has been demonstrated that melatonin efficiently attenuates this hyperphosphorylation [14, 15]. Neuroinflammation is a common factor in AD, and melatonin has been shown to significantly reduce the pro-inflammatory response [11]. Additionally, in AD patients, levels of melatonin are lower compared to other aged-matched control subjects [16–19].

Nowadays, AD treatment is mainly symptomatic offering only temporary benefits. There are only three acetylcholinesterase inhibitors (AChEI) (donepezil, rivastigmine, and galantamine) approved to treat AD symptoms. Moreover, the combination with memantine, a *N*-methyl-D-aspartate receptor antagonist, limits glutamate excitotoxicity besides the cholinergic deficit. Some clinical studies with AD patients following this cholinergic treatment, with or without memantine, introduced a combination therapy with prolonged-release melatonin that significantly improved cognitive performance, sleep efficiency, and also insomnia [20]. Combination therapy thus is an alternative strategy to address the complex pathophysiology of AD at several points, offering a superior efficacy. However, this combination of molecules with metabolic or pharmacokinetic differences involves new challenges to solve. The design of complex molecules able to interact with two or more targets, the so-called multi-target-directed ligands (MTDLs), has emerged as a powerful drug design alternative and runs parallel to drug combination in the treatment of AD.

The new melatonin-dibenzyl amine hybrids are an example of this promising type of molecules. López-Iglesias et al. explored the therapeutic potential of these hybrids evaluating in vitro its neuroprotective activity, showing that at low-micromolar concentrations, these molecules could promote auto-repair processes from neural stem cells in the CNS, protect neurons from mitochondrial oxidative stress, increase patient memory, and reduce the formation of

amyloid plaques [21]. Also melatonin-tacrine heterodimers were reported more potent AChEI than tacrine, showing potent antioxidant capacity, and due to its low in vitro toxicity and ability to penetrate across the blood-brain barrier, it is expected that they may be able to increase patient cognition, diminish the oxidative damage caused by mitochondrial free radicals, and delay the degenerative process related to the excessive deposition of Aβ [22, 23]. In several experiments, transgenic (Tg) murine models of AD have been used to assess a possible therapeutical beneficial effect of melatonin. In this line, melatonin was found to be effective, inhibiting Aβ deposition in APP 695 Tg mice, alleviating behavioral impairments and memory deficit deficiencies following long-term administration of melatonin at a daily dose of 10 mg/kg [24]. In APP/PS1 Tg mice, treatments with melatonin from 2 to 2.5 months reduced cognitive impairments, decreasing Aβ deposition and inflammatory cytokines in the hippocampus and entorhinal cortex [25]. APP/PS1 Tg mice were also used to confirm the efficacy of a tacrine-melatonin hybrid to inhibit amyloid-induced cell death and reduce Aβ toxicity, suggesting the possibility for a new potential therapeutic strategy in AD [26].

The expression of the MT2 melatonin receptor in the hippocampus is decreased in AD patients [27]. Some studies address an interaction between valproic acid and the melatoninergic system upregulating MT1 and MT2 melatonin receptors, providing a new target in the strategy for attenuating the loss of hippocampal neurons and synaptic connection, also enhancing the beneficial effects of endogenous or administered melatonin [27–29].

In neuroblastoma models using Aβ peptides or okadaic acid as model of AD, melatonin inhibits free radical production, measured as lipid peroxidation, by scavenging free radicals generated by these two neurotoxic agents [30, 31]. In this study, either melatonin or vitamin C administration prevented the effects of okadaic acid. The reduction of free radical damage was greater with melatonin, due to that melatonin increased the levels of glutathione S-transferase and glutathione reductase. Melatonin is also able to reverse the GSK-3 activation induced by calyculin A, supporting that melatonin actions are not only based on its scavenging effect [15]. In AD patients, a 20–50 % reduction of α7 subtype of nicotinic acetylcholine receptors (nAChRs) has been observed in specific brain areas [32]. Hence, it is important to maintain an intact cholinergic function to avoid cognitive impairments. Melatonin is a modulator of nAChRs, as demonstrated in studies in vas deferens contraction by acetylcholine in rats [33, 34]. Recently, it has been proposed that melatonin should be administered at night to increase the number of α7 nAChRs and allow a reduction in anticholinesterase dose [35]. In this sense, it has been demonstrated that combination of subeffective concentrations of melatonin and galantamine protects against oxidative stress in neuroblastoma cells [36], which opens a new pathway to explore, finding synergic combinations of melatonin with other molecules, and may reduce the possible toxicity of the molecules at high concentrations and increase the efficacy at low concentrations.

Several clinical trials have been documented on the possible application of melatonin to improve some of the symptoms of AD, such as sleep difficulties and cognitive impairment. Melatonin (3 mg/day; nine patients vs. placebo; 11 patients) for 4 weeks significantly prolonged the sleep time and decreased locomotor activity at night, in a double-blind study. Melatonin treatment also caused an improvement in cognition [37]. In earlier stages of the AD in patients with mild cognitive impairment, this indolamine also showed significantly better performance concomitantly with an improvement in wakefulness and sleep quality [37–39]. However, in two multicenter, randomized, placebo-controlled clinical trials, melatonin (2.5 or 10 mg slow release; 8.5 mg immediate release and 1.5 mg sustained release) did not show significant differences compared to placebo in objective sleep measures and circadian rhythmicity [40, 41]. In chronic insomnia, the main problem is the circulation half-life time of melatonin, between 20 and 30 min or less, promoting sleep initiation, but sleep maintenance is only partially solved [42].

Haloperidol, used in AD patients who develop psychotic features and disruptive behaviors [43],

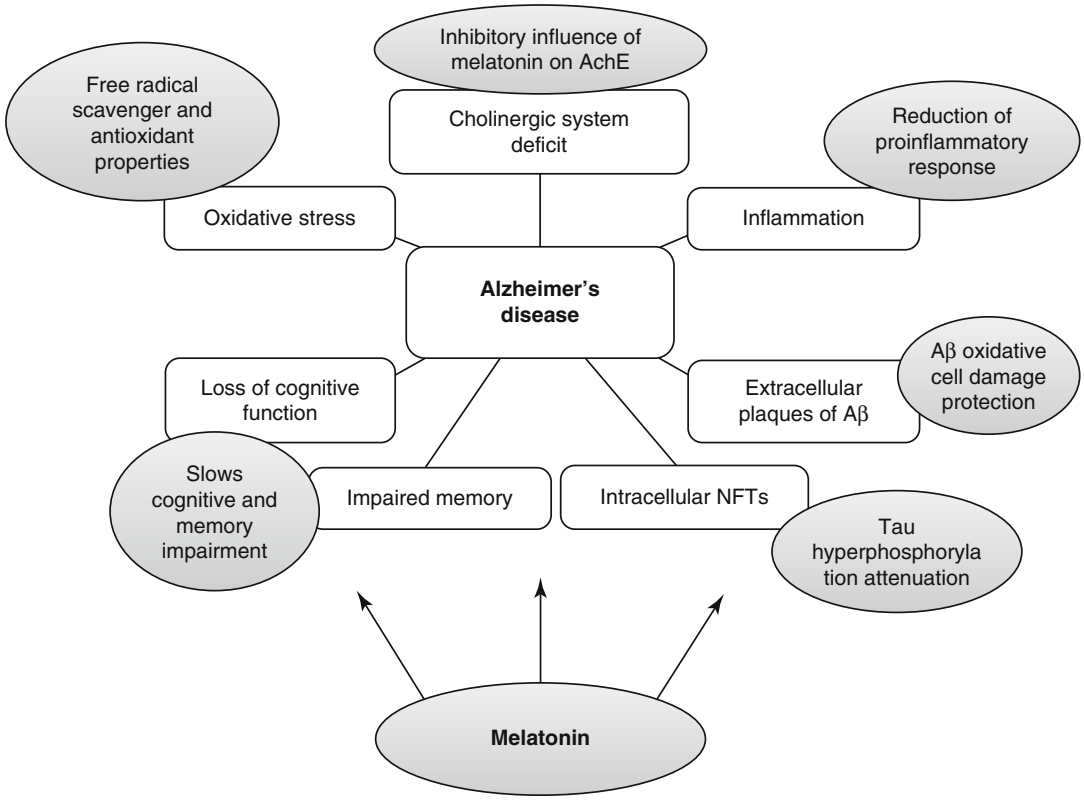

Fig. 7.1 Schematic representation of melatonin effects on AD main factors

in a combination therapy with melatonin, seems to prevent the antipsychotic side effects, which may be due to its dopamine modulatory effects and to the oxidative neurotoxicity prevention [44]. It has been described that clonazepam, also used to treat REM sleep behavior disorder, which often appears in late stages of neurodegenerative disorders, in a co-treatment with melatonin, may be helpful in some cases, even when clonazepam alone had failed [45] (Fig. 7.1).

7.3 Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after AD affecting particularly the elderly. PD is characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta. Clinical symptoms appear when about 3/4 of dopaminergic cells are lost in this region. Tremor, rigidity, bradykinesia, and postural instability are the main symptoms of PD [46]. There are other non-motor symptoms that can appear before the onset of PD, like REM-associated sleep behavior disorder (RDB), depression, or hyposmia, which should not be underestimated, given that they reduce patient quality of life and lead to a worse prognosis of the disease. Biochemical studies suggest that reactive oxygen/nitrogen species are important mediators in the pathogenesis of PD, being oxidative stress the major cause of dopaminergic neuronal death. Hence, the antioxidant properties of melatonin could be useful for the treatment of PD patients. There are many in vitro and in vivo studies showing melatonin neuroprotective effect against toxicity induced in PD models like neurotoxin1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) [47, 48] and 6-hydroxydopamine [49, 50]. In these models, melatonin significantly prevented the increased lipid peroxidation and also increased the levels of

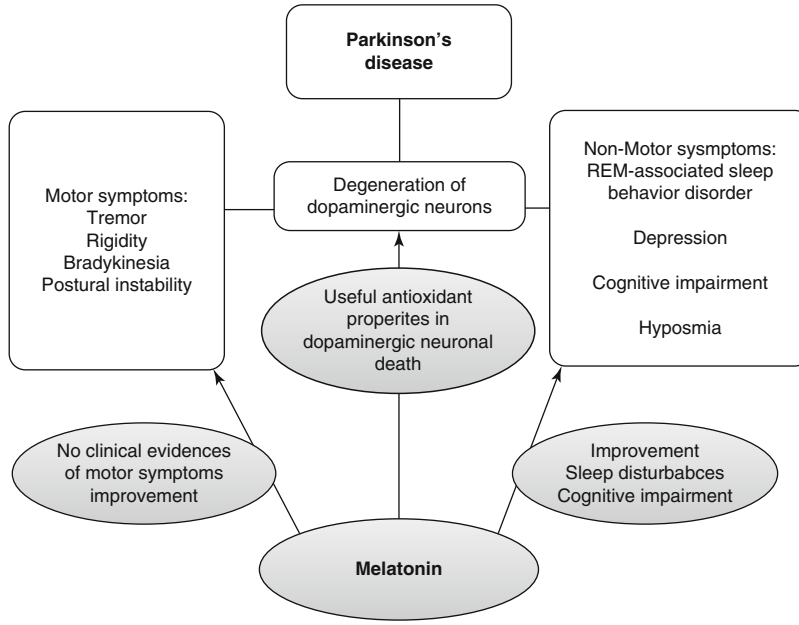

Fig. 7.2 Schematic representation of melatonin effects on PD progressive loss of dopaminergic neurons and associated clinical symptoms

antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase. Moreover, MPTP could inhibit mitochondrial complex I of the electronic transport chain (ETC). It has been suggested that melatonin, by increasing the activity of complex I and IV of the ETC, could exert a beneficial effect on MPTP-induced damage [51].

Studies carried out in patients with PD assess the possible role of melatonin in the modification of symptoms associated with this disease, such as sleep difficulties, cognitive impairment, or motor dysfunctions combined with a stable PD medication [52–54]. A double-blind, placebo-controlled crossover trial enrolled 40 subjects with PD who complained of sleep disturbances. All subjects were taking stable doses of antiparkinsonian medications during the course of the study; a significant improvement in total nighttime sleep duration was observed during the 50 mg melatonin treatment. Furthermore, there was also a significant improvement in subjective sleep quality and quantity and daytime sleepiness during the 5 mg melatonin treatment compared to placebo [55]. In another study, 18 PD patients were randomized to receive melatonin (3 mg) or placebo 1 h before bedtime for 4 weeks while taking levodopa with therapeutic benefit. In patients with lower doses of levodopa, a more severe sleep fragmentation was observed; although melatonin significantly improved subjective quality of sleep, it could not improve motor dysfunction [53] (Fig. 7.2).

7.4 Huntington's Disease

Huntington's disease (HD) is a hereditary disease characterized by a progressive motor impairment, cognitive decline, and psychiatric disturbances and, at the moment, with no effective cure. Some therapeutic strategies are focused on the antioxidant defense, and multiple studies in vitro and in vivo report a beneficial effect of melatonin as a protector of neuronal damage.

Sleep disturbances are reported in 80% of HD patients [56], and it can substantially impair their quality of life. It has also been observed that melatonin concentrations are reduced in HD [57, 58]. Furthermore, diurnal melatonin levels strongly correlated with both motor and func-

tional impairment, indicating that melatonin levels in HD may progressively decline with advancing disease [57]. As it is known, and as a result of its major role in regulating the circadian rhythms, melatonin has been widely demonstrated to alleviate sleep disturbances. Melatonin administration was reported to defer the clinical signs of the disease in an animal model of HD likely involving its antioxidative properties [59]. In a more recent study, melatonin delayed disease onset and prolonged lifespan in the R6/2 transgenic mouse HD model. The importance of the MT1 receptors in the protective effect of melatonin has also been demonstrated in this genetic model of HD [60].

7.5 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal disease that devastates CNS and the most common form of motor neuron disease. It is typically characterized by a progressive degeneration of the upper and lower motor neurons, excitotoxicity, free radical injury, inflammation, ischemia, and eventual activation of proteases that are the cell death mechanisms involved in HD [61, 62].

The cellular and extracellular alterations seem to be related to the oxidative stress, inflammation, and apoptosis, a series of facts that suggest that molecules like melatonin are a possible therapeutic agent, given that its mechanism of action involves protection at all these events [63]. However, there are only a few studies with melatonin in ALS. In 2002, the first study with melatonin was initiated, with only three ALS patients included; they were taking their ALS regular medication (riluzole, vitamins C and E, creatine, and amitriptyline), and melatonin was then coadministered at a dose of 30–60 mg for 13 months, obtaining promising results, but it was not enough to definitively guarantee the efficacy of melatonin [64]. This group continues testing the possible melatonin role in ALS neuroprotection in vitro and in vivo. In cell-cultured motoneurons, melatonin was able to attenuate glutamate-induced death and orally administered in a genetic mouse model of ALS; melatonin delayed disease progression by 25 % and extended survival. In 31 ALS patients, melatonin (5 mg/kg) was applied nightly as a suppository during 2–24 months. Melatonin was well tolerated with some patients, reporting improved sleep quality and reduced protein carbonyls in blood, for up to 4 months, compared to levels before treatment onset [65]. It has been suggested that MT1 and MT2 melatonin receptors are involved in the neuroprotection of motoneurons from glutamate excitotoxicity [66, 67], which explains how melatonin exerts its neuroprotection against this cell death factor.

Conclusion

The efficiency of melatonin counteracting the toxic reactions of drugs and other processes that generate free radicals and associated reactants is almost daily demonstrated in new publications. Commonly, it is heard that "melatonin helps against everything," maybe ironically but perhaps due to the exceptional pleiotropy of this neurohormone and its beneficial effects in several diseases. Mechanistically, melatonin has a variety of actions, such as a direct free radical scavenger activity, indirect antioxidant properties, and anti-inflammatory and neuromodulatory effects. Considering melatonin diverse actions via both receptor and receptor-independent actions and given melatonin high lipophilicity and how easily it crosses morphophysiological barriers, melatonin appears as a good candidate in the development of combination therapy for neurodegenerative diseases. Furthermore, considering the effectiveness of the results obtained with a combination therapy of melatonin and other drugs added to the safety of melatonin and the lack of an actual effective pharmacological therapy in most of the neurodegenerative diseases, the possibility of a combined therapy with melatonin reaches a prominent place in further clinical trials.

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8 Melatonin, a Neuroprotective Agent: Relevance for Stress-Induced Neuropsychiatric Disorders

Piyarat Govitrapong, Kasima Ekthuwapranee, Nootchanart Ruksee, and Parichart Boontem

8.1 Introduction

The term neuropsychiatric disorder commonly refers to psychiatric disease caused by brain disorders and is classified as an organic mental disorder by the International Classification of Diseases Version 10 (ICD-10). The major components of neuropsychiatric symptoms are cognitive impairment and disturbance of consciousness. Additional common neuropsychiatric symptoms include depression, anxiety, paranoid-hallucinatory states, and behavioral and personality changes. Depression is one of the most common neuropsychiatric symptoms that frequently occur in Alzheimer's patients, affecting as many as 50 % of patients and causing a significant burden on *individuals*, families, and society. In addition to patients with Alzheimer's disease, patients with Parkinson's disease and cerebral stroke also reported a depressive symptom. Depression is associated with various symptoms, including depressed mood, change in sleep or activity, guilt, loss of energy or fatigue, and suicidality. An epidemiological study showed a relationship between environmental factors such as stressful life events and an increased risk for depression [1]. However, there is a *difference* in *individual stress vulnerability*; most individuals present normal outcomes even after exposure to stress [2].

P. Govitrapong (✉)
Research Center for Neuroscience,
Institute of Molecular Biosciences,
Mahidol University, Salaya, Thailand

Center for Neuroscience and Department
of Pharmacology, Faculty of Science,
Mahidol University, Salaya, Thailand
e-mail: piyarat.gov@mahidol.ac.th

K. Ekthuwapranee • P. Boontem
Research Center for Neuroscience,
Institute of Molecular Biosciences,
Mahidol University, Salaya, Thailand

N. Ruksee
Research Center for Neuroscience,
Institute of Molecular Biosciences,
Mahidol University, Salaya, Thailand

National Institute for Child and Family Development,
Mahidol University, Salaya, Thailand

8.2 Stress-Induced Neuropsychiatric Disorder

Chronic stress is a major factor in depressive disorders. Dysregulation of the HPA axis is a common characteristic of depression [3, 4]. A previous study showed that depressed patients present an abnormal response in the dexamethasone suppression test. This test is used to measure the ability of an exogenous glucocorticoid receptor agonist to suppress endogenous stress hormone release [5]. In depressed patients, an impaired negative feedback regulation of stress response indicated a downregulation of

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glucocorticoid receptors [6]. Depression or stress induces neuronal atrophy of the adult hippocampus, which may contribute to the molecular changes observed in the pathophysiology of depression [7, 8]. Furthermore, the chronic mild stress showed that depression-like behaviors are accompanied by inflammation, neuronal cell damage, decreased neurogenesis, and apoptosis in the hippocampus [9]. Chronic high glucocorticoid levels, such as in long-term stress, could affect several brain regions. The hippocampus is one of the brain areas susceptible to the deleterious effects of stress.

The hippocampus not only plays an important role in learning and memory but also processes information from several brain regions, including the amygdala and prefrontal cortex to regulate emotional information [10, 11]. Several groups of investigators have reported alterations in the circadian rhythm in the neuroendocrine system during depression. Polymorphisms in clock-related genes have been shown to be associated with circadian rhythm disorders such as familial sleep syndrome and delayed sleep phase syndrome, both of which present a higher risk for depression [12].

Our previous study showed that mice treated with a high dose of dexamethasone, a synthetic glucocorticoid receptor agonist, for 21 consecutive days displayed immobility times that were significantly longer than those in the control group, using the forced swimming test [13]. This finding indicates that chronic dexamethasone administration induced depressive-like behavior. In addition, using the Morris water maze, we showed that dexamethasone-treated mice had significantly impaired spatial memory. The dexamethasone-treated mice had prolonged water maze performance latencies and spent less time in the target quadrant compared with the control group [14]. In addition, an impaired memory performance has been demonstrated in humans with high glucocorticoid levels [15–17]. Thus, there is consistent evidence in several models and species suggesting that stress or high glucocorticoid levels have a negative effect on hippocampal functioning. The hippocampus contains a high density of glucocorticoid receptors and is vulnerable to stress-induced damage, which impairs both plasticity and function.

8.3 Melatonin and Stress-Induced Neuropsychiatric Disorders

Melatonin is a hormone mainly secreted in the pineal gland. After being released from the pineal gland, melatonin is discharged into the blood vascular system and possibly also into the cerebrospinal fluid (CSF) of the third ventricle. Both blood and CSF melatonin have access to the suprachiasmatic nucleus (SCN) neurons, where they act on melatonin receptor 1 (MT1) and melatonin receptor 2 (MT2). This phenomenon indicates that melatonin influences the firing rate of the SCN neurons, thereby resetting the circadian pacemaker and regulating circadian processes such as sleep.

The secretion of melatonin by the pineal gland could possibly be associated with the release of cortisol, although the mechanism underlying this relationship is still unclear [18]. Recently, melatonin has been observed to directly inhibit adrenocorticotropic hormone (ACTH) responses in the human adrenal gland [19], presumably contributing to the lowest release of cortisol at night, when melatonin is secreted at its maximum.

Melatonin has pleiotropic neurobiological actions mediated through three known binding sites. The first site is a cytosolic enzyme that has been described as quinone reductase 2 (QR2/MT3), which is involved in toxification and detoxification processes. Melatonin type 1 (MT1) and melatonin type 2 (MT2) receptors are seven transmembrane G-protein-coupled receptors. The exact roles of MT1 and MT2 are unclear. The ratios of the expression of these two receptors might be important for some melatonin functions. Alterations in the ratio of MT1 to MT2 receptors in the brain have been indicated in neurodegenerative disorders such as Alzheimer's disease [20]. Melatonin treatment in

subventricular zone (SVZ) progenitor cells showed an increase in the expression of MT1, whereas this treatment did not exert any effect on the expression of MT2 [21]. In animals, the effects of fluoxetine, desipramine, and clomipramine on MT1/MT2 mRNAs were specific to the brain. In the hippocampus, protracted treatment with these antidepressants increased the amount of MT1 mRNA but decreased the MT2 mRNA content. In the striatum, antidepressants decreased the MT1 mRNA level [22].

Melatonin is widely used as a dietary supplement in various countries. Melatonin is a chronobiotic drug that improves sleep propensity, decreases sleep latency, reduces alertness and neurocognitive functioning, and lowers the core body temperature [23]. In animal models, melatonin exerts certain antidepressant-like actions. A recent study showed that melatonin significantly reduced the risk of depressive symptoms in women with breast cancer, during a 3-month period after surgery [24]. Chronic nighttime melatonin supplement significantly attenuated stress-induced behavioral disturbances, especially cognitive impairment and depressive behaviors [25]. Fonken et al. showed that mice exposed to light at night showed depression-like responses and a lower performance in a hippocampus-dependent learning and memory task. These results indicate that exposure to the light at night can alter mood in mice [26].

The Srinivasan group indicated that agomelatine is very effective in attenuating depression-like symptoms and showed an early onset of action with a good tolerability and safety profile. Agomelatine improved sleep capability and ameliorated circadian rhythm disruption [27]. Combined melatonin and exercise therapy in humans indicated that melatonin and exercise are useful independent adjunct therapies for bipolar disorder [28]. A study in maternally separated rats showed that melatonin supplementation ameliorates the negative impact of stress on the circadian system [29]. Compared with other antidepressant drugs, agomelatine has significantly higher efficacy and potential acceptability compared with SSRIs and SNRIs, when treating depression [30]. Ramelteon, a drug that binds to MT1 and MT2 receptors with high affinity, has been approved by the US Food and Drug Administration (FDA) for the treatment of sleep disturbance particularly that associated with delayed sleep onset.

Our recent study using the force swimming test showed that mice treated with melatonin followed by dexamethasone had a significant decrease in immobility time compared with dexamethasone-treated mice. This outcome indicated that chronic administration of dexamethasone-induced depression-like behavior, which could be prevented by pretreatment with melatonin [13]. In addition, the Morris water maze was performed, which showed that dexamethasone-treated mice had significantly higher escape latency than the control and the group pretreated with melatonin prior to dexamethasone administration had a significantly reduced escape latency compared with the dexamethasone-treated group. The duration in the target quadrant was used as an index of memory. The decrease of the duration in the target quadrant due to the dexamethasone treatment was significantly attenuated by the melatonin treatment [14].

8.4 Melatonin as a Neuroprotective Agent During Stress

Melatonin administration was associated with diminished overall glucocorticoid (GCs) secretion and increased sensitivity to glucocorticoid feedback. Melatonin significantly prevented the stress-induced decrease in glucocorticoid receptor (GR) expression and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) protein levels in the hippocampus, two molecules implicated in depression, and improved the modulation of the HPA axis [31]. Moreover, melatonin inhibits the nuclear translocation of the GR in mouse thymocytes [32]. Using different animal models, it has been shown that stress [33] or glucocorticoids [34–36] cause a downregulation of

brain-derived neurotrophic factor (BDNF) protein levels and mRNA expression in the hippocampus [37], whereas reductions in GCs by adrenalectomy upregulate exon IV BDNF transcript [38]. Several results indicate that the expression of BDNF decreases in various mental disorders such as schizophrenia, bipolar disorder, and major depression [39–41]. Thus, it is possible to conclude that both BDNF and GCs are associated with synaptic function and the pathophysiology of depression in the hippocampus [37]. Furthermore, an in vitro study indicated that melatonin promotes the viability and neuronal differentiation of neural stem cells and increases BDNF production [42].

The reduction of melatonin in patients suffering from depression is most likely caused by a disturbance of the noradrenergic transmission [43, 44]. Furthermore, the results obtained from Ramirez-Rodriguez's experiments are in accordance with those regarding the neurotransmitter content [45]. This outcome indicates that after treatment with melatonin, serotonin increased to a higher extent than norepinephrine and dopamine; an upregulation in serotonergic level resulting from melatonin treatment is presumably predominant in antidepressant action. Moreover, a chronic melatonin treatment attenuated the glucocorticoid-induced decrease in the hippocampal progenitor cell proliferation, similar to antidepressant action that improves the balance of GC level. In addition, melatonin decreases the GR translocation to the nuclei in HT22 cells [46]. Thus, melatonin is an interesting molecule, because it is an endogenous modulatory factor and may be able to restore disturbed physiological balances. A study by Kim in 2002 indicated that various types of stress, such as immobilization, restraint, and psychosocial conflict, can damage brain structures and impair brain activity [47]. The hippocampus is a brain area that has recently received significant attention in mood disorder research. Long-term stress and prolonged GC exposure cause structural and functional changes in the hippocampus, including atrophy of apical dendrites of CA3 pyramidal neurons [48].

8.5 Antioxidant Effect of Melatonin

Several experiments indicated that melatonin functions as an antioxidant, directly acting as a potent free radical scavenger [43, 49], and has protective effects on neuronal cells [50]. Shen and colleagues have postulated that melatonin's ability to improve cognitive functions is related to its antioxidant action [51]. Melatonin receptors have been found in several brain regions, as well as in the hippocampus, and they play an important role in learning and memory function in mice [52]. Melatonin alters electrophysiological processes associated with memory, such as long-term potentiation (LTP). Furthermore, the MT1/MT2 agonist ramelteon is capable of increasing the neuronal BDNF protein content [53]. In the hippocampus, melatonin acts as a neuroprotective agent and might be beneficial to improving memory in neurological diseases. Melatonin modulates the expression of cell adhesion molecules and the release of serotonin, enhances short-term memory in rats, and prevents oxidative stress caused by aluminum in the rat hippocampus [54]. Melatonin regulates the complex interaction of the dendrites of new neurons in the adult hippocampus [55]. Administration of melatonin to humans with mild cognitive impairment is more effective in controlling cognitive, sleep, and mood decay than that in patients with fully expressed Alzheimer's disease [56]. This outcome demonstrated the role of melatonin from a clinical perspective.

Glucocorticoids inhibited BDNF-induced synaptic maturation and excitatory neurotransmitter glutamate release [57, 58]. With respect to the effect of BDNF on behavior, mice that are genetically deficient in the BDNF-TrkB system fail to show antidepressant behavioral outcomes, indicating that hippocampal BDNF regulates neurogenesis and behavior in the mice [59]. In vitro, melatonin promotes the viability and neuronal differentiation of neural stem cells and increases the expression of BDNF [42]. In rodents, a downregulation of both BDNF mRNA and BDNF protein was found in several brain

regions following stressful paradigms [60, 61]. Interestingly, an impairment of the regulation of GC homeostasis is considered to be associated with the symptoms of depressive disorder [62, 63]. Because GCs are known to control stress responses, and the BDNF expression is changed during stress, it is possible that BDNF is a functionally significant downstream target in regulating behavior. A recent investigation found that pretreatment with dexamethasone reduced BDNF [64, 65].

The role of the interaction between BDNF and N-methyl-D-aspartate (NMDA) receptors in the mechanism of learning and memory is a subject of widespread attention. Previous studies reported that the relationship between BDNF/TrkB signaling and NMDA receptors is very important for spatial memory formation [66]. The NMDA receptor is involved in a wide variety of processes in the central nervous system, including synaptogenesis and synaptic plasticity. Calcium flux through NMDA receptors is believed to play a critical role in synaptic plasticity, which is a cellular mechanism for learning and memory. Long-term potentiation, the most intensively studied cellular and molecular model for learning and memory, in the hippocampus is generally dependent on NMDA receptor activation [67]. BDNF increases the expression of NR1, NR2A, and NR2B NMDA receptor subunits in cultured hippocampal neurons [68]. A recent study found that BDNF-enriched synaptic proteins, including NR2A, NR2B, GluR1, and synapsin I, were suppressed by dexamethasone [57]. An alteration of NMDA receptor expression in the hippocampus and cerebral cortex by pharmacological doses of melatonin suggests that the hippocampus may be the area in which melatonin regulates cognitive processes [69]. Melatonin modulates the expression of NMDA receptor and may regulate neuronal plasticity [70]. Previous studies showed that melatonin (10 mg/kg) significantly increased both NMDAR 2A and NMDAR 2B expression levels compared with those in the control group [71].

Our previous study demonstrated that dexamethasone treatment caused morphological changes in the hippocampus and decreased the number of CA3 pyramidal neurons. These effects may be due to oxidative stress and neurotoxicity enhancement. The melatonin pretreatment prevented dexamethasone-induced impairment of the CA3 region neurons, which suggests that melatonin counteracts dexamethasone-induced oxidative stress [14]. Reiter et al. recently reported that melatonin and its metabolites act as powerful neuroprotective agents [72]. We additionally detected the expression levels of the proteins, BDNF, NR2A/B, CaMKII, and synaptophysin in the hippocampus and prefrontal cortex exposed to chronic dexamethasone administration. We found that all of these proteins were decreased in the prefrontal cortex, and all, except synaptophysin, were decreased in the hippocampus. Pretreatment with melatonin prevented these reductions.

Several studies have indicated that dexamethasone can regulate apoptotic cell death and oxidative damage [73–75]. In our recent study [76], we have shown that dexamethasone induces an increase in reactive oxygen species (ROS) formation and reduces mitochondrial calcium homeostasis in SH-SY5Y cells, which can be abolished by melatonin. Melatonin administration was associated with decreased overall corticosterone secretion and increased sensitivity to glucocorticoid feedback. These findings suggest that long-term melatonin treatment may protect several regulatory components of the HPA axis [77]. Melatonin inhibits dexamethasone action by inhibiting the GR [78] and reduces transcription activity [79]. Melatonin plays a protective role by inhibiting the translocation of the GR into the nucleus by impairing HSP90 dissociation [32]. Melatonin significantly prevented stress-induced decrease in the GR protein levels in the hippocampus [80]. Therefore, hyperactivity of the HPA axis involves impairment of the feedback inhibition of glucocorticoids, possibly caused by the diminished regulation or dysregulation of the GR. Moreover, excessive glucocorticoids might decrease the TrkB-bound GR and decrease BDNF signaling and BDNF-induced glutamate release [81]. MT1 and MT2 melatonin receptors

were downregulated by dexamethasone treatment [82]. Altogether, our results suggest that the influence of melatonin and dexamethasone on different levels of protein expression appears to be via oxidative stress and the GR-dependent pathway.

8.6 Chronic Stress and Neurogenesis

In the adult rat, 9,000 new neurons are born per day and survive with a half-life of 28 days [83]. The dentate gyrus (DG) of the hippocampus is able to generate new neurons throughout life, with neural progenitor cells dividing in the subgranular zone and migrating to the granule layer [84]. Various stressful experiences increased plasma corticosterone levels, leading to a significant inhibition of hippocampal cell proliferation [85]. Chronic stress and high GC levels can suppress neurogenesis in the DG. Stress-induced cellular changes in the hippocampal area, such as dendritic debranching, neuronal and glial cellular shrinkage, apoptosis, reduced gliogenesis, and reductions in dentate neurogenesis, could significantly contribute to the hippocampal volume. A study by McEwen showed that stress induced the remodeling of hippocampal dendrites and reduction of DG neurogenesis, which lead to cognitive impairment and behavioral alterations [86]. Recently, chronic stress decreased neurogenesis in the subgranular zone of the hippocampus in the stages of proliferation, survival, and differentiation of progenitor cells [44]. In rodents, antidepressants enhance hippocampal neurogenesis [87]. In addition, the decrease in DG neurogenesis may be involved in the etiology of depression [88, 89]. Moreover, stress exposure suppresses cell proliferation and increases cell death in the DG [46].

8.7 Melatonin and Neurogenic Hypothesis of Depression

The neurogenic hypothesis of depression is a model that has been suggested for explaining how depression occurs and how it may be treated. Recent human studies indicate that a major class of antidepressant such as tricyclic antidepressants (TCA) and monoamine-oxidase inhibitor (MAOI) enhances cell proliferation levels beyond that of SSRIs [90]. These results indicate a role for hippocampal neurogenesis in successful antidepressant treatment.

Adult hippocampal neurogenesis has been reported to exhibit circadian variation in cyclic light-dark conditions. Fujioka et al. observed that the number of bromodeoxyuridine (BrdU)-labeled proliferating cells and that of BrdU and class III β-tubulin double-labeled cells [newborn neurons] in the granule cell layer of the group constantly exposed to light was significantly lower compared to the same in the group exposed to light and darkness. These results demonstrate that constant light conditions inhibit hippocampal neurogenesis and suggest a critical role for an environment with light-dark cycles in maintaining the hippocampal system [91]. These findings are consistent with the study of Tamai et al. which showed that the M-phase cell number significantly increased during the night but was constant throughout the day, indicating the daily rhythm of progenitors [92]. It appears that molecules secreted during the night, such as melatonin, might regulate the generation of newborn neurons.

Administration of glucocorticoids inhibits the proliferation of progenitor cells in adult hippocampus [93]. Long-term exposure to mild stress leads to a reduction in the neurogenesis in ault animals [94]. Adrenal steroid inhibition in aged animals can lead to the recovery of neurogenesis similar to that observed in adult rats, suggesting that aging animals retain the capacity for undergoing neurogenesis at the same rate observed in adults [95]. An in vitro study using corticosterone and dexamethasone, glucocorticoid receptor agonists, found that corticosterone decreases the number of BrdU-labeled cells and caused dendritic atrophy in mature neural cells [96]. Other studies found that dexamethasone exerts a deleterious effect on proliferation, but not differentiation. It is summarized that corticosterone elicits its effects on neurogenesis, including proliferation and differentiation, whereas glucocorticoid

receptor stimulation is sufficient to decrease only proliferation [97]. These results are consistent with the study by Kim and his colleagues, which showed that glucocorticoid receptor activation suppresses only proliferation, but not differentiation of hippocampal neurogenesis [98]. In contrast, other studies showed that activation of glucocorticoid receptor by dexamethasone inhibits neuronal differentiation [99].

A study using the California Verbal Learning Test in younger depressive adults indicated that individuals with mild to moderate unipolar depression showed a decline in verbal memory performance compared with the healthy group, suggesting that memory impairment is associated with depression [100]. A chronic unpredictable stress model of rat showed a significant decrease in locomotive activity and learning ability. Moreover, the synaptic plasticity of hippocampus neurons is also decreased, whereas the level of serum cortisol increased, suggesting the association between depression and learning inhibition [101]. A study by Yun et al. suggested that chronic restraint stress decreases hippocampal neurogenesis, leading to an impairment of hippocampus-dependent fear memory in mice [102]. The study in prenatal stress in rats showed a reduction of neurogenesis in the dentate gyrus, an impairment in hippocampal-related spatial tasks, and inhibition of learning-induced neurogenesis [103]. Chronic social stress leads to an impairment in neurogenesis in the adult hippocampus, weakens spatial learning, and strengthens habit-like responses [104].

While a correlation between the decrease in the hippocampal neurogenesis and depression is indicated, it is still unclear whether decreases in neurogenesis are a cause or consequence of depression. Several groups of studies reported that the major antidepressants stimulated proliferation in the dentate gyrus of the hippocampus [105]. Additionally, these drugs showed no effect on proliferation in the subventricular zone or the primary motor cortex, indicating that neurogenesis in the hippocampus alone may play a role in depression [106]. A study using rivastigmine indicated that rivastigmine can increase neurogenesis and improve depression-like behaviors. This result concluded that rivastigmine treatment restores the normal function of the hippocampal system, an activity that possibly ameliorates depressive behaviors in patients with Alzheimer's disease [107]. Beta-asarone treatment has been found to increase the expression of BDNF at the levels of transcription and translation and hippocampal neurogenesis and can reverse chronic unpredictable mild stress-induced depression-like behaviors, studied using both sucrose preference tests and the forced swim. Moreover, beta-asarone can attenuate the reduction of ERK1/2 phosphorylation and CREB phosphorylation in chronic unpredictable mild stress rats. These data demonstrated that adult neurogenesis is involved in antidepressant-like behavioral effects [108]. A study by Hayashi suggested that mice with decreased postnatal neurogenesis exhibit a susceptibility to stress and may present decreased neuronal activity and cognitive deficits in the adult [109]. These results are consistent with those from a different study that suggested that deficits in adult neurogenesis lead to a behavior indicative of anxious and depressive-like mood states [110]. A long-term experiment to allow full differentiation and integration of new neurons in the neuronal circuitry indicated that the neurons in the hippocampus are a key factor for the long-term spontaneous recovery from depressive-like behavior, as well as for the action of antidepressants [111]. A study in humans participating in long-term aerobic exercise, which has been shown to increase neurogenesis, revealed that long-term aerobic exercise can improve the visual pattern separation task, using the Beck Depression Inventory (BDI) scales. This study supports the hypothesis that neurons born in the adult brain in the DG contribute to the orthogonalization of incoming information [112]. These results were in accordance with those from another study that indicated the important role of hippocampal neurogenesis to counteract stress.

A recent study demonstrated the role of neurogenesis in depression, using transgenic mice with functionally impaired radial glia neural stem cells. According to this study, the genetically modified mice showed a lack of both astrocytes and immature neurons expressing

doublecortin in the dentate gyrus. On the first day of restraint, the transgenic mice had elevated levels of corticosterone relative to the wild-type group, suggesting impaired negative feedback control of glucocorticoid release [113]. To investigate the role of neurogenesis in the hippocampus, X-ray irradiation was used to reduce neurogenesis in the hippocampus while sparing subventricular zone neurogenesis. Corticosterone levels were elevated during the recovery from the restraint stress in the irradiated mice compared with the control, indicating that the impairment of adult dentate gyrus neurogenesis enhances the secretion of glucocorticoids in response to stress. These results are consistent with another X-ray irradiation study, which induced a deficit in hippocampal neurogenesis and showed that prevention of neurogenesis affected the behavioral effects of antidepressants. These findings indicate that the behavioral effects of chronic antidepressants may be regulated by the stimulation of neurogenesis in the hippocampus [114]. Moreover, neurogenesis-deficient mice present impaired inhibition of endogenous glucocorticoids, using the dexamethasone suppression test. Furthermore, the results showed that new neurons buffer the effect of prior stress in the novelty-suppressed feeding test, buffer the effect of unavoidable stress in the forced swim test, and induce reward-seeking behavior independent of stress in the sucrose preference test. All of these results are in accordance with other studies indicating the role of neurogenesis in the stress response system [115, 116]. Computational evidence provided a novel function for adult hippocampal neurogenesis, which plays a crucial role in the shutting off of stress response, suggesting that the newborn neurons play a critical function in restoring stress hormone systems back to normal and preventing the progression of stress-related mood disorders [113, 117]. Altogether, the results explain the mechanism underlying the HPA axis dysfunction observed with aging – a process that occurs with reduction in neurogenesis.

8.8 Melatonin and Neurogenesis in Stress-Induced Depression

Our strong evidence indicated the role of melatonin in the neurogenesis. Melatonin increased proliferation and neurogenesis in adult mouse progenitor cells obtained from the subventricular zone [21] and in adult hippocampus [118]. A study in pinealectomized rats showed a decrease in neurogenesis in the dentate gyrus, which can be reversed by a melatonin supplement [119]. A study in aging mice found that melatonin supplementation promoted the survival of new cells in the dentate gyrus and increased the expression of doublecortin-labeled cells, indicating that melatonin delays the reduction of adult hippocampal neurogenesis [120]. Chern and his colleagues suggested the effects of melatonin to stimulate the increase of endogenous neurogenesis in stroke mice [121]. An in vitro study showed that melatonin enhances proliferation and neurogenesis [42]. A recent study indicated that melatonin increased proliferation, the number of new neurons, and the expression of the MT1 receptor in the subventricular zone [21, 118]. These effects are dependent on the activation of melatonin receptors, as it could be blocked by the application of the receptor antagonist luzindole.

Melatonin attenuates the negative effects of dexamethasone, a glucocorticoid receptor agonist, on neurogenesis by activating BDNF and ERK1/2 cascades and can counteract the effects of chronic dexamethasone treatment on depression-like behavior [13]. A recent study indicated that the combination of melatonin and citalopram, a widely used antidepressant, exerts synergism to induce an antidepressant-like action that could be related to the regulation of adult hippocampal neurogenesis [122]. A study in chronic corticosterone-treated mice indicates a relationship between behavior and neurogenesis and confirms the hypothesis that neurogenesis contributes to the effects of melatonin as an antidepressant [123]. A study using chronic agomelatine treatment, agomelatine being a melatonin

analog, revealed the role of agomelatine in enhancing hippocampal cell proliferation and survival in stress and reducing immobility, observed using the tail suspension test [124]. A study by Paizanis et al. showed that glucocorticoid receptor-impaired mice, a transgenic model for affective disorders with HPA axis feedback control deficit, exhibit a downregulation of GR and BDNF expression and decrease hippocampal neurogenesis, whereas chronic agomelatine can reverse these effects [125].

Our recent study to determine whether melatonin exerted an effect against stress-induced changes in neurogenesis during the development of depression [13] revealed that chronic administration of dexamethasone-induced depressive-like behavior and that this could be reversed by pretreatment with melatonin. Moreover, the number of BrdU-immunopositive cells and doublecortin [the neuronal-specific marker] protein levels was significantly reduced in dexamethasone-treated mice. Pretreatment with melatonin was found to renew BrdU-labeled cells and doublecortin expression in the dentate gyrus. Furthermore, pretreatment with melatonin prevented dexamethasone-induced reduction in the GR and ERK1/2 in the hippocampal area. Melatonin may protect hippocampal neurons from damage and reverse neurogenesis after chronic dexamethasone treatment by activating BDNF and ERK1/2 cascades. These results revealed that melatonin pretreatment prevented the reduction of cell proliferation, immature neuron precursor cells, and GR and ERK1/2 expression. This indicates that melatonin attenuates the dexamethasone-induced depressive-like behavior, supporting the notion that melatonin possesses anti-stress and neurogenic actions [13]. This might be useful for using melatonin to stimulate adult hippocampal neurogenesis in neuropsychiatric disease, dementia, or cognitive aging.

To further understand the underlying mechanism of melatonin in restoring learning and memory impairment and stress-induced depressive behavior, we used an in vitro model to study the direct effects of melatonin and dexamethasone on the proliferation of adult hippocampal progenitor cells in culture [126]. We found that the addition of dexamethasone to hippocampal progenitor cells obtained from adult rats resulted in a reduction in the number of neurospheres, and pretreatment with melatonin abolished these effects. A reduction of ERK1/2 phosphorylation and G1–S-phase cell cycle regulatory molecules, cyclin E and cdk2, in dexamethasone-treated progenitor cells was prevented by pretreatment with melatonin. These effects were mediated via glucocorticoid and melatonin receptors. In this model, we also demonstrated the cross talk and cross regulation between glucocorticoid receptors and melatonin receptors. Considering the novel function of new neurons in stress response regulation, melatonin could possibly be used as a therapeutic agent to protect the decrease of neurogenesis, which in turn could restore the stress response system to prevent long-term stress-induced depression.

Conclusion

Several attempts have been made to elucidate the mechanism by which melatonin protects the adult mouse brain from the effects of stress-induced decrease in GR, BDNF, NR2A/B, CaMKII, and synaptophysin expression in the hippocampus and prefrontal cortex and its influence on spatial memory (Fig. 8.1). Moreover, the data presented here support the effect of melatonin on adult hippocampal neurogenesis. This effect restores depressive behavior via the upregulation of ERK1/2 and may protect hippocampal neurons from additional damage in response to chronic stress exposure. Moreover, melatonin restored HPA axis dysfunction via the upregulation of MT1, which could be beneficial for the treatment of depression behavior. Herein, we support the role of melatonin in neurogenesis. However, the precise mechanism by which melatonin and other signaling molecules affect cognitive function requires further investigation.

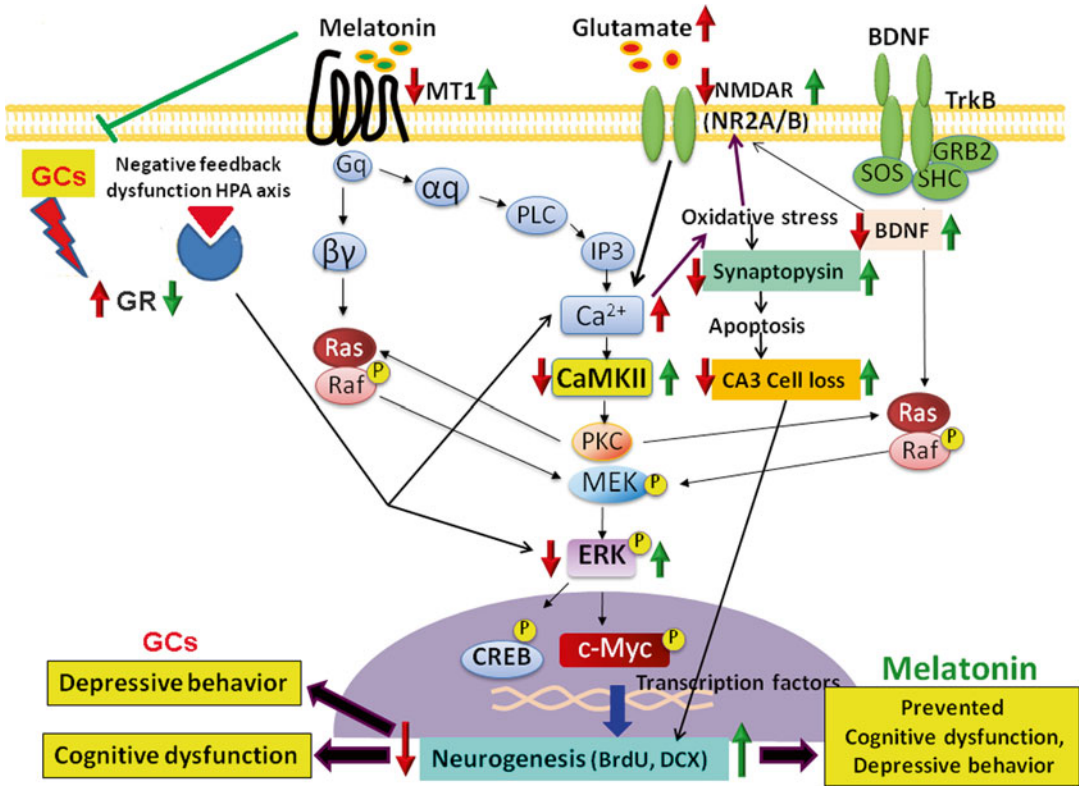

Fig. 8.1 Schematic diagram illustrating the mechanism of melatonin on glucocorticoid-induced depressive behavior and molecular neuroplasticity associated with the cognitive pathway (*red arrow* represents glucocorticoid effects and *green arrow* represents melatonin effects)

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Melatonin, Sleep, Circadian Rhythm, and Mood Disorders

9

Venkataramanujam Srinivasan†,
Domenico de Berardis, Michele Fornaro,
Francisco López-Muñoz, Timo Partonen,
and Rahimah Zakaria

9.1 Introduction

The clinical picture of mood disorders such as major depressive disorder (MDD) and bipolar disorder (BD) and their form with a seasonal pattern known as seasonal affective disorder (SAD) are dominated by pathological mood and psychomotor disturbances. The cluster of signs and symptoms seen in these disorders is sustained over a period of weeks to months, and they recur often in a period or cyclical fashion [1].

As mood disorders happen to be cyclical also, disturbances in circadian rhythms have been implicated in these disorders [2]. By definition, circadian rhythms are endogenous and are of "self-sustaining" in nature and will generate their rhythm and persist in the absence of any external input [3]. Lesion experiments conducted in hypothalamic tissue have shown that the suprachiasmatic nucleus (SCN) of the hypothalamus acts as the major circadian pacemaker [4] and this master clock has been located in the SCN of humans

†Author was deceased at the time of publication.

V. Srinivasan, PhD, MAMS
Sri Sathya Sai Medical Educational and Research Foundation, International Medical Sciences Research Study Center "Prasanthi Nilayam",
40 Kovai Thirunagar, Goldwins, Kovai Coimbatore 641014, Tamil Nadu, India

D. de Berardis
NHS, Department of Mental Health,
Psychiatric Service of Diagnosis and Treatment,
Hospital "G. Mazzini", ASL 4, Teramo, Italy

Department of Neurosciences and Imaging and Clinical Sciences, University "G. D'Annunzio",
Chieti, Italy
e-mail: dodebera@aliceposta.it

M. Fornaro
Department of Education Science,
University of Catania, Catania, Italy

Polyedra Clinical Group, Teramo, Italy

F. López-Muñoz (✉)
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences,
Portucalense University, Porto, Portugal
e-mail: francisco.lopez.munoz@gmail.com; flopez@ucjc.edu

T. Partonen
Department of Mental Health and Alcohol Research,
National Public Health Institute, National Institute for Health and Welfare, Helsinki, Finland
e-mail: timo.partonen@thl.fi

R. Zakaria
Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia,
Kubang Kerian, Kelantan, Malaysia

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as well [5]. Numerous studies conducted on depressive patients have shown that functioning of the circadian timekeeping system is markedly affected in patients with BD [6–8].

Malfunctioning of the circadian timekeeping system is said to be the reason for marked disturbances in the timing and distribution of rapid eye movement (REM) and non-rapid eye movement (NREM) sleep stages seen in patients with MDD, and these are documented as "primary characteristics" of MDD [9]. Both epidemiological and electroencephalographic studies implicate "sleep disturbances" as a frequent underlying factor for mood disorders [10]. Polysomnographic studies on patients with MDD or BD have found objective findings of sleep disturbances like difficulty in falling asleep, staying asleep, and early-morning awakenings [11]. In addition, decreases in sleep efficiency (SE) and slow-wave sleep (SWS) and increases in sleep onset latency (SOL) and nocturnal awakenings have all been reported in patients with MDD [12]. REM sleep abnormalities are considered as specific symptoms of MDD [13], although similar findings characterize patients with narcolepsy. Changes in the timing of the NREM-sleep-to-REM-sleep cycle within the night-time sleep in patients with depressive disorders are interpreted as a consequence of disorganized pathways regulating sleep-wake cycle [7, 14].

As the circadian system, or the SCN, is involved in timing of the sleep propensity and wakefulness and consolidation of these two states [15], the SCN is considered as significant in regulating sleep-wake rhythm [16, 17]. As neurons of the SCN express high concentration of both MT_1 and MT_2 melatonin receptors and melatonin acts on these receptors to alter the neuronal firing rate [18], or phase shift the neuronal firing rates in SCN [19], the hormone has important role in the regulation of both sleep-wake cycle and circadian rhythms. Desynchronization of these two is considered as one of the key triggers for development of depressive disorders [19]. Existence of disturbed sleep-wake cycles coupled with abnormalities of circadian rhythms, sleep disturbances, the cyclical nature of depressive disorders, and the diurnal variations in its symptomatology implicates dysfunction of the circadian timekeeping system as the major precipitating factor for depressive illness [20].

9.2 Sleep Abnormalities in Mood Disorders

Sleep disturbances constitute one of the core features of depressive symptomatology as evidenced from the fact that more than 80 % of depressed patients suffer from poor quality of sleep [21]. Whether sleep disturbances in depression are "trait-like" feature, it remains as a controversial issue [22]. In the early studies, it was demonstrated that changes in sleep architecture often preceded changes in clinical state and even predicted the relapse [23]. Indeed, sleep has been included as one of the diagnostic criteria for MDD [24]. Patients with depressive disorders experience all major symptoms of insomnia including (a) difficulty in falling asleep, (b) difficulty in staying asleep, and (c) early-morning awakenings [11]. Sleep studies of slow-wave activity (SWA) in patients with MDD reveal that δ wave counts decreased as compared with sleep architecture of normal controls. Both fast-frequency β activity and elevated α activity have been noted in sleep recordings from depressive patients [9].

The temporal distribution of REM sleep is altered in patients with MDD. Decreased REM onset latency, which is the period from the onset of sleep to the appearance of first REM period, is the most common feature observed in MDD [13, 25, 26]. It is suggested that reductions in NREM sleep, especially SWS, are the cause for reductions in REM sleep latency [27]. A correlation between the reduced SWS and the abnormal temporal distribution associates with the increased severity of depressive symptoms and the increased risk of suicide [9]. Many of the current antidepressants produce REM sleep suppression with the increase in REM onset latency prior to ameliorating the symptoms of depression [12].

It has been suggested that an ideal antidepressant should shorten the sleep latency, decrease the number of awakenings after sleep onset, and increase the level of alertness during daytime [28], showing thereby the importance of "sleep disturbances in the pathogenesis of depressive disorders." But most of the conventional antidepressants that are in clinical use today act by elevating the daytime mood of depressed patients by activating the brain with rather nonspecific mechanisms, and persistence of this effect in the night will result only at the expense of sleep quality [29].

The status of sleep abnormalities as a diagnostic test for differentiating MDD from other psychiatric disorders was made in one meta-analysis and literature review. Using 31 publications, the authors concluded that sleep studies for the detection of MDD appear replicable with a moderate effect size and that additional studies are required for defining sensitivity and specificity [30]. In a study on patients with schizophrenia and those with MDD, it was found that REM latency and REM sleep parameters had a significant relationship to clinical symptoms in MDD as assessed with the Hamilton Depression Rating Scale (HDRS) scores. Moreover, patients with MDD also differed from healthy controls in SOL, the number of awakenings after sleep onset, SWS, REM latency and NREM sleep stage 1 [31, 32]. Abnormalities in REM density and NREM sleep have been suggested to be potential biomarkers that persist even beyond remission in patients with MDD [33]. Although unipolar and bipolar types of depression can be clearly distinguished, no significant differences are observed between these two groups in terms of nocturnal sleep patterns [34–36]. Polysomnographic studies on patients with bipolar disorder with either depression or mania have shown that shortened REMOL and disturbed sleep continuity occur during manic episodes [37]. Bunney and his associates have found that patients suffering from bipolar depressive disorder exhibited marked reductions in sleep during the night before they switched from depression to mania [38] and this finding was confirmed in the other study also [7]. These studies were taken as evidences to propose that bipolar disorder symptoms are the result of internal desynchronization of circadian rhythms [39].

9.3 Circadian Rhythm Disturbances in Mood Disorders

Circadian clock malfunctioning has suggested to be a contributory factor for mood disorders such as MDD, BD, and SAD [39]. Mood and circadian rhythms have a reciprocal relationship and share common features in neurobiology, clinical presentation, and treatment methods. An interrelation between sleep, circadian rhythms, and mood is illustrated in Fig. 9.1. It is modified from the model presented by Foley et al. [40]. Disruptions of the circadian timekeeping system are said to result in neurobiological dysfunction due to changes in melatonergic system and those in the circadian clock functions, ultimately manifesting as a depressive episode and in subsequent as illness [39]. The early evidence linking mood regulation and circadian rhythms was based on the phase-shift hypothesis, according to which mood disturbances arise from either a phase advance or a phase delay of the master circadian pacemaker with the rhythms of melatonin, cortisol, core body temperature, REM sleep, and other circadian rhythms and with the sleep-wake cycle [41]. Kripke and his coworkers pro-

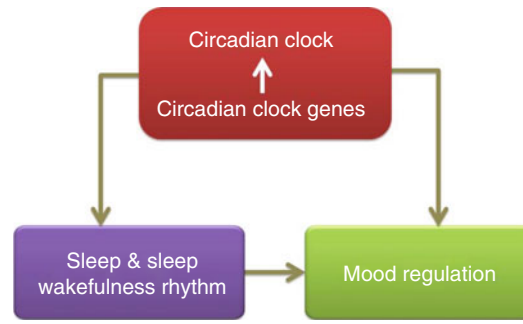

Fig. 9.1 Basic relationship of circadian rhythm, sleep, and mood regulation

posed in 1983 [42] that depression is due to the internal desynchronization of circadian oscillators, with a strong oscillator being linked to phase advances. Phase advances of core body temperature and REM sleep cycles in relation to the rest-activity cycles were reported in both unipolar and bipolar depressed patients [43]. Similarly, a phase advance of (3–6 h for) the peak of 3-methoxy-4-hydroxyphenylglycol excretion was reported in both manic and depressed patients [44]. Abnormalities in the timing and distribution of REM and NREM sleep stages are suggested as primary characteristics of MDD [9]. Hence, the proper study and understanding of the mechanisms involved in sleep and circadian rhythms disturbances will be helpful for explaining the pathophysiology that underlies mood disorders [45].

Some studies demonstrate that suppression of REM sleep results in improvement of mood and subsequently resorted to sleep deprivation (wake) therapy. However, the therapeutic effects of sleep deprivation therapy are influenced by the patient's characteristics and the diurnal variation in mood that contribute to the prognosis after sleep deprivation therapy [46]. The relationship between mood adjustment and the circadian clock systems that regulate "diurnal preference" should also be considered while treating the patient. In this context, it has been found that the evening type of preference to the daily activities increases the susceptibility for development of mood disorders [47, 48]. Hence, the individual genetic effects that control the molecular mechanisms of circadian clocks are involved in mood disorders, and the assessment of these effects and response to treatment are important and need attention while treating patients with mood disorders.

A bidirectional relationship between regulation of daytime affect and night-time sleep exists, and disturbances during the day affect the night-time sleep and circadian functions [49]. Most of the core symptoms of MDD exhibit circadian rhythmicity and are governed by the molecular clocks present in the SCN. The diurnal mood variation has its lowest point in the morning hours in most of the patients with MDD [41, 50]. A poor coupling of the circadian oscillators to the external or the internal rhythms has been demonstrated in certain mood disorders.

9.4 Circadian Clock Genes and Mood Disorders

Polymorphisms in the core oscillator genes *CRY2*, *PER2*, *ARNTL*, and *NPAS2* have been found to associate with SAD [51, 52]. Sequence variations in these genes that form the functional unit at the core circadian clock have been shown to predispose to SAD, in specific to winter depression [51]. Moreover, a detailed pattern of circadian gene expression was carried out with high-quality post-mortem human brain samples obtained from six cortical and limbic regions (dorsolateral prefrontal cortex, anterior cingulate cortex, hippocampus, amygdala, nucleus accumbens, cerebellum) derived from 55 controls with no history of psychiatric or neurological illness and from 34 patients with MDD [53]. Among the top-ranked rhythmic genes that were covered in this study were the known clock genes *ARNTL*, *PER2*, *PER3*, *NR1D*, *DBP*, *BHLHE40*, and *BHLHE41*. Findings of this study revealed the cyclical patterns for most of the known circadian genes and offered empirical evidence for molecular dysregulation of circadian rhythms across six brain regions of clinically depressed patients. Patients with MDD exhibited an abnormal phasing of circadian gene expression and disrupted phase relationships between circadian genes, which account for the disruption of regulation of a range of neural processes and behavior that are manifested as MDD [53]. Results of this study could pave the way for identification of novel biomarkers and treatment targets for mood disorders [53].

In studies carried out on *CRY2* gene expression in depression, the effect of total sleep deprivation on the circadian oscillation of *CRY2* mRNA in human peripheral blood mononuclear cells was assessed, and it was observed that one-night total sleep deprivation led to an increase in *CRY2* mRNA levels in controls, whereas in

depressed patients with BD, there was a decrease in *CRY2* mRNA levels [54]. Further in this study, *CRY2* genetic variants were found to be significantly associated with winter depression or SAD [54]. A recent study carried out on Chinese population involving 105 subjects with MDD and 485 controls reveals that MDD patients have significantly higher frequencies of C-allele and CC-genotype in *CRY1* rs2287161 and those of T-allele and TT-genotype in *TEF* rs738499 than controls [55]. *CRY2* is a circadian gene that participates in regulation of the evening oscillator, and its link to vulnerability for depression has been reported earlier in depressed bipolar patients [56]. However, it needs to be pointed out that the Psychiatrics Genomics Consortia for schizophrenia, BD, and MDD found no evidence for association of genes linked to control of the circadian rhythms and suggested that genes encoding components of molecular clock are not good candidates for harboring common variants that increase the risk to BD, schizophrenia, or MDD [57]. However, the genome-wide association studies that combine samples from different populations may not cover all the circadian genes and their variants and might thus have lost the relevant information.

The current understanding is that proteins encoded by the core circadian clock genes *ARNTL*, *ARNTL2*, *CLOCK*, and *NPAS2* form heterodimers and bind to sites, or "boxes," to initiate the transcription of their target genes among which are the core circadian clock genes *CRY1*, *CRY2*, *PER1*," and *PER2*. In turn, proteins encoded by these latter genes form heterodimers and act as repressors of the transcription-translation feedback loops of the circadian clocks. Sleep-related chronotherapies such sleep deprivations and sleep phase advances are effective in resetting the abnormal clock gene machinery and thereby correcting the abnormal circadian rhythms in terms of the phase, period, and amplitude. This fact and the finding that these chronotherapies lead to improvement in mood suggest that "altered clock gene machinery" is likely to represent a core pathophysiological defect in a subset of patients with mood disorders [58].

Certain polymorphisms of *ARNTL* are said to associate with the predisposition to BD [59]. Other circadian clock genes with a polymorphism reported to associate with BD include *CLOCK* [58], *NR1D1*, and *Per3* [60–63]. Meta-analysis of integrating data obtained from genome-wide association studies, as well as human and animal model studies, points out that other circadian genes like *RORA* and *RORB* are also associated with BD [64]. Genetic variations in *ARNTL* are in addition found to associate with SAD [65]. Activation of circadian gene transcription varies with the time of the day also. In day-active animals, *PER1* and *PER2* get activated in the morning hours, while *CRY1* and *CRY2* get activated in the evening hours [66]. Both the dawn and dusk components, *PER2* and *CRY2* genetic variants, respectively, are associated with SAD in particular [6, 56]. A schematic diagram of polymorphisms of clock genes and mood disorders is presented in Fig. 9.2.

There may be hundreds of genes in the human brain that are likely to be involved in the daily rhythmic events including the sleep-wake cycle. Daily rhythms in these genes are dysregulated in patients with mood disorders, suggesting thereby that mood disorders are due to dysregulation of circadian functions. This has been sufficiently substantiated by the abnormal rhythms in core body temperature, cortisol, melatonin, 3-methoxy-4-hydroxyphenylglycol, REM sleep latency, and decreases in total sleep time and sleep efficiency, as has been discussed in the earlier paragraphs.

Studies of the physiological and molecular mechanisms underlying disrupted circadian rhythms and dysregulated sleep-wake mechanisms will greatly help not only in understanding the pathophysiology of mood disorders but also will help to treat these disorders more effectively. Insomnia occurs in nearly 60–80 % of patients with MDD [67]. Antidepressant drugs that are commonly prescribed for treatment of depression may actually worsen insomnia and thus impair and postpone the full clinical remission from the illness. Tricyclic antidepressants, selective serotonin reuptake inhibitors, and serotonin-norepinephrine reuptake inhibitors all cause not

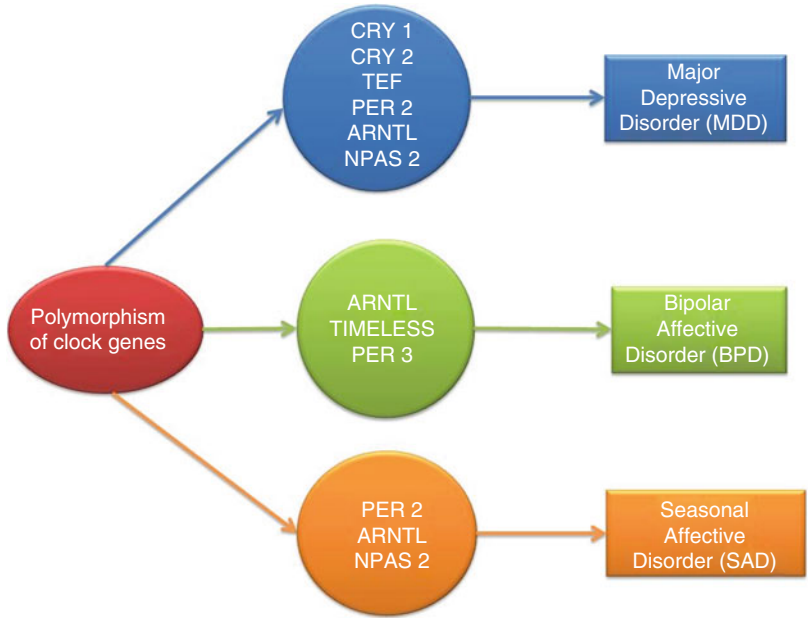

Fig. 9.2 Polymorphisms of clock genes and mood disorders

only REM sleep suppression but also may thereby aggravate insomnia of depressed patients [68]. A comprehensive program of therapy for depression should address not only its clinical and behavioral signs and symptoms but should focus its attention on concomitant symptoms like sleep and circadian rhythm disturbances [45, 67].

9.5 Agomelatine Use for Mood Disorders

As depressive disorders are linked to disturbances of circadian rhythms, an antidepressant that benefits sleep quality and resets the disturbed circadian rhythms will have a superior efficacy [68, 69]. An ideal antidepressant should decrease SOL, decrease the number of awakenings after sleep onset, increase sleep quality during the night, and improve alertness during the day [70]. Close to such ideal drug is the newly introduced melatonergic antidepressant agomelatine (Servier), an MT_1 and MT_2 melatonergic receptor agonist with 5-HT_{2C} antagonistic properties. This drug got its approval in November 2008 by the European Medicines Agency. A number of clinical trials involving multicenter and multinational studies have documented the clinical efficacy of agomelatine in MDD and found it having the efficacy superior over placebo in terms of response during the acute phase of treatment. The efficacy of agomelatine over sertraline, fluoxetine, and escitalopram in MDD has been well documented. Agomelatine's treatment efficacy based on HDRS, CGI, and Montgomery-Åsberg Depression Rating Scale (MADRS) has all been reviewed, and its efficacy is equal to any other antidepressant [71–73].

Unlike other antidepressants, agomelatine improves sleep continuity and sleep quality and increases sleep efficiency and the total amount of SWS [74]. It also improves early NREM and REM sleep [75]. A greater reduction in SOL during the night with a reduced daytime sleepiness was seen in agomelatine-treated depressed patients as compared with patients treated with escitalopram.

Agomelatine normalizes NREM sleep in depressed patients [76]. Since changes in NREM sleep precede the clinical improvement, as assessed with the HDRS, it has been suggested that agomelatine's antidepressant effect is medi-

ated through its ability to normalize sleep architecture [77]. EEG analysis of the effects of agomelatine on sleep in patients with MDD shows that agomelatine (25 mg per day for 6 weeks) increases sleep efficiency with a decrease in awakenings, starting from day 7 onwards. Both SWS duration and percentage of time spent in SWS (sleep stages 3 and 4) increase significantly [77]. As agomelatine did not influence REM latency or the amount of REM sleep, its antidepressant effect has been attributed to its action on correcting the abnormalities of the homeostatic system of sleep regulation [77].

Agomelatine's potential superiority over other antidepressants of the 5-HT_{2C} antagonists has been attributed to its effect of improving sleep at night and alertness during the day that is not seen with the use of other antidepressants. With agomelatine's combined mechanism of actions of preserving sleep quality at night-time and elevating mood during daytime, depressed patients experience a better quality of life [78]. Agomelatine's sleep-promoting melatonergic action counteracts its antihypnotic actions mediated through its 5-HT_{2C} antagonism. 5-HT_{2C} receptors are concentrated in the frontal cortex, amygdala, hippocampus, and corticolimbic structures that all are involved in the regulation of mood and cognition [78].

As we have discussed in the earlier paragraphs, disruptions of circadian rhythms also correlate with the clinical severity of depression, a finding that is attributed to disturbances of sleep-wake cycle. As agomelatine is a specific agonist of MT_1 and MT_2 melatonergic receptors located in the SCN, it exerts its action by resetting the disturbed circadian rhythms including the sleep-wake rhythms seen in patients with MDD, and this action should be considered as an important component of its antidepressant effects. Agomelatine's chronobiotic effect was studied in healthy older men where this drug (50 mg per day) caused phase advances by 2 h on average in core body temperature profiles and in the temporal organization of cortisol secretion [79]. Phase delays of the circadian rhythms relative to the timing of the habitual sleep-wake cycle are an important contributing factor for the pathophysiology of SAD. Treatment with agomelatine (25 mg per day for 14 weeks) for acutely depressed patients with SAD by using *circascreen* (a self-rating scale for the assessment of sleep and circadian rhythm disturbances) demonstrated that the drug alleviated symptoms significantly from the second week of treatment onwards and suggested that its hypnotic and resetting effects are important in the treatment of SAD. In a recent multicenter observational CHRONOS study conducted on 6,276 depressed patients, agomelatine caused reduction in HAMD – score from a mean of 22.5 p6.9 at baseline to 4.7p4.7 at the end of 8 week treatment period. Improvements in sleep-wake cycle were confirmed in this study by the marked improvements in all three insomnia-related HAMD-17 items [80]. Rates of response and remission were high both in the overall population and in the subgroup of patients with severe depression. As benefits of treatment observed in randomized studies do not always translate into clinical practice, the authors of the present study evaluated the antidepressant efficacy and tolerability of agomelatine in the naturalistic study and found agomelatine caused rapid onset of benefit across all HAMD-17 items showing agomelatine as highly effective in the treatment of depressive episodes in a "real-world" clinical setting [80].

Conclusions

Disturbed sleep and disrupted circadian rhythms are two cardinal features seen in patients suffering from all three major types of mood disorders, including MDD, BD, and SAD or winter depression. Sleep disturbance and depression have reciprocal relationships and influence each other. In MDD, sleep disturbances are a part of the diagnostic criteria. Besides exhibiting all major symptoms of insomnia like decreases in total sleep time and sleep efficiency, depressed patients also exhibit alteration in the temporal distribution of REM sleep. Polysomnographic studies on patients with MDD or BD yield objective findings of sleep disturbances, and these features are even used as "biologic markers" for identifying the specific type of mood disorders.

The task of a given antidepressant should be not only to improve the clinical and behavioral features but also to improve the concomitant sleep disturbances. Hence, the effects of antidepressant on sleep should be given a main importance while prescribing a drug for treatment of depressive disorders. Second, as depression is linked to disturbances of circadian rhythms, an ideal antidepressant should reset the disrupted circadian rhythms in addition to correct the abnormalities of sleep-stage dynamics. The currently used antidepressants while effective in producing a clinical remission also may exacerbate insomnia and may thus not be effective enough in tackling circadian and sleep-wake rhythm disturbances. In this context, the recently introduced melatonergic antidepressant agomelatine, with its dual mechanism of action on MT_1 and MT_2 melatonergic receptors in the SCN and other brain regions associated with sleep and circadian rhythm regulation, promotes sleep and resynchronize the disrupted circadian rhythms back to normal. These actions by themselves improve the clinical state of depressed patients having MDD, BD, or SAD. In addition, agomelatine's action through 5-HT_{2C} antagonism in the prefrontal cortex, corticolimbic structures, hippocampus, and amygdala, which are the key brain regions involved in mood regulation, relieves symptoms of depression, and helps to achieve the clinical remission. All these events reinforce the hypothesis of direct involvement of melatonergic system in the etiology of sleep and depressive disorders.

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10 Melatonin Induces Antidepressant-Like Behavior by Promotion of Adult Hippocampal Neurogenesis

Gerardo Bernabé Ramírez-Rodríguez

10.1 Introduction

Melatonin is the main product synthesized by the pineal gland [1]. This hormone exerts antioxidant and neuroprotective actions [2]. Additionally, melatonin is related to the control of circadian rhythms [3], and the dysregulation of the circadian production of melatonin has been associated with neuropsychiatric disorders such as depression, in which patients show disrupted circadian rhythms and low levels of melatonin [4–6] (*for a review*, *see* Chaps. 8 and 9, *respectively*).

Depression is a neuropsychiatric disorder in which several alterations at the neurochemical, morphological, and behavioral levels are present [4, 7, 8]. This disorder is highly influenced by stress, which acts as a key player to precipitate depression [9]. Thus, stress contributes to this multifactorial neuropsychiatric disorder in which the hippocampus is involved and altered [7, 10] (*an in-depth review of this topic is presented in* Chaps. 8 and 9, *respectively*).

G.B. Ramírez-Rodríguez, PhD
Laboratory of Neurogenesis, Division of Clinical Investigations, National Institute of Psychiatry "Ramón de la Fuente Muñiz",
Calzada México-Xochimilco 101, Colonia San Lorenzo Huipulco, Delegación Tlalpan,
C.P. 14370 México, DF, México
e-mail: gbernabe@imp.edu.mx

The hippocampus is one of the brain regions belonging to the limbic system, and it is affected by depression [7]. This brain structure expresses a high density of glucocorticoid receptors, making it a stress-sensitive region. Thus, the hippocampus presents changes at the cellular and morphological levels after stress that strongly impact behavior [7, 10–13] (Fig. 10.1).

10.2 Adult Hippocampal Neurogenesis

Among the cellular alterations caused by stress, it is known that stress alters the plasticity of the hippocampus [7]. In this regard, changes at the level of the dendritic spines and astrocytic and neuronal cells and in the neuropile occur after stress [7]. In addition to that, the generation of new neurons is also altered by stress [13–15].

Interestingly, the hippocampus is one of the brain regions in which constitutive generation of new neurons (also known as neurogenesis) occurs as a lifelong process [16]. Specifically, the dentate gyrus incorporates new neurons formed from neural stem cells (NSCs) located in the subgranular zone (SGZ) [16]. Once the NSCs are activated, they go through several cellular events to finally form mature granular cells [16]. The different events involved in the hippocampal neurogenic process can be identified by the temporal expression of protein markers [16, 17].

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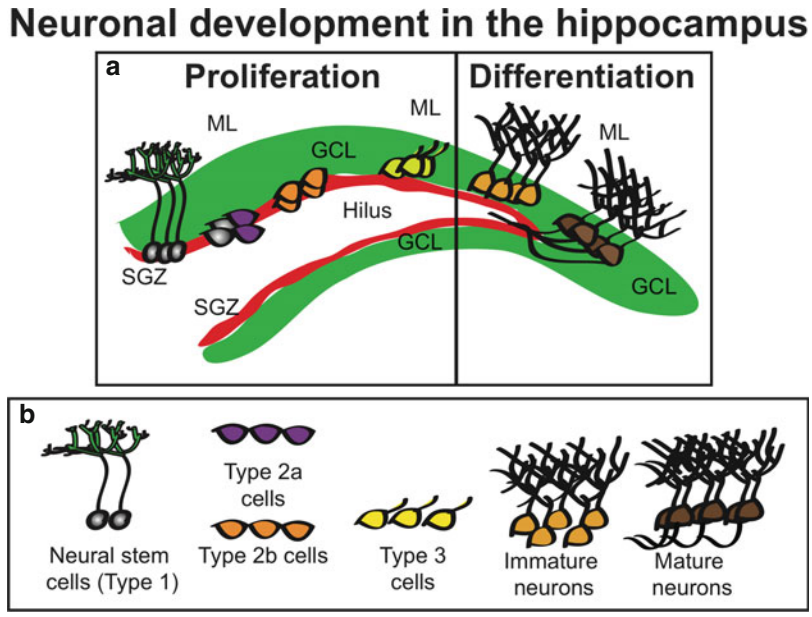

Fig. 10.1 Schematic representation of the adult hippocampal neurogenic process. (**a**) Adult hippocampal neurogenesis involves cell proliferation, migration, survival and differentiation. (**b**) Representative drawings of the cell populations participating in the generation of mature and functional new neurons in the hippocampus

In detail, NSCs, also known as type 1 cells, express the glial fibrillar acidic protein (GFAP) and the sex-determining region Y-box transcription factor (Sox2). Once type 1 cells undergo asymmetric division, the type 2a (GFAP-Sox2+) cellular population is formed. Thus, type 2a cells symmetrically divide to give rise to type 2b (Sox2+doublecortin (DCX)+) cells which will form DCX-expressing neuroblasts (type 3 cells). Once type 3 cells exit the cell cycle, immature neurons develop more mature dendrites. During the transition to maturation, the DCX stage shows co-labeling with a calcium-binding protein, calretinin, that later will be switched to another calcium-binding protein called calbindin that is expressed in the more mature stage of adult hippocampal neurogenesis [16–20] (Fig. 10.1).

The neurogenic process is highly regulated, and among the known regulator neurotransmitters (such as GABA, glutamate, and serotonin), growth factor, neurotrophins, and hormones (such as estrogens, growth hormone, and melatonin) play a significant role in the generation of new neurons. In addition, physical activity and housing in an enriched environment promote neurogenesis with increased levels of growth factors and neurotrophins [17, 21]. Additionally, antidepressant drugs positively affect hippocampal neurogenesis [13, 15, 22]. Moreover, negative regulators, such as abuse of drugs, sleep deprivation, and stress, alter different events involved in hippocampal neurogenesis [23] (Fig. 10.1).

10.3 Adult Hippocampal Neurogenesis Is Affected by Stress

Hippocampal neurogenesis is affected by several types of stressors such as, but not limited to, acute stress (i.e., forced swim test, FST) or chronic stress (i.e., chronic mild stress, CMS) that impact cell proliferation and survival and maturation of newborn cells in the dentate gyrus of the hippocampus [12, 13, 24]. To counteract the negative effects of stress on hippocampal neurogenesis, several strategies have proven their efficacy [25–27]. Among them, antidepres-

sant drugs revert the alterations caused by stress on the hippocampal neuronal development [15, 22, 28, 29]. Surprisingly, adult neurogenesis is a process that occurs over 4 weeks, progressing from NSCs to newborn mature neuron formation [16]. Similarly, the behavioral benefits of antidepressant drugs also occur in a 4-week period. Thus, antidepressant drugs also use the generation of new neurons in the hippocampus as one of their targets to restore neuroplasticity [15, 27]. In humans, at the present time, the influence of antidepressant drugs has only revealed an effect on cell proliferation, without discarding changes at the levels of survival and maturation of new neurons [30, 31].

The mechanism through which stress affects hippocampal neurogenesis is unknown. However, some reports have provided information about the increased levels of cytokines such as interleukin-1β (IL-1β) in different areas of the brain after stress exposure. Additionally, the administration of IL-1β produced similar effects of altering hippocampal neurogenesis and decreasing the mediators of neurogenesis such as the brain-derived neurotrophic factor (BDNF). Interestingly, the IL-1β receptor, IL-1RI, is expressed in the precursor cells [30, 31]. Additionally, IL-6 and TNFα are increased after stress exposure [32]. Thus, the proinflammatory cytokines act as negative mediators of stress to decrease hippocampal neurogenesis [30, 32].

10.4 Melatonin Antidepressant Effects

Several studies have given information about the possible antidepressant-like effects of melatonin based on the fact that disturbances in circadian rhythms are associated with depression, that melatonin's secretion at night is a marker for circadian rhythms, and that plasma levels of melatonin change during depression [4, 33]. In this sense, preclinical reports show that chronic treatment with melatonin prevented several behavioral alterations caused by CMS in C3H/He rodents [34–36]. Similarly, in Balb/C and C57Bl/6 mice, pretreatment with melatonin (2.5–10 mg/kg) decreases despair behavior tested in the FST [37]. A similar beneficial effect of melatonin was also reported in rats exposed to the FST after corticosterone treatment [38, 39]. In an additional model, the tail suspension test, melatonin presented an antidepressant-like effect in rodents [40]. More recently, in a mouse model of seasonal affective disorder, melatonin induced an antidepressant-like effect concomitantly to changes in the levels of the serotonin content in the amygdala [41]. Moreover, melatonin also decreased immobility-associated despair in the FST in a neurodegenerative disease mouse model [42].

10.5 Melatonin and Neurogenesis

Considering that drugs with antidepressant-like actions might act as regulators of adult hippocampal neurogenesis [43], several groups have explored the association of melatonin's antidepressant-like effects with adult hippocampal neurogenesis. In this part of the chapter, I will mention some of those works that have explored the influence of melatonin on some events of adult hippocampal neurogenesis.

Under physiological conditions, it has been shown that the chronic administration of melatonin to intact C57Bl/6 female mice specifically influenced the cell survival of newborn cells and consequently the net neurogenesis in the dentate gyrus of the hippocampus [44]. Additionally, in the same strain of mice, melatonin promotes the dendrite maturation of newborn neurons [45]. Moreover, in Balb/C mice, the chronic administration of melatonin influenced cell proliferation, survival, and the early immature newborn neurons, identified by the expression of doublecortin [46]. However, melatonin by itself was not able to affect cell proliferation in C3H mice [47], a strain of mice in which just the combination of melatonin with physical exercise showed a significant increase in cell proliferation [47]. The differences observed in the effects of melatonin on several events of the hippocampal neurogenic process may depend on the differences in the

genetic backgrounds of the strains of rodent used [48]; thus, the effects observed in one strain of mice cannot be extrapolated to other strains because of the existence of several key regulators presented in the hippocampal neurogenic niche that can be influenced by melatonin.

10.6 Melatonin: Antidepressant-Like Effects and Adult Hippocampal Neurogenesis

Regarding the influence of melatonin on animal models of stress, maternal separation-stressed rats recovered cell proliferation after melatonin supplementation [49]. From this study, several works reported the beneficial effect of melatonin on different events of hippocampal neurogenesis that are more related to its antidepressant-like effects.

Recently, we reported that concomitantly to the increase in adult hippocampal neurogenesis, melatonin caused an antidepressant-like effect in female C57Bl/6 mice tested in the FST [44]. Additionally, in a depression animal model created by the chronic administration of corticosterone, melatonin decreased the depressant-like behavior tested in the FST, and at the cellular level, melatonin reverted cell proliferation and doublecortin impairment caused by high levels of corticosterone [50]. Similarly, but in ovariectomized mice, melatonin decreased depressive-like behavior in the tail suspension test and reverted alterations at the hippocampal neurogenic level [51]. These three studies have revealed that, in addition to modulating neurogenesis, melatonin is able to cause antidepressant-like effects, at least in mice (Fig. 10.2).

10.7 Combinations of Melatonin with Antidepressant Drugs to Overcome Depression

However, the pharmacological characteristics of melatonin have caused the field to find analogues of melatonin with higher potencies to promote antidepressant-like effects, and agomelatine is a prototype of this type of alternative [52–54]. Interestingly, agomelatine also has shown positive effects on neurogenesis and as an antidepressant [52, 55–58] (*an in-depth review of the effects of agomelatine is presented in* Chaps. 18, 19, 20, and 21). In this regard, the combination of antidepressant drugs with melatonin has gained attention in overcoming depression [59–61]. In a preclinical study, the combination of imipramine and melatonin caused a reduction in depressive-like behavior [62]. Regarding adult hippocampal neurogenesis, the combination of buspirone and melatonin showed positive regulation at the level of hippocampal neurogenesis and decreased depressive-like behavior in rodents, but it also showed antidepressant effects in subjects diagnosed with major depressive disorder [63]. Recently, we reported the identification and dose optimization of the combination of melatonin and citalopram to decrease depressive-like behavior and to favor adult hippocampal neurogenesis in rodents [64]. Altogether, these studies have provided information about the beneficial

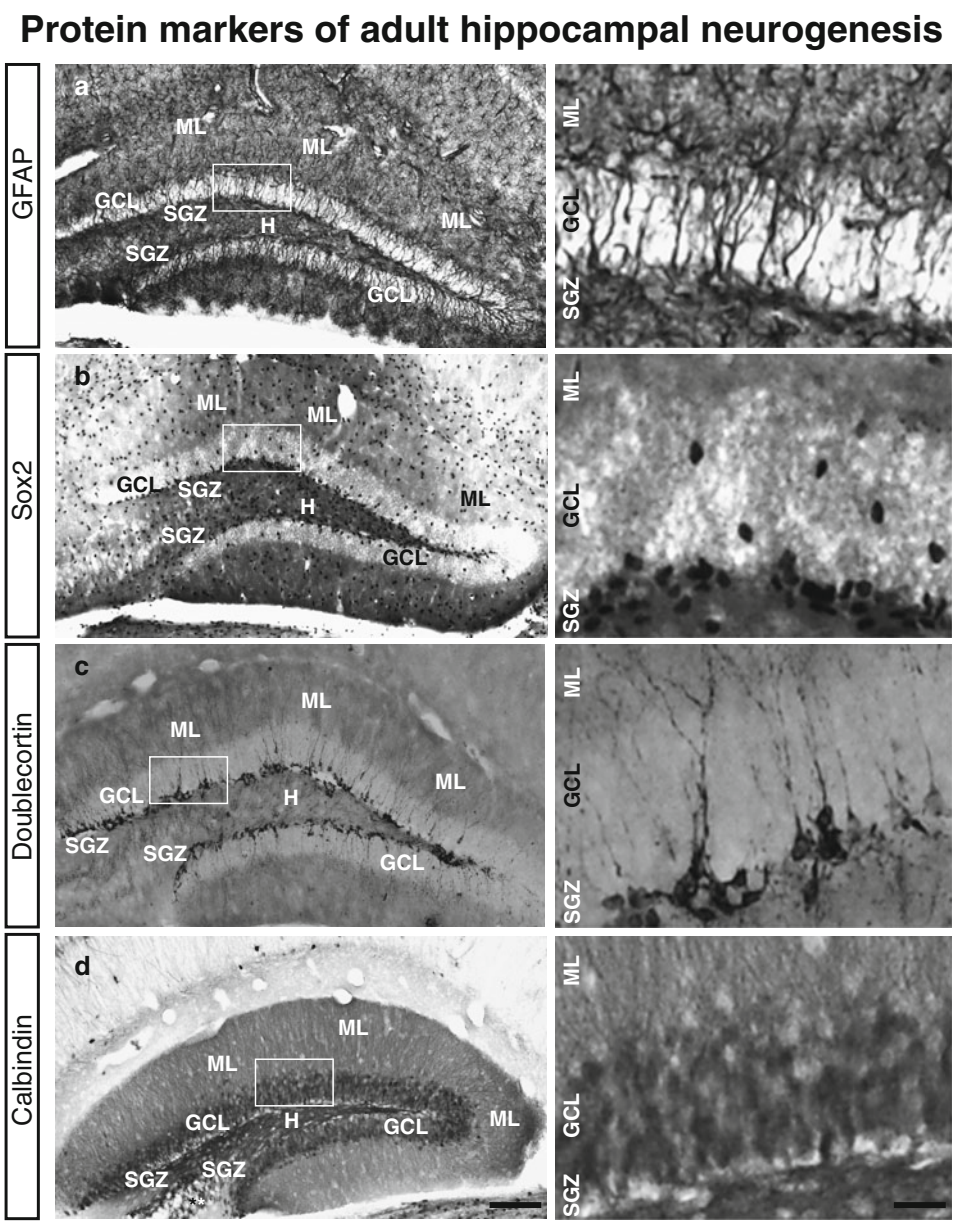

Fig. 10.2 Adult hippocampal neurogenesis. Representative pictures of radial glial cells positive for the glial fibrillar acidic protein (*GFAP*, **a**), Sox2-positive cells (**b**), doublecortin neuroblasts and immature neurons (**c**), and mature granular cells positive for calbindin (**d**). Inserts show high-power pictures. Scale bar in (**d**) = 40 μm, scale bar in high-power pictures = 80 μm. Images also show the granular cell layer (*GCL*), the subgranular zone (*SGZ*), the molecular layer (*ML*), and the hilus (*H*)

effects of the combination of melatonin with antidepressant drugs to overcome some depression-related behaviors with an increase in adult hippocampal neurogenesis (Fig. 10.2).

Conclusion

The information in this chapter attempted to present an overview of the effects of melatonin as an antidepressant-like factor, as related to its effects on neuroplasticity. This is not limited to adult hippocampal neurogenesis and may include changes at the level of other types of plasticity such as formation or remodeling of dendrite spines. Although there is strong evidence about the participation of melatonin as a key regulator of adult hippocampal neurogenesis, there are relevant aspects of this process that have not yet been explored that deserve to be studied to clearly state the effects of melatonin as an antidepressant. In addition, the mechanisms through which melatonin influences hippocampal neurogenesis have to be explored. In this regard, the role of melatonin membrane receptors in the context of adult hippocampal neurogenesis is supported only by controlled in vitro systems, not by in vivo studies [65].

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Association Between Melatonin and Neuroimmune Diseases

11

Murat Terzi, Mehmet Emin Onger, Aysin Pınar Turkmen, Sefa Ersan Kaya, Arife Ahsen Kaplan, Berrin Zuhal Altunkaynak, and Suleyman Kaplan

11.1 Anatomy and Physiology of the Pineal Gland

Pineal gland has been defined by Herophilus in the third century BC in 1958. Pineal gland is located in the central part of the brain at the roof of the third ventricle. It is surrounded by the pia mater. Despite its tiny size in proportion to the body, pineal gland is exposed to a high volume of blood flow. Pineal gland consists of pinealocytes and glial cells (Fig. 11.1).

Sympathetic system dominates the stimulation of pineal gland, and nerve fibres originated from the superior sympathetic ganglion reach to the gland through nervi conarii [13]. Apart from the sympathetic innervation, parasympathetic and serotoninergic nerve fibres to a small extent innervate it. Lerner et al. have observed that the skin colour of amphibia has been blanched after administration of tissue extracts from pineal gland, and they named this hormone as melatonin [5]. Pineal gland is not the only organ responsible for melatonin production. Although many peptide hormones are synthesised in the pineal gland, the main hormone is melatonin. After a decade, brightness and darkness have been known to be important roles in functions of pineal gland. Being one of the hormones that comes to mind with respect to circadian rhythm, the melatonin hormone involves in many functions in the organism while it is emphasised with its feature of being a potent antioxidant [13]. Being a highly lipophilic and hydrophilic hormone, melatonin is rapidly mixed with the blood and body fluids without being stored in the body [5]. Circadian rhythm of melatonin release becomes irregular with the age, and it is observed to deteriorate not only due to physiological conditions but also due to several clinical disorders including mood disorders [1, 7]. Melatonin has also been shown to be synthesised in the APUD (amine precursor uptake and decarboxylation) cells, which are recognised as part of diffuse neuroendocrine system [5]. These cells are located in the retina, lacrimal glands, bronchus and other regions of the brain, liver, kidneys, adrenal glands, gastrointestinal system, thymus, placenta, ovary, testicle and endometrium [5, 13]. Melatonin has also been shown to be synthesised in mast cells, leucocytes and natural killer cells (Fig. 11.2).

M. Terzi
Departments of Neurology, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

M.E. Onger • A.P. Turkmen • S.E. Kaya
A.A. Kaplan • B.Z. Altunkaynak • S. Kaplan (✉)
Department of Histology and Embryology, Medical School, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey
e-mail: skaplanomu@yahoo.com

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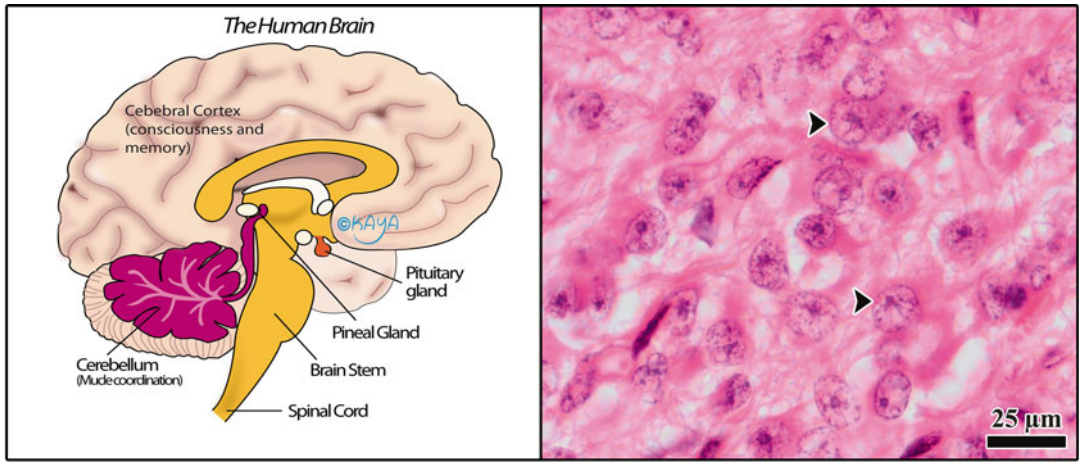


Fig. 11.1 Anatomical localisation and microscopical structure of the pineal gland are seen. *Arrowheads* sign pinealocytes (Redrawn from Brzozowska et al. [6])

11.2 What Is Melatonin?

Melatonin is a hormone synthesised in animal cells directly from the amino acid tryptophan, which follows a serotonin pathway in the pineal gland [5]. Their synthesis and releasing begin by nocturnal period and end up in the diurnal period. Prolongation of diurnal period or sudden exposure to light stops the production of melatonin [3]. Therefore, melatonin is also referred to as "biochemical identifier of darkness", a symbolic name. The release of melatonin in humans reaches to peak levels at midnight and ends in the morning. Serum melatonin levels vary based on the age. Newborns have very low levels of melatonin. The secretion of melatonin becomes rhythmic after month 3. The maximum melatonin production detected between the third and the fifth years of life [3]. Melatonin secretion decreases with the age. Gender is reported to have no effects on melatonin release [13]. Although it has been shown that melatonin is also synthesised in the organs other than pineal gland such as the retina and intestines, this synthesis has very little effect on the plasma levels of melatonin [5]. Melatonin is highly lipophilic and hydrophilic and thus is rapidly mixed with the blood and body fluids without being stored in the body. Seventy percent of the melatonin is bound to albumin in the blood. Melatonin is mainly metabolised in the liver and then in the kidneys into 6-hydroxymelatonin sulphate and 6-hydroxymelatonin glucuronide. These compounds are excreted via urine. Due to the rhythmic release, more metabolites are present in the urine during nocturnal period [3] (Figs. 11.3 and 11.4).

11.3 Functions of Melatonin and Their Receptors

Pharmacologically, two different cell membrane-bound melatonin receptors are reported in mammals, MT1 (high affinity) and MT2 (low affinity) [9]. Melatonin receptors have been found in T- and B-lymphocytes in rats, while melatonin receptors have also been reported to be present in human T-lymphocytes [17]. MT2 receptor is reported to have important contributions in strengthening the immune function. Melatonin exhibits antioxidant activity by not only their strong free radicals' scavenger effect but also blocking the nitric oxide synthase activity [11]. It has also been reported to prevent oxidant damage in the tissues caused by neutrophil activation in inflammation models such as burn damage, sepsis and ischaemia-reperfusion injury. Due to the melatonin having inhibitory effect on the immune response as an antioxidant, it has been

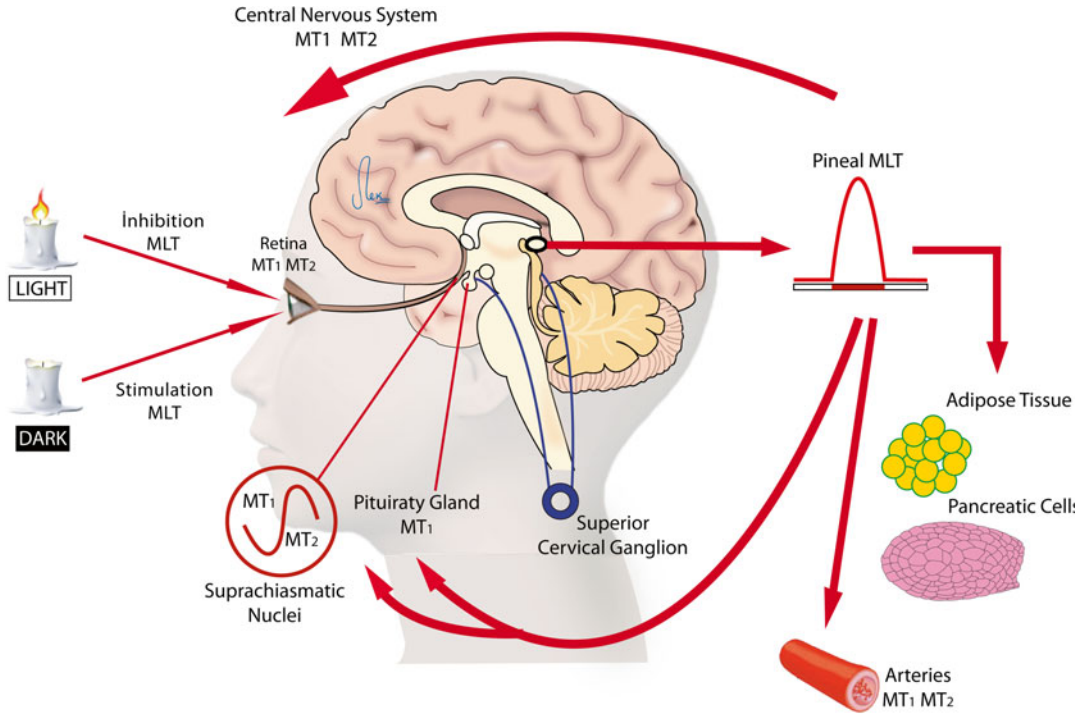


Fig. 11.2 The brain circuitry by which light inhibits the melatonin production in the pineal gland (Redrawn from Konturek et al. [20])

suggested that melatonin may be helpful in organ transplantation. The absence of toxicity also supports the safe use of this agent in transplantation. Another effect of melatonin on immune system is its anti-inflammatory activity. Previous researches suggested that melatonin might be effective on the virus-carrying and infectious diseases such as HIV, bacterial infections and cancer [17, 23]. Melatonin is a strong scavenger of hydroxyl and peroxyl radicals. As its scavenging activity on peroxyl radical is lower than that on vitamin E, it has been reported that melatonin has a lower neutralising activity against lipoperoxyl radical [24, 28].

Melatonin has also been demonstrated to have indirect effects on free radicals. Melatonin exhibits antioxidant activity also by activating the glutathione peroxidase enzyme, which metabolises the hydroperoxides, and it leads to increase in the SOD activity that catalyses conversion of O_2 radical to H_2O_2. Also melatonin prevents from the reduction in catalase activity during oxidative stress and inhibits the nitric oxide synthase (NOS) enzyme and so NO formation [28, 30]. Recent studies have shown that melatonin is associated with oxidative stress and exhibits antioxidant activity both as direct radical scavenger and with its indirect effects [26, 28, 30]. The most important advantage of melatonin is its highly lipophilic features. So it easily acts as a free radical scavenger. Melatonin needs no binding site and receptor for its free radical scavenger activity.

Although serotonin, the precursor of melatonin, doesn't cross, melatonin easily crosses the blood-brain barrier due to their lipophilic activity. Vitamin E, another antioxidant, couldn't cross the barrier. Thanks to this activity, melatonin is accepted as a superior antioxidant [10]. Melatonin is a very powerful antioxidant eliminating hydroxyl, the most harmful radical (OH) [10]. Another advantage of melatonin is the absence of toxic side effects even at application with very high doses (300 mg/day) and in long-term usage (up to 5 years) in contrast to other antioxidants (Figs. 11.3 and 11.4).

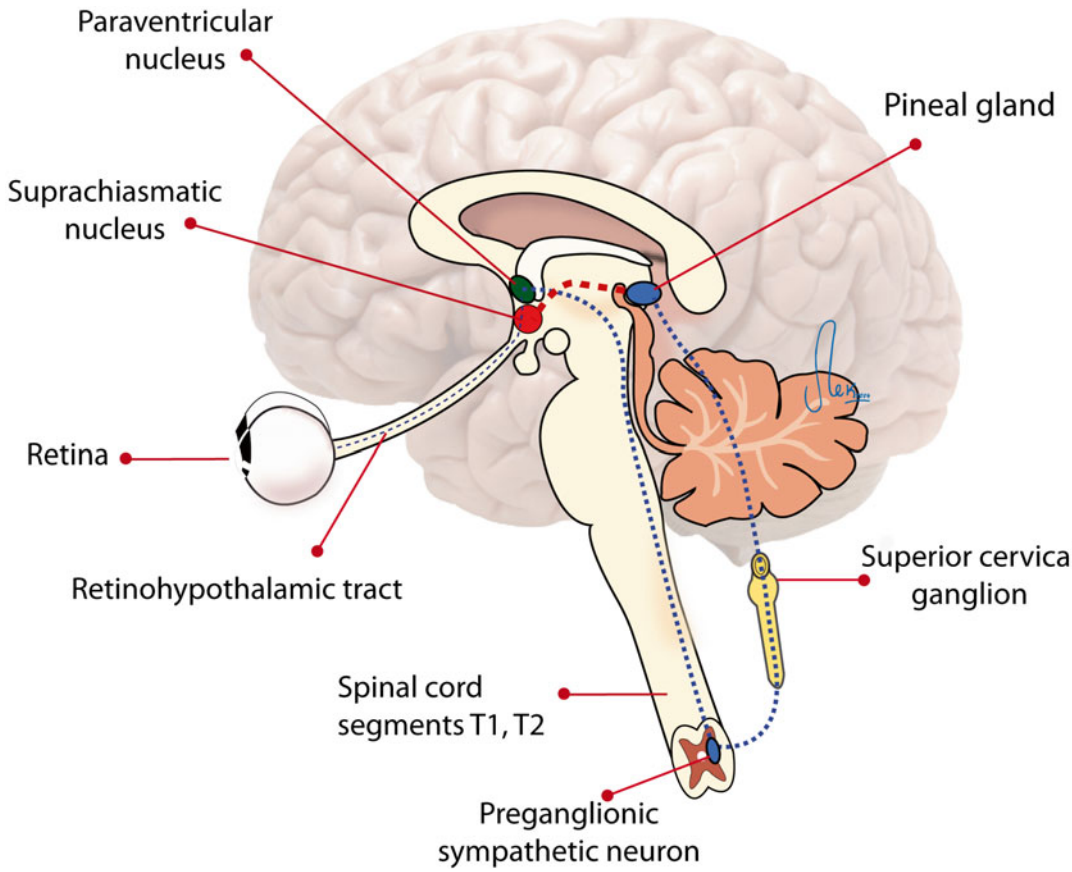


Fig. 11.3 Circadian regulation of the melatonin and functions of the pineal gland (Redrawn from Dubocovich et al. [8])

11.4 Melatonin and Body Regulation

The causes of anatomical and functional degeneration occurred in the organs during the ageing process include decreased antioxidant capacity and damage caused by free radicals, and these are attributed to melatonin hormone that decreases with the age [1, 13]. Nocturnal melatonin peak is moved to an earlier time with the increasing age [13]. As is known, melatonin stimulates the antioxidant enzymes, reduces the lipid peroxidation and protects the brain tissue from oxidative damage, and studies have indicated free radicals that are increased due to decreased melatonin levels as one of the reasons of neurodegenerative damages in the brain [19]. The loss in melatonin, an important hydroxyl radical scavenger, with the increasing age, renders brain tissue more vulnerable to oxidative attacks, and it has been reported that melatonin therapy can prevent this. Possibly, ageing adversely affects the pineal gland and reduces the melatonin production due to decreased number of β-adrenergic receptors on the membrane of the pinealocytes. As is known, β-adrenergic receptors initiate a series of events, which cause an increase in melatonin synthesis by mediating release of norepinephrine from sympathetic neurons into the pineal gland [19].

Sleep is essential for people. Key mechanism for sleep regulation is diurnal-nocturnal cycle, and light exposure leads to stimulation of a nerve pathway extending from the retina to the hypothalamic area in the brain. Suprachiasmatic nucleus in the hypothalamic area acts as a "biological clock" that is responsible for regulation of

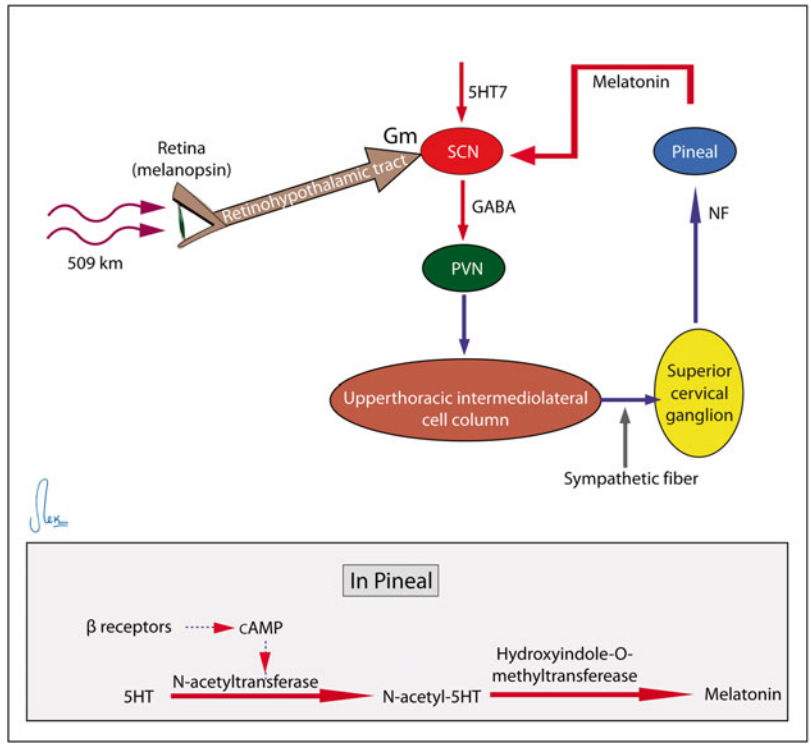


Fig. 11.4 Effects and receptors of the melatonin are seen (Redrawn from Reiter et al. [31])

activities affecting the whole body by initiating signals that control hormones to other areas of the brain, body temperature, sleep or wake feelings [1, 3]. Until nocturnal period, the release of hormones associated with sleep such as melatonin is suppressed, as the pineal gland is inactive. Since the suppressive signals created by suprachiasmatic nucleus that prevent the release of melatonin disappear when the sun goes down, the pineal gland is stimulated and melatonin production begins. With the increasing levels of melatonin, less stimulation is perceived and sleep feeling increases. Melatonin has been found to be associated with the onset, latent phase and the sleep quality rather than the total sleep duration [3, 32]. These effects of melatonin contribute to hypothermic activity and thermoregulation of the body on sleep.

Although the suprachiasmatic nucleus is the primary regulator of sleep-related variables, some researches showed that the melatonin released from the pineal gland by stimulation of this region is also associated with sleep [3]. As is known, sleep disorder is a common problem in depressive patients. Factors including waking up early in the morning due to diurnal variation, shortening of REM sleep latency with the intensity shifting to the first one-third period of the night, body temperature and phase shifting in cortisol secretion prove the association between depression and circadian rhythm disorder [7].

Seasonal depression is characterised by depression attacks that begin during autumn-winter and improve during spring-summer. Depressive symptoms include fatigue, hypersomnolence, hyperphagia, carbohydrate starvation, weight gain and loss of libido, and the disorder occurred during short days of winter is defined as seasonal affective disorder [7, 22, 27]. While the disorder continues during the short days, the severity of symptoms is reduced when the days become longer. The prevalence is usually higher in people living in regions at the north latitude and varies depending on the ethnicity.

Although the presence of circannual rhythm has been reported in addition to circadian rhythm, it has also been demonstrated that mood disorders may be triggered in case of problems with

adaptation to long days (photoperiod) and seasonal variations. Two aetiologic mechanisms have been defined for seasonal depression: photoperiodic hypothesis which causes circadian disorganisation and thus triggering of mood disorder and circadian phase-shifting hypothesis [22, 27].

According to photoperiodic hypothesis, differences in diurnal period during summer and winter as well as in the duration of nocturnal melatonin release cause development of depression attacks in susceptible individuals [7, 22, 27]. Whereas patients with seasonal depression had either significant seasonal differences in melatonin release or more diurnal melatonin release during winter, control group had no significant seasonal fluctuations in their melatonin levels as observed in those patients. Phase-shifting hypothesis is based on the opinion that shorter exposure to sunlight during the winter delays the circadian rhythm and disrupts the synchronisation between the suprachiasmatic nucleus regulating body rhythm and the sleep-wake cycle. Phase-shifting hypothesis is supported by the fact that morning phototherapy results in phase advance, while the evening phototherapy is not sufficiently effective.

In the past, seasonal depression was believed to be associated with abnormal melatonin mechanism; however, over time, this disorder was thought to be possibly associated with serotonin function. Association is possible between the changes in melatonin levels and the seasonal depression, revealed that the administration of high doses of melatonin to healthy subjects reduces the dizziness and lack of concentration and shortens the long reaction time, and the administration of melatonin prevents the worsening of depressive symptoms [7, 27].

Phototherapy as well as pharmacologic therapy is used for seasonal mood disorders. Phototherapy is as effective as antidepressants and can be used as first-choice therapy [7]. Phototherapy is administered through a light box with standard 10,000 lux power, which is placed vertically 1 m away from the user. The administration begins 30–90 min after waking up in the morning and lasts for about 30 min. It has been demonstrated that phototherapy can be used for treatment of conditions associated with circadian rhythm disorder such as other depressive disorders, delayed or advanced sleep phases as well as seasonal depression.

Less exposure to light during spring and winter due to long nocturnal period increases the duration of melatonin release. The increase in the duration of melatonin release results in more sleep and food intake, and phototherapy reverses the effect by shortening the duration of melatonin release [7]. Another mechanism of phototherapy acts through phase delay. Phase delay in endogenous circadian rhythm is advanced by morning phototherapy resulting in antidepressant activity [7, 27]. Morning phototherapy results in phase advance with higher antidepressant activity while evening phototherapy causes phase delay (Fig. 11.5).

11.5 Melatonin and Immune System Organs

There are limited researches about the effects of melatonin on immune system organs or cells. Laboratory studies investigating the correlation between photoperiod and immune functions report that the functions are more effective in short days than long days because of increased melatonin production in short days [1].

Immune system is suppressed in result of removal of pineal gland or experimental inhibition of melatonin synthesis. In such a case, the administration of melatonin reactivates the suppressed immune system. Melatonin can prevent immune deficiencies developed secondary to stress, viral diseases and bacterial disorders and during corticosteroid use or drug therapy [1, 4, 5]. Melatonin also helps to treat suppression of immune function following a trauma or a haemorrhagic shock. Melatonin is reported to increase immune response in immune deficiencies developed particularly due to ageing. Studies in poultry animals including quail, turkey and chicken have shown that melatonin improves the cellular and humoral immune responses [4]. Melatonin receptors have shown on the lymphocyte surfaces and macrophages. It has been observed that thymus morphology is impaired, and cellular and humoral immune responses are decreased in animals subjected to pinealectomy [28]. The increase in melatonin leads to increase in pro-inflammatory cytokines from macrophages.

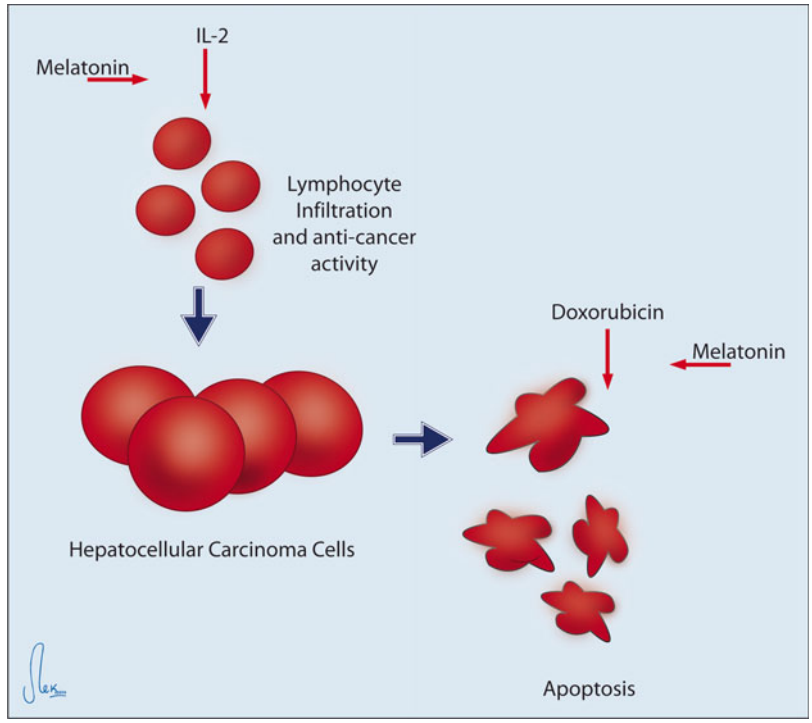


Fig. 11.5 Effects of the light on the melatonin release and pineal gland (Redrawn from Gómez et al. [16])

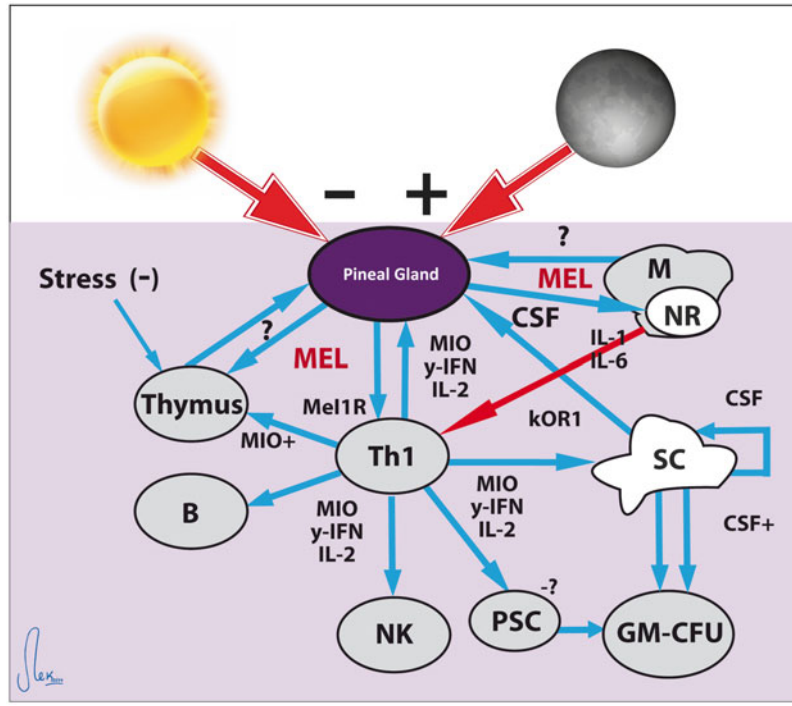
Melatonin leads to enlargement in sizes of the thymus, spleen and other tissues directly associated with immune function in mammals. Atrophy has been observed in the thymus gland of animals whose pineal glands were experimentally removed and who were given drugs that inhibit pineal function, and hyperplasia has been observed in the gland when these animals were administered with melatonin [5]. However, studies in birds suggested that melatonin caused to reduce in the masses of the spleen and bursa of Fabricius [4]. This difference may be attributed to the fact that the thymus hormones of mammals are different than those of poultry animals (Figs. 11.6 and 11.7)

11.6 Interactions Between Melatonin and Immune Cells

Melatonin activates the immune system cells either directly through melatonin receptors or indirectly by changes in steroid hormones. The activity of natural killer cells is reported to decrease after removal of pineal gland in mice. However, another study in mice has shown that the number of monocytes and natural killer cells have increased after 7 and 14 days of addition of melatonin to feed [17]. Melatonin receptors have been identified on some monocytes and macrophages. Binding of melatonin to these receptors increases the production of granulocyte-macrophage colony-stimulating factor, and macrophage production and function are improved.

Photoperiod and melatonin facilitate the lymphocyte proliferation in many rodents. Lymphocyte proliferation is increased in mice during the days with short diurnal period [1]. The administration of melatonin to mice leads to the same effect. A hamster study demonstrated that melatonin affects the immune cell function directly through the lymphocytes. This activity of melatonin occurs via MT2 receptors. Melatonin is reported to inhibit apoptosis during the formation of B-lymphocytes in mouse bone marrow [17].

Antibody production is also affected by the administration of melatonin. Mice given with luzindole (an MT2 receptor antagonist) have produced lower levels of IgG compared to mice that did not administered with this agent [24]. Pharmacologic inhibition of melatonin in mice



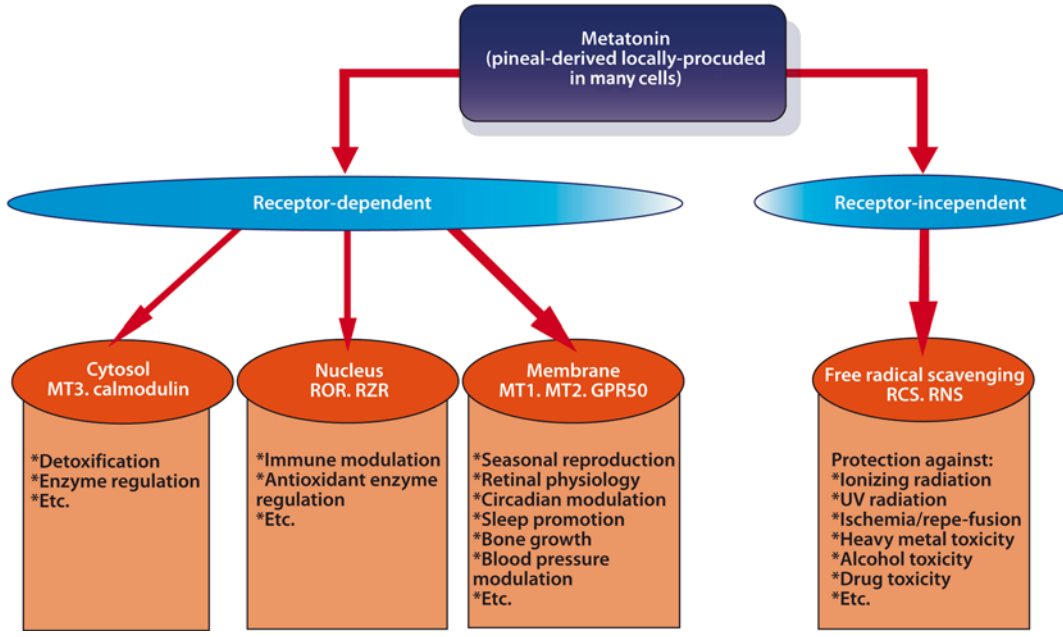


Fig. 11.6 The effects of melatonin on the immunological cells. Melatonin activates antigen-presenting cells (APCs), naive CD4+ cells, natural killer (NK) cells and T-helper lymphocytes (Redrawn from Szczepanik [33])

Fig. 11.7 The melatonin-immune-haematopoietic and lymphoid system association. Melatonin activates the melatonin receptors on the membranes of the pinealocytes (Mel1) and T-helper lymphocytes (Th1) or the receptors (NR) in the nuclei of the macrophages (M), and melatonin leads to secretion of some cytokines like IFNs, IL-1, IL-2, etc. Indeed, these cytokines may contribute to immune regulation and protect from secondary immune deficiencies and infection diseases and cancers. *PSC* pluripotent stem cells, *NK* natural killer cells, *B* B-lymphocytes (Redrawn from Maestroni [25])

reduced the immunological response to red blood cells of the sheep. A study in healthy young individuals suggests that a 10-day oral administration of 10 mg/day melatonin significantly increases the IgA levels in saliva [17].

Melatonin receptors have also been detected on the cells of the spleen. It is reported that melatonin treatment leads to an increase in the proliferation of the cells in the spleen of the rodents such as mice, voles and hamsters and MT2 receptors implicate the immunological stimulating effect of melatonin [3, 9]. Melatonin therapy also increases the T-cell proliferation [14]. In addition, melatonin increases the expression of large tissue compatibility complex class II molecules. This facilitates the ability of macrophages to supply antigen to T-cells [14]. Also, the number of studies demonstrating that it has effects on immune system has increased in recent years. It was reported that melatonin has antioxidative, anti-inflammatory and immunomodulatory effects. In addition, the ratio of TH1/TH2 changes in favour of TH1 and proinflammation is increased. Recently, melatonin is considered as an antioxidant that is released from pineal gland [19, 23]. There are studies indicating that melatonin may exert its effects on immune system via TH17 [21]. TH17 subpopulation has been recently identified as a distinct T-helper cell. TH17 plays an important role due to its effects on immune regulation in neuroimmune diseases, and thus it has a critical association with melatonin. It may be perceived as having a modulating role in the course of neuroimmune diseases due to its important role on T-regulator/TH17 balance. Melatonin directly interacts with T-lymphocytes and affects cellular immunity [29]. Highly sensitive binding sites have been identified on mice T-helper lymphocytes. Melatonin has increased the attenuated T-helper cell activity in immunosuppressed mice, and an increase has been observed in endogenous opioids released by T-helper lymphocytes in result of stimulation of T-lymphocytes by melatonin [17]. These endogenous opioids have stimulating effects on immune function. In mice, endogenous opioids resulted from T-helper lymphocytes increase the antibody synthesis. The administration of melatonin in aged mice results in thymus gland function and T-cell-mediated immune function that are similar to those observed in young mice [17]. CD4 and CD8 and T-lymphocytes and also B-lymphocytes express melatonin receptors on their membranes [9, 14, 17, 29]. In the literature, it was reported that following the melatonin treatment, the expressions of interleukin IL-2 and interferon (IFN)-γ are reduced, but the expressions of IL-4 and IL-10 are increased [14, 17]. Melatonin reduces the apoptosis of T-cells and increases the T-cell-mediated cytokine expression. Melatonin stimulates T-lymphocytes to produce IFN-γ, IL-2, IL-6 and IL-12 and indirectly IL-4 and IL-10 [29]. Melatonin administration increases the release of IL-2, IL-6, IFN-γ, IL-1 and IL-12 from human monocytes [29]. These cytokines may prevent the stress-related immunosuppression or secondary immune deficiencies (Fig. 11.7).

11.7 Melatonin: Relation to Body Functions and Diseases

Melatonin involves in regulation of many biological functions including sleep, circadian rhythm, adrenal gland and thyroid gland functions, reproduction, ageing, antioxidant system and immunity [23, 24]. Recent studies showed that seasonal changes might be a regulator for the immune functions by nocturnal melatonin release that is a biological signal for photoperiod [1, 11]. Melatonin is a molecule level of which is increased during night-time and decreased to the lowest limits during daytime. Its production is increased due to short diurnal period during winter and decreased due to long diurnal period during summer. Impairment of these functions may form the basis for several diseases and increase the rate of getting infections, autoimmune diseases and cancers.

Studies have shown that pro-inflammatory cytokines are increased during winter as compared to summer [7]. This seasonal change affects the neuroimmune system and may explain the seasonal feature of behavioural and emotional contagion [7, 22, 27]. Individuals working at shift works at an early age with effects on diurnal

rhythm have been observed to more likely to have MS [32]. This association suggested that MS may be developed due to effects of shift work on melatonin levels.

Melatonin also regulates the production of nitric oxide. Melatonin decreases the nitric oxide synthesis, which plays a pro-inflammatory role [10, 30]. Melatonin treatment was tried in many animal models of autoimmune neurological disease and examined in patients with autoimmune disease. Obtained results from the studies revealed that an endogenous melatonin level is important in the occurrence of the autoimmune neurological diseases and exogenous melatonin treatment may be useful in autoimmune neurological diseases.

Melatonin has inhibitory effects on cancer development. The effects of seasons on the development of cancer are associated with melatonin [1]. During the winter where nights are long, melatonin production is increased, and thus the tumour development slows down [3]. During the spring and summer where nights are short, melatonin production is decreased, and thus the risk of breast cancer is increased. Cancer suppresses the immune reactions. It is not clear by which mechanisms melatonin modifies the immune function and prevents the development of cancer. Melatonin is used with IL-2 in treatment of cancer. Interleukin 2 exerts its anticarcinogenic activity at very high doses and has serious toxic effects when used alone. For combination of melatonin and IL-2, it has been desired to observe that melatonin increases the effect of IL-2 and so reduce effective dose of IL-2 [11]. This combination has been administered in metastatic kidney and lung cancers, metastatic colorectal carcinoma, hepatoma and gastric carcinomas, metastatic endocrine and pancreatic tumours and also in breast cancer; so it was found to be satisfactory. Melatonin also inhibits the formation of apoptosis in healthy cells (Fig. 11.8).

Multiple sclerosis (MS) is an acute, neuroinflammatory and neurodegenerative autoimmune disease of the CNS and characterised by demyelination and axonal loss [12]. Jean-Martin Charcot, a neurologist, has first suggested the clinical and pathological definitions of the disease in 1898. The frequency is twofold more in women than men. Multiple sclerosis is the major non-traumatic neurological disease in young adults. It is the most prevalent inflammatory demyelinating disease in the central nervous system. The mechanisms of neuronal damage in MS are poorly understood but recent studies suggested that mitochondrial injury might be a reason in demyelination process and axonal loss [12, 18]. Mitochondrial dysfunction may be observed in MS, and this may be associated with clinical disability. In mouse models of MS, the administration of intraperitoneal melatonin has been demonstrated to ensure remission in axonal damage and demyelination and improvement in mitochondrial dysfunction [18]. Melatonin is a hormone, which reduces the levels of antioxidant and free radicals. This study suggested that oxidative stress is decreased by melatonin, and thus melatonin has a positive role in immunopathogenesis of MS. However, new studies investigating the role of melatonin in the oxidative stress mechanism and mitochondrial respiratory enzyme chain are required.

Multiple sclerosis is associated with increased levels of depression, which may lead to decreases in melatonin levels [32]. It is known that depression is also linked to sleep dysregulation. Moreover depression is associated with decreased levels of serotonin, which is a precursor for melatonin [22]. Melatonin can be used for treatment of insomnia, which may be seen in patients with MS [32]. It may also be useful for effects on the sleep quality due to circadian rhythm disorders which may be seen in MS. Therefore, more investigations are needed to ascertain the role of melatonin in the treatment and pathophysiology of MS.

Experimental autoimmune encephalomyelitis (EAE) is the unique animal model for MS, due to their many clinical and immunopathological similarities to MS. Melatonin has a modulating effect on immune system with a T-helper ratio of 1/2. This effect leads to positive impacts on immunopathogenesis of autoimmune neurological diseases including MS [28]. Melatonin, thanks to this effect, can play an active role in clinical course of neuroimmune disorders.

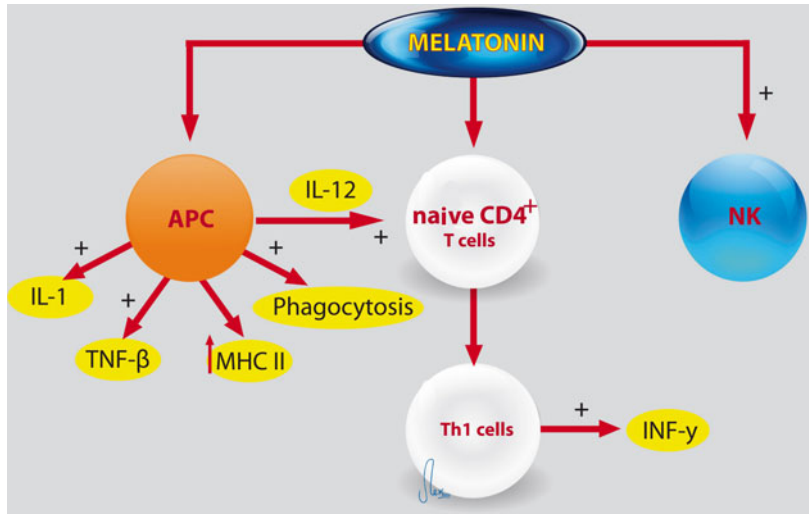


Fig. 11.8 Anticancer effects of the melatonin are evaluated in two different ways. Firstly, melatonin stimulates IL-2 release from macrophages and so lymphoid infiltration to cancer site. Secondly, melatonin treatment with anticancer drug or alone contributes to tumour cell apoptosis (Redrawn from Glenister et al. [15])

The number of studies demonstrating melatonin has positive effects on immunopathogenesis of MS and EAE, the animal model of MS, has increased in recent years. Inflammation, oxidative stress, demyelination and axonal loss involve in immunopathogenesis of disease [18]. Oxidative stress is critical especially for neurodegeneration. Recent studies have shown a reduction in antioxidant levels with the increase in oxidative stress. This indicates an association with the increase in number of attacks and the disability. Conducted studies indicate an association between the decreased levels and dysregulation of synthesis of blood melatonin and the symptoms of MS patients including fatigue, depression and impaired sleep quality [32]. These studies report that abnormal changes in the melatonin levels may lead to the pathogenic changes of MS. Melatonin plays a positive role in neurodegeneration observed in MS with its inhibitory effect on free radicals and antioxidant activity. Also neuroprotective effect of melatonin may be caused from their antioxidant, anti-inflammatory, anti-apoptotic and anti-excitotoxic activities [14, 17]. Thanks to these effects, melatonin can be regarded as having an important role especially in neuroprotective effects in chronic autoimmune neurological diseases such as MS.

The administration of melatonin in animal models showed a reduction in the levels of ICAM in experimental autoimmune encephalomyelitis [18]. Recent data show that melatonin triggers a reduction in the levels of ICAM-1 and VCAM. Natalizumab is an immunomodulator therapy that has been commonly used in treatment of multiple sclerosis in recent years. It is successfully used to prevent attacks and disability, which may be observed in the course of MS, and to prevent occurrence of new cranial demyelinating lesions. Both melatonin and natalizumab have been associated to an effect on adhesion factors such VCAM and intercellular adhesion molecule 1 (ICAM-1) [2].

There are studies demonstrating that MS patients have lower melatonin levels than healthy individuals [2, 12, 18]. However, the number of studies indicating a relation the low melatonin levels with the high levels of pro-inflammatory cytokines is limited. These studies suggest that the decrease in melatonin level may cause a decrease in antioxidant levels and increase in pro-inflammatory levels and may be a predisposing factor for autoimmune neurological diseases such as MS. Studies investigating the blood melatonin levels in MS patients receiving natalizumab have shown an increase in melatonin levels, and this increase has been suggested to cause a decrease in oxidative stress [2]. It may be suggested that the use of natalizumab may lead to an increase in neuroprotective effect in MS, thanks to this melatonin-mediated interaction.

Gaining an increased interest since the 1950s, melatonin has been reported to have several physiological effects on the neuroimmune system. Particularly, mood disorders and some neuroimmunological diseases such as MS accompanied by circadian rhythm disorder are closely associated with plasma melatonin levels. The regulation of circadian rhythm with phototherapies as well as pharmacologic agents is maybe helpful in improving manifestations of mood disorders and neuroimmunological diseases.

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Melatonin in Clinical Status of Patients with Fibromyalgia Syndrome

12

Andrei Pereira Pernambuco, Marina de Barros Pinheiro, and Débora d' Ávila Reis

Abbreviations

4P-PDOT	4-phenyl-2-propionamidotetralin
5-HT2C	Serotonin receptors
6-SMT	6-Sufatoxymelatonin
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CNS	Central nervous system
COX	Cyclooxygenase
ELISA	Enzyme-linked immunosorbent assay
FIQ	Fibromyalgia Impact Questionnaire
FM	Fibromyalgia
GABA	Gamma-aminobutyric acid
MT	Melatonergic receptors
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
PSQI	Pittsburgh Sleep Quality Index
QR2	Quinone reductase type 2
ROR	Retinoid-related orphan receptor
SF36	Medical Outcomes Study Questionnaire 36-Item Short-Form Health Survey
SS	Symptom Severity Scale
SWLS	Satisfaction with Life Scale
WPI	Widespread Pain Index

12.1 Introduction

Fibromyalgia (FM) is a complex musculoskeletal condition characterized by the presence of chronic widespread pain. Patients experience pain on palpation of specific anatomical areas

A.P. Pernambuco, MSc, PhD (✉)
Department of Morphology, Cell Biology Institute, Federal University of Minas Gerais, Av. Antônio Carlos, 6627, Campus Pampulha, 31270-910 Belo Horizonte, MG, Brazil

Department of Health Sciences, University Centre of Formiga (UNIFOR-MG) – Centre for Research and Extension (CEPEP), Av. Dr. Arnaldo de Senna 328, Água Vermelha, 35570-000 Formiga, MG, Brazil

Department of Health Sciences, University of Itaúna, Mg 431Road, Km 45, s/n. White Campus, 35680-142 Itaúna, MG, Brazil
e-mail: pernambucoap@ymail.com

M. de Barros Pinheiro, MSc
Arthritis and Musculoskeletal Research Group – AMRG, Faculty of Health Sciences, The University of Sydney, Room S227, S Block, Cumberland Campus, 2141 Sydney, NSW, Australia
e-mail: marinadebarros@hotmail.com, mdeb9852@uni.sydney.edu.au

D. d' Ávila Reis, MSc, PhD
Department of Morphology, Cell Biology Institute, Federal University of Minas Gerais, Av. Antônio Carlos, 6627, Campus Pampulha, 31270-910 Belo Horizonte, MG, Brazil
e-mail: debsdavila@gmail.com

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and other symptoms such as fatigue, non-restorative sleep, depression, anxiety, morning stiffness, and headache [1].

The prevalence of FM is estimated at 2–4 % of the general population, and it is more prevalent among women, corresponding to 90 % of the cases [2, 3]. To date, the pathophysiology of FM is not completely understood, hindering the development of effective therapeutic approaches for this condition [3, 4]. The lack of effective treatments for FM contributes to persistent physical symptoms and often psychological sequelae, including even suicidal thoughts [3]. Moreover, as the prevalence of FM increases, the cost to individuals and society associated with this condition is on the rise [5].

The incomplete understanding of the pathophysiology of FM and the absence of effective management strategies have prompted the development of studies investigating the role of melatonin in the pathophysiology and treatment of this condition. Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone produced mainly by the pineal gland. Its production is regulated by light and its release is the main event indicator of the evening to the body [6]. Melatonin is synthesized from tryptophan and serotonin, and, when released, part of the melatonin crosses the blood-brain barrier and diffuses in the cerebrospinal fluid, while another part is released into the bloodstream [7]. Metabolization of melatonin occurs in the liver in two steps: (i) hydroxylation transforms the hormone into 6-hydroxymelatonin and (ii) the association of melatonin with sulfuric or glucuronic acid results in the major melatonin metabolite, 6-sulfatoxymelatonin (6-SMT) which is excreted in the urine [8–10]. The 6-SMT is the main metabolite of melatonin found in urine, and its levels are highly related to the levels of melatonin produced by the pineal gland. Therefore, 6-SMT is one of the main ways used by researchers to indirectly measure levels of melatonin in the body [9, 11].

The studies that have investigated the role of melatonin on FM syndrome can be divided into two main areas: (i) the role of melatonin as a potential etiologic agent of FM and (ii) the use of melatonin as a potential therapeutic agent to treat this condition. The current interest of melatonin in FM syndrome is justified mainly by the fact that low levels of this hormone have been observed in patients with FM [11, 12]. Furthermore, melatonin has chronotropic [13], analgesic [14], and anxiolytic properties [15] that could clinically benefit patients with FM.

12.2 The Fibromyalgia Syndrome

The diagnostic criteria for FM were first established in 1990 by the American College of Rheumatology. The diagnostic criteria include the presence of chronic and widespread pain (presence of pain greater than 3 months above and below the waist line, on both sides of the body, affecting at least one component of the axial skeleton) and pain on palpation in at least 11 out of 18 tender points assessed in the physical examination [1].

Pain is often accompanied by many other symptoms including sleep disturbances, stiffness, fatigue, depression, and mood disturbance [1, 16].The evolution of clinical research has revealed that these other symptoms associated with the clinical features of FM are as important as pain itself. As a result, the American College of Rheumatology has since reformulated the diagnostic criteria for FM, since these associated symptoms were not included in the previous diagnostic criteria established in 1990 [1]. The new diagnostic criteria for FM, published in 2011, are based on two key indices: the Widespread Pain Index (WPI) and Symptom Severity (SS) Scale [16]. The WPI indicates the presence of pain in 19 parts of the body. The SS evaluates the presence of other symptoms including affected sleep, fatigue, cognitive symptoms, and multiple visceral symptoms. According to the new criteria, the diagnosis of FM is confirmed when WPI score is greater or equal to 7 and the SS scale is greater or equal to 5 or when the WPI score ranges from 3 to 6 combined with SS scale of greater or equal to 9 (Table 12.1). After the publication of the new diagnostic criteria, FM is no longer considered a condition purely characterized by pain, but an algic condition associated with other significant symptoms [16].

The pathophysiology of FM remains uncertain. Several mechanisms have been proposed,

for example, central or peripheral sensitization, genetic changes, behavioral disorders, oxidative stress, immunological changes, and neuroendocrine disorders [4, 16]. However, these factors in isolation could not explain the wide variety of symptoms that compose the clinical feature of FM. Therefore, it is believed that the pathophysiology of FM is the result of the interaction between these multiple etiologic factors [17, 18].

The multifactorial etiology of FM combined with the large heterogeneity of clinical characteristics impedes the development of effective therapeutic strategies for this condition [3, 4]. Currently, several pharmacological and non-pharmacological treatments are available, such as tricyclic antidepressants, anti-inflammatories, analgesics, anxiolytics, anticonvulsants, acupuncture, physiotherapy, cognitive-behavioral therapy, aerobic exercises, and health education programs [3, 4]. However, none provide complete resolution of the symptoms [19]. Therefore, clinicians and researchers agree that the best treatment option for FM consists of a combination of pharmacological and non-pharmacological therapies, as this combination has resulted in improved outcomes, such as the relief of symptoms and improvement in the quality of life and function of patients with FM [18, 19].

The difficulty in the treatment of FM results in substantial burden to individuals and society and higher costs related to this condition. Assuming that FM affects up to 3 % of the population, it is estimated that the total expenditure of FM reaches up to €12 billion per year in a population of 80 million people, based on data from several countries, such as Canada, United States, United Kingdom, and Holland [5]. Costs with medication account only for a small proportion of the total cost (€960 million, 8 %). The remaining costs are related to other factors such as work absenteeism, care seeking, and compensation claims [5].

12.3 Involvement of Melatonin with the Main Symptoms of FM

The main symptoms experienced by patients with FM are widespread pain, sleep disturbances, fatigue, and cognitive-behavioral disturbances, such as anxiety and depression. The manifestation of these symptoms is so prevalent among patients with FM that they are part of the criteria for the diagnosis of this condition [16]. According to the melatonin properties previously described, such as chronotropic [13], analgesic [14], and anxiolytic properties [15], it is likely that melatonin may interfere with the expression of each of these symptoms [11, 12]. As reduced levels of melatonin may cause the development of these symptoms, therefore in contrast, the therapeutic intake of melatonin may result in the relief or even the remission of these symptoms [20].

12.4 Mechanisms of Action of Melatonin on Pain

Melatonin has the potential to reduce pain in patients with FM [21]; however, the mechanisms of action of this hormone in promoting analgesia are still unclear and they are debatable. It is likely that the analgesic effect of melatonin is related to opioid receptors; benzodiazepines; α1- and α2-adrenergic, serotonergic, cholinergic, and N-methyl-D-aspartate (NMDA) receptors; opioids coupled to G-protein receptors; or gamma-aminobutyric acid (GABA)-B receptors [21, 22]. Although being able to signal through all of these receptors, melatonin binds preferentially to melatonergic receptors (MT), and there is evidence that these receptors are highly involved in antinociceptive mechanisms [14].

Currently, three melatonergic receptors have been described: MT1, MT2, and MT3. MT1 and MT2 are coupled to the G-protein; this connection activates multiple intracellular mediators such as cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), or [Ca^{2+}] [23]. The MT3 receptors have only been recently identified and their physiological importance has not yet been fully determined; however, it is known that the binding of MT3 receptors is associated with a quinone reductase protein type 2 (QR2), an antioxidant enzyme. In this case, the melatonin may act as a co-substrate for the activation of QR2, and therefore the MT3 receptors may be primarily involved in processes other than analgesia [24].

Table 12.1 Diagnostic criteria for fibromyalgia established by the American College of Rheumatology

Fibromyalgia diagnostic criteria (1990)	Fibromyalgia diagnostic criteria (2011)
Widespread pain: presence of pain above and below the waist line, on both sides of the body, affecting at least one component of the axial skeleton (cervical, thoracic, or lumbar spine or anterior part of the thorax)	*Widespread Pain Index (WPI)*: the number of painful areas in the patient (0–19) is bilaterally assessed: right shoulder girdle, left shoulder girdle, right arm, left arm, right forearm, left forearm, right hip, left hip, right thigh, left thigh, right leg, left leg, right jaw, left jaw, thorax, abdomen, and lumbar, thoracic, and cervical region
Tender points: pain on digital palpation with a force of 4 Kg/cm^2 applied for four seconds) in at least 11 out of 18 specified locations (nine bilateral points): occiput, low cervical region, trapezius muscle, supraspinatus muscle, second rib, lateral epicondyle, gluteal, greater trochanter, knee	*Symptom Severity (SS)*: fatigue, non-restorative sleep, and cognitive symptoms are considered. A score is given for each symptom according to the severity of the symptom in the past week, using the following scale: 0 = no problem, 1 = slight or mild problems (generally mild or intermittent), 2 = moderate (considerable problems, often present and/or at a moderate level), and 3 = severe (pervasive, continuous, life-disturbing problems)
Diagnosis: the above criteria must be met. Widespread pain should be present for at least 3 months. The presence of other medical conditions does not exclude the diagnosis of fibromyalgia	Presence of somatic symptoms, assessed with the following scale ¥:
	0 = no symptoms, 1 = few symptoms, 2 = a moderate number of symptoms, and 3 = a great deal of symptoms
	The SS final score is the sum of the fatigue, non-restorative sleep, and cognitive symptom scores in addition to the "other symptom" score. The final result ranges between zero and 12
	Diagnosis: the following three conditions must be met: (1) WPI ≥7 and SS score ≥5 or WPI from 3 to 6 and SS ≥9. (2) Symptoms have been present at a similar level for at least 3 months. (3) The patient does not have a disorder that would otherwise explain the pain
¥ *Somatic symptoms that might be considered*: muscle pain, irritable bowel syndrome, fatigue/tiredness, thinking or remembering problem, muscle weakness, headache, pain/cramps in the abdomen, numbness/tingling, dizziness, insomnia, depression, constipation, pain in the upper abdomen, nausea, nervousness, chest pain, blurred vision, fever, diarrhea, dry mouth, itching, wheezing, Raynaud's phenomenon, hives/welts, ringing in the ears, vomiting, heartburn, oral ulcers, loss of/change in taste, seizures, dry eyes, shortness of breath, loss of appetite, rash, sun sensitivity, hearing difficulties, easy bruising, hair loss, frequent urination, painful urination, and bladder spasms	

Fonte: Wolfe et al. [1, 16]

The evidence regarding the role of MT1 and MT2 receptors in the mechanism of analgesia is compelling and can be confirmed by the use of antagonists to melatonergic receptors, such as luzindole, 4-phenyl-2-propionamidotetralin (4P-PDOT), or K-185 [25]. The MT1 and MT2 receptors are widely distributed throughout the central nervous system (CNS), including the thalamus, hypothalamus, trigeminal tract, trigeminal nucleus, and dorsal horn of the spinal cord, particularly in the laminae I–V and X, and as these regions are crucial for pain control, the presence of MT1 and MT2 receptors in these structures supports the role of melatonin in nociceptive modulation [26, 27]. The binding of melatonin on MT1 and MT2 receptors negatively modulates the formation of cAMP and intracellular Ca^{2+}. In sequence, potassium channels are activated and Ca^{2+} channels are blocked, possibly by the activity of Gβγ subunits of the receptor. All these changes contribute to the reduction of nociception [28].

NMDA receptors also play an important role in the transmission of pain sensation, mainly through the phenomenon known as wind-up. This phenomenon is the perceived increase in pain intensity over time when a given painful stimulus is delivered repeatedly [29]. It is caused by repeated stimulation of group C peripheral nerve fibers, leading to progressively increasing electrical response in the corresponding posterior horn of the spinal cord [29]. Melatonin is able to reduce the wind-up phenomenon in a dose-dependent manner, with high doses being able to eliminate this phenomenon. This effect is explained by the action of melatonin on the paths of generation of intracellular nitric oxide (NO)

dependent of NMDA receptors [30]. It is known that NO can interact with NMDA receptors and cyclooxygenase (COX) resulting in hyperalgesia; however, NO may also exert antinociceptive and antiallodynic effects through K+ channels sensitive to cGMP-PKG-ATP, which has led researchers to consider using NO in the treatment of pain [31].

Several studies in animals and humans have investigated the effectiveness of melatonin as an antinociceptive agent in different types of pain, such as acute, chronic, inflammatory, and neuropathic. In most cases, the results are satisfactory and support the use of melatonin as a pharmacological agent for pain reduction [14, 20, 32, 33]. According to this evidence, the reduced production of melatonin observed in FM patients may increase nociception, which could manifest clinically as hyperalgesia and/or allodynia [11, 12, 14]. This sequence of events supports the hypothesis that disturbances in melatonin production could be related to the pathophysiology of FM; moreover, this evidence supports the investigation of melatonin supplementation in the treatment of patients with FM [11, 12, 14].

12.5 Melatonin and Fatigue

Melatonin appears to directly influence the manifestation of chronic fatigue, another symptom frequently observed in patients with FM. The underlying neurological mechanisms associated with the perception of fatigue are poorly understood. However, monoaminergic abnormalities, changes in sleep-wake cycle, and prefrontal dysfunctions are recognized as factors that contribute to the onset and/or persistence of chronic fatigue [34].

Some studies suggest that feelings of fatigue may be related to a change in the circadian rhythm of melatonin, resulting in a delayed secretion of this hormone by the pineal gland [28, 35]. Furthermore, Masruha et al. demonstrated that subjects with high concentrations of 6-SMT reported lower levels of fatigue [9]. Similar results have already been published by Akerstedt et al., and they found a strong negative correlation between melatonin levels and fatigue in healthy individuals [36].

Knowledge of the involvement of melatonin in fatigue has led to the development of clinical trials evaluating the treatment of chronic fatigue through melatonin supplementation. The results of these trials are so far encouraging; however, the relationship between melatonin and the perception of fatigue is still unclear [22, 37].

Pardini et al. [34] have investigated the action of agomelatine in reducing the perception of fatigue. Agomelatine is a melatonergic antidepressant, a potent agonist of melatonin in the MT1 and MT2 receptors and antagonist of serotonin receptors (5-HT2C), and it has also being tested as a therapeutic alternative to FM [38]. According to this study, agomelatine can modulate not only the melatonergic system but also the dopaminergic and noradrenergic systems, through 5-HT2C receptors [14, 34]. Thus, by acting as an antagonist on 5-HT2C receptors, agomelatine would lead to an increase in dopaminergic and noradrenergic tone in the prefrontal cortex, resulting in the reduction of fatigue perception. This is partially explained by the fact that the perception of fatigue is directly related to reduced dopaminergic and noradrenergic activity in the prefrontal cortex. Further supporting this hypothesis is the effect of bupropion, commonly used for the treatment of fatigue, in increasing noradrenergic and/or dopaminergic tone [34]. The findings of this study may provide clues about the mechanisms of melatonin in reducing the perception of fatigue.

To date, there is no strong evidence that melatonin reduces the perception of fatigue in patients with FM through mechanisms previously described or through different mechanisms from those used for agomelatine since the affinity of melatonin to 5-HT2C receptors is much more limited than that of agomelatine [38]. However, the melatonin may also signal through serotonergic, adrenergic, and cholinergic receptors [14]. Nevertheless, further studies are required to investigate the mechanisms of both melatonin and agomelatine in reducing perceived fatigue in patients with FM.

12.6 Melatonin and Sleep

Melatonin also has the potential to improve sleep quality by reducing the sleep onset latency, increasing the duration of sleep, improving sleep efficiency, and regulating the sleep-wake cycle [38]. These effects are brought about by melatonin binding with the MT1 and MT2 membrane receptors located in the region of suprachiasmatic nucleus of the hypothalamus. The result is the reduction of neuronal stimulation in this area of the brain [38]. The suprachiasmatic nucleus is the area considered responsible for controlling the "biological clock," which adjusts the circadian rhythm under the influence of melatonin [39].

The MT1 and MT2 receptors found in the suprachiasmatic nucleus region have distinct functions. The MT1 receptors are involved in the onset of sleep, while the MT2 receptors are associated with the phase setting of the circadian rhythm [40, 41]. Also important is the binding of melatonin with MT1 receptors, resulting in the reduction of neuronal stimuli in the suprachiasmatic nucleus region, whereas the binding of melatonin with MT2 receptors acts in resetting the "biological clock" [38].

The binding of melatonin to these receptors, MT1 and MT2, involves complex signaling pathways. The first route discovered involved the reduction of cAMP in a dose-dependent manner to the agonist [42]. Nowadays, there are other known melatonin receptor signaling pathways, such as the activation of phospholipase C, through the binding of melatonin with MT2 receptors, and the control of inward rectifying K+ channels (Kir channels) with secondary effects on the voltage-controlled Ca^{2+} channels, arising from melatonin binding to the MT1 receptors. These signaling pathways lead to the circadian phase changes (MT2) and reduction of neuronal stimuli in the suprachiasmatic nucleus (MT1) [43]. Such effects are known as hypnotic effects of melatonin. Therefore, melatonin presents different mechanisms of action when compared to conventional drugs used to induce sleep (e.g., benzodiazepines) and/or Z-drugs (e.g., zolpidem, zaleplon, and zopiclone), which induce a generalized depression of the nervous system via $GABA_A$ receptors [42]. However, melatonin is also capable of influencing even indirectly GABAergic mechanisms in sleep-related regulation through downstream routes related to the suprachiasmatic nucleus [38].

Due to its influence on sleep, melatonin and other melatonin receptor agonist agents, such as agomelatine and ramelteon, are being tested in clinical trials. However, despite its great therapeutic potential, considerations are necessary [44, 45]. Firstly, it must be acknowledged that MT1 and MT2 receptors are not found exclusively in the suprachiasmatic nucleus. These receptors have also been identified in structures of the immune system, gastrointestinal tract, blood vessels, structures of the CNS, and various hormone subsystems [43]. The widespread distribution of these receptors implies that melatonin does not act only on sleep but instead acts in a pleiotropic manner, affecting various physiological functions [43]. Furthermore, melatonin may bind to other receptors, such as opioid, benzodiazepines, and α1- and α2-adrenergic, serotonergic, cholinergic, NMDA, and retinoid-related orphan receptors (ROR) [43]. These receptors are widely distributed throughout body tissues, leading to the potential of melatonin to interfere with physiological functions and increasing the risk of developing unpredictable effects [43].

In spite of the pleiotropic characteristics of melatonin, studies evaluating its use for the treatment of sleep disorders have shown satisfactory results, and the researchers are considering the use of melatonin to be safe and effective for the treatment of sleep disorders [13, 38, 44]. However, there are still some uncertainties to be resolved, such as the ideal dose for the different sleep disorders (e.g., chronic primary insomnia, jet lag, sleep, and circadian rhythm disruption); the most suitable time for drug intake, since the pharmacokinetics of melatonin may differ significantly when used at night or morning periods; as well as the ideal drug release profile, since extended-release drugs act differently from immediate-release drugs, and each of them seems to have different effects on the types of sleep disorders [46].

12.7 Melatonin and Cognitive-Behavioral Changes

Depression and anxiety are the two most commonly cognitive-behavioral problems found in patients with FM. It is estimated that up to 70% of patients with FM report depressive symptoms [47] and up to 50% have anxiety symptoms [48]. According to studies conducted in humans and animals, alterations of melatonin levels might contribute to the development of both symptoms [49, 50].

Anxiety disorders are characterized by excessive and uncontrollable concerns with trivial situations with disproportionate fears of catastrophic consequences. Anxiety often occurs with physical signs and symptoms, such as nausea, headache, fatigue, restlessness, and sweating [51]. The interference of melatonin on anxiety disorders is likely explained through binding of melatonin with GABA receptors that are widely distributed in the brain, including the suprachiasmatic nucleus [52]. These receptors are known to be involved in the regulation of behavior functions [53]. The binding of melatonin to GABA receptors activates the GABAergic system resulting in a dose-dependent increase of GABA concentration in the CNS, resulting in the production of an anxiolytic effect [54, 55]. This anxiolytic effect can be obtained even independently of benzodiazepine receptors, since when the melatonin is concurrently taken with flumazenil, an antagonist of benzodiazepine receptors, the anxiolytic effect of melatonin can still be observed [56].

Evidence shows that not only the GABA receptors but also the MT2 receptors are involved in the modulation of anxiety [15, 57]. This evidence shows that the use of a selective agonist for this type of receptor (UCM765) results in high anxiolytic effects in animals [15]. Moreover, the use of luzindole, an antagonist of MT1 and MT2 receptors, has been shown to be capable of blocking the effects of melatonin on anxiety [15, 50]. Further evidence confirming the involvement of these receptors in anxiety is the observation in knockout mice to MT2 ($MT2^{-/-}$) showing complex changes in anxiety behavior [58]. These findings, although promising, still need to be further explored, particularly the neurobiological and cellular mechanisms by which the MT2 receptors can modulate anxiety.

The anxiolytic effects of the melatonin appear to be related to both the activation of the GABAergic system and the melatonergic system that mutually act to modulate the anxiety disorders [59, 60]. This hypothesis is supported by the fact that almost all GABAergic thalamic reticular neurons express MT2 receptors [61]. Additionally, the administration of melatonin significantly increases the levels of GABA in certain areas of the brain, such as the hypothalamus, cerebellum, and cerebral cortex, resulting in decreased anxiety levels [62].

Research with animals and humans has been conducted to investigate the role of melatonin in reducing anxiety disorders [15, 55]. The results are promising and demonstrate satisfactory anxiolytic effects, with minimal adverse effects and good tolerance by patients. These studies show that melatonin may be considered an effective therapeutic strategy for treating anxiety disorders and may replace other commonly used drugs such as the benzodiazepines [15, 55, 60, 63].

Depression is another cognitive-behavioral change commonly found in patients with FM. Depression is characterized by reduced mood, low self-esteem, and loss of interest in activities previously considered pleasurable (anhedonia). These symptoms may also be accompanied by fatigue, anxiety, and changes in weight and/or sleep [51]. It is likely that melatonin is also associated with depressive symptoms experienced by patients with FM, as people with major depression display lower levels of melatonin [64, 65]. Several studies have investigated the use of melatonin in the treatment of depression; however, most have found that melatonin alone does not significantly affect symptoms of depression [66]. Nevertheless, there is a study that demonstrated an improvement in depressive symptoms after the use of melatonin [67]. The chronotropic effects provided by the binding of melatonin with MT1 and MT2 receptors appear to be insufficient to generate a significant reduction in depressive symptoms [44, 66].

Although melatonin does not appear to have a large effect on depressive symptoms, agomelatine has shown excellent results as an antidepressant

drug [68, 69]. These results are likely due to the chronotropic effects resulting from stimulation of MT1 and MT2 receptors and inhibition of 5-HT2C receptors [68, 70]. Studies have shown that agomelatine is effective for treating depression, safe and well tolerated by patients [69]. Agomelatine may be considered more efficient than currently used antidepressants [70], due to its different mode of operation particularly in regard to circadian phases of light-dark cycle, inducing sleep at night and keeping the person awake during the day. Additionally, agomelatine has fewer adverse effects than the currently used drugs [68].

12.8 Melatonin Levels in Patients with FM

The strong evidence linking melatonin with the main symptoms of FM encouraged several research groups to investigate the levels of this hormone in patients with FM [12, 35]. In the first study comparing melatonin levels between patients with FM and healthy controls, the authors evaluated the levels of 6-SMT in the urine of 39 women with FM and 39 healthy women [71]. The results revealed slightly increased levels of 6-SMT in the urine of patients with FM; however, the difference found was not statistically significant. The levels of 6-SMT found in women with FM also did not correlate significantly with the time of onset of FM, reproductive status, or behavioral and sleep disorders presented by patients [71].

Another study identified abnormal levels of melatonin secretion in patients with FM during dark hours [12]. In this study, the secretion of melatonin in patients with FM was 31% lower than in healthy controls. Moreover, the peak serum melatonin level in patients with FM was also significantly lower when compared to the control group. It is important to note that only eight patients with FM and eight controls matched for age and body mass index (BMI) were used [12]. When these data were published, several hypotheses were proposed in an attempt to explain the findings [72]. One hypothesis describes the possible difficulty of intestinal absorption of tryptophan associated with the presence of anti-serotonin antibodies in patients with FM, and this combination of factors could contribute to reduced melatonin synthesis, since both tryptophan and serotonin are precursors of melatonin. Neurodegenerative disorders (reduction of β-adrenergic stimulation) and neuroendocrine changes (increase in prolactin, endorphins, and enkephalins, reduction of TSH, T3, and T4) were also speculated as possible causes of reduced production of melatonin by patients with FM. According to this evidence, all these changes are related to the phenomenon of stress response, and therefore it could be influencing the normal function of the pineal gland [72].

An additional study investigated 27 women, divided into three groups: FM ($n=9$), chronic fatigue syndrome ($n=9$), and healthy controls ($n=9$). All women stayed in a hospital for 24 h, and blood samples were collected via an intravenous catheter every 10 min. The results showed significantly elevated serum levels of melatonin between 11 pm and 6:50 am in patients with FM compared to healthy controls [73]. However, no significant changes in regard to the pattern of release of this hormone were found between the groups [73]. The results of this study are isolated, since no other research has found high levels of melatonin in patients with FM.

Another study evaluating some markers of circadian rhythm, including melatonin levels, in women with FM ($n=10$) and healthy women ($n=12$) was published [74]. The investigators have controlled the analysis for variables such as luminosity levels, posture, sleep-wake cycle, meals, and physical activity. The results showed similar levels of melatonin in patients and controls, as well as other markers of the circadian rhythm, which led the authors to conclude that circadian abnormalities should not be considered key factors in the pathophysiology of FM [74].

Similar results were found when 25 women with FM who had not yet gone through menopause and 20 healthy women matched by age were included in a study that investigated serum levels of melatonin by the enzyme-linked immunosorbent assay (ELISA) test [35]. The results revealed similar levels of melatonin in patients

and controls. Melatonin levels in patients with FM also did not correlate with the time of onset of symptoms and sleep disorders and not even with the scores of fatigue and pain. According to the authors, further studies should be conducted to elucidate the true importance of melatonin in FM [35].

Different results were found by Pernambuco et al., who have evaluated the levels of 6-SMT and the intensity of symptoms in patients with FM and healthy controls matched for gender, age, and BMI. The authors found that the excretion of 6-SMT from 8 pm to 8 am was 36 % lower in patients with FM when compared to healthy individuals. Moreover, the levels of 6-SMT in the urine of patients with FM were below the median value found in healthy controls (12.18 ng/ml) in 67 % of cases. However, the values of 6-SMT found in the urine of patients with FM were not significantly correlated with the scores obtained in any of the following questionnaires: the Fibromyalgia Impact Questionnaire (FIQ), Pittsburgh Sleep Quality Index (PSQI), Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36), or Satisfaction with Life Scale (SWLS). The authors of this study could not rule out the involvement of melatonin in the onset and/or evolution of FM; however, they observed that other neuroendocrine changes may be associated with disturbances of melatonin production interfering directly and negatively in the manifestation of symptoms in patients with FM [11].

As previously highlighted, the evidence regarding melatonin levels in patients with FM is conflicting. It is believed that these differences may be associated with the large heterogeneity of clinical characteristics of FM [75]. Furthermore, there is considerable methodological variability between the studies previously mentioned, for instance, methods used to collect and analyze samples (ELISA or radioimmunoassay), the type of marker used (6-SMT or melatonin), the type of sample (urine, serum, or blood), as well as other variables such as the type of food supply and the duration of exposure to light, which are variables that are not controlled in all studies [9, 74, 76–78]. It is also important to emphasize that melatonin levels correlate inversely with age, but this does not seem to be a plausible explanation for the differences found in the studies, since in all of them, patients with FM and controls were matched for age [9, 74, 76–78].

As noted, the discussion related to the role of melatonin in the pathophysiology of FM remains ongoing. More studies of high methodological quality are needed to clarify this important issue.

12.9 The Use of Melatonin as a Therapeutic Agent in FM

A number of studies investigating the therapeutic role of melatonin used alone or in combination with other drugs for the management of FM have been published, and in most cases, the results are encouraging [20, 22, 79].The first study investigating the use of melatonin as a therapeutic agent for FM was a pilot study comprising 21 patients [79]. The patients received capsules containing 3 mg of melatonin and were instructed to take it 30 min before going to sleep. The study duration was 4 weeks and 19 patients completed the protocol. The final results revealed that the intake of 3 mg of melatonin resulted in significant reduction of tender points and pain intensity, improvement of an overall assessment performed by the physician, and better sleep quality. Although this was a pilot study that employed weak methodological design, the authors concluded that melatonin was safe for patients with FM [79].

A study investigated the effect of daily doses of 6 mg of melatonin on symptoms presented by patients with FM [37]. Only four patients were enrolled in this study, one man and three women. Fifteen days after the beginning of the study, all patients developed a sleep-wake pattern considered normal by researchers and by patients, based on their perception. After treatment with melatonin, which for some patients lasted 3 months and others up to 15 months, the authors found evidence that strongly supported the use of this substance in the management of FM, once all four patients normalized their sleep-wake cycles and showed significant improvement in pain, fatigue, and behavior [37]. However the low number of

patients recruited suggests these results be interpreted with caution.

A review article on the role of melatonin as a therapeutic agent in FM was published, and at this time, only two original studies had been conducted: one by Citera et al. [79] and a second one by Acuna-Castroviejo et al. [36]. As both studies included in the review demonstrated positive results, the review also supported the use of melatonin in the treatment of FM [80].

The study that investigated the largest number of FM patients being treated with melatonin to date was a double-blind randomized controlled trial [22]. One hundred and one patients with FM were enrolled in this study and were randomly divided into four different treatment groups. Participants assigned to group A ($n=24$) received daily doses of 20 mg of fluoxetine; group B ($n=27$) received daily doses of 5 mg of melatonin; group C ($n=27$) received daily doses of 20 mg of fluoxetine in combination with 3 mg of melatonin; and group D ($n=23$) received daily doses of 20 mg of fluoxetine plus 5 mg of melatonin. Doses of fluoxetine were always taken in the morning while melatonin at night, during a period of 8 weeks. Prior to treatment commencing, all patients reported pain, stiffness, fatigue, sleep disorders, anxiety, depression, reduced quality of life, and high scores on the FIQ, indicating that FM was severely impacting the daily lives of these patients. The findings of this study revealed that the use of melatonin, alone or in a combination with fluoxetine, was effective in the treatment of patients with FM. The groups receiving melatonin in combination with fluoxetine (groups C and D) showed greater improvements in the FIQ score (individual domains and total score), quality of life, pain levels, fatigue symptoms, sleep quality, morning stiffness, anxiety, and depression. These findings indicate that the use of melatonin in combination with fluoxetine seems to be an effective alternative for the treatment of FM, even more effective than the use of melatonin or fluoxetine in isolation [22].

Studies evaluating the effect of agomelatine on FM have also been published. Bruno et al. investigated the effect of this agomelatine on pain, depression, anxiety, and cognition in patients with FM. Over 12 weeks, 15 women with FM who were not using other medications received daily doses of 25 mg of agomelatine. The treatment resulted in significant improvement of depression, anxiety, and pain; however, the results for cognition were not significant. The authors concluded that the use of agomelatine is safe and well tolerated by patients with FM, recommending its use for the treatment of patients with FM [81]. In another investigation, 23 participants with FM and symptoms of depression were investigated. Participants received daily doses of 25–50 mg of agomelatine, and the outcome measures were depression, pain severity, sleep quality, and impact of FM on quality life. Participants demonstrated significant improvements in depression, impact of FM on quality life, and pain intensity. However, no improvement was observed in sleep quality. According to the authors, agomelatine was well supported by patients, since only mild and transient side effects were observed [82]. However, the low methodological quality of this study should be taken into consideration when interpreting these results.

The latest study published so far on this topic is a double-blind randomized controlled trial phase II. The study included 63 women who were divided into three groups. Participants in group A ($n=21$) received 25 mg of amitriptyline, group B ($n=21$) received 10 mg of melatonin, while group C ($n=21$) received 10 mg of melatonin and 25 mg of amitriptyline. The aim of the study was to evaluate the effectiveness of different treatments on the clinical symptoms of pain, pain threshold, and sleep quality in patients with FM. The findings of this study strongly support the use of melatonin, either alone or in combination with amitriptyline, for the treatment of FM, as both treatments were more effective than the use of amitriptyline alone regarding pain symptoms and threshold. These results are in accordance to the findings of Hussain et al. [22]. In relation to number of tender points and sleep quality, the three types of treatment were similarly effective. According to the authors, melatonin can improve the endogenous pain inhibition system and therefore should be used for the treatment of pain in patients with FM [20].

According to the evidence listed above, the use of melatonin for the treatment of patients with FM is a feasible and promising alternative. Melatonin appears to produce beneficial effects on the main symptoms of FM: pain, anxiety, and sleep disorders. Although melatonin does not produce large effects on depression, agomelatine emerges with great therapeutic potential for the treatment of depression and other symptoms that composes the clinical features of FM. However, there is still a paucity of good evidence as only few studies, and most of them with low methodological quality, have investigated the clinical application of these drugs. Moreover, the ideal dosage and dose regimes for its use have not been well established, and whether melatonin should be used alone or in combination with other drugs still remains unknown. Further research is needed to answer these questions and establish the role of melatonin in the treatment of FM.

Conclusion

Research investigating altered melatonin levels in patients with FM is conflicting. Studies that identified reduced levels of melatonin in patients with FM failed to demonstrate any relationship between melatonin levels and the manifestation of symptoms. However, the involvement of melatonin in the pathophysiology of FM should not be disregarded. It is likely that melatonin plays an important role in the onset and persistence of symptoms in patients with FM, but the multifactorial nature of this condition makes it difficult to measure the impact of melatonin alone. The involvement of melatonin with FM syndrome is more easily observed when analyzing the well-established properties of this hormone and the relationship with some of the main symptoms presented by patients with FM, including pain, fatigue, and anxiety, and sleep disorders. Another important aspect to be considered is the successful use of melatonin and/or its agonists in the treatment of FM and in reducing the impact on quality of life for individuals with FM. However, as current evidence is scarce and overall have low quality, more studies are needed to establish the efficacy of melatonin in the treatment of FM.

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Melatonin's Beneficial Effects in Hepatic Injury

13

Pınar Atukeren and Hafize Uzun

13.1 Melatonin at a Glance

Melatonin (N-acetyl-5-methoxytryptamine) is a lipophilic major neurohormone, and it is secreted during the dark hours at night by the pineal gland, thus referred as the endogenous signal of darkness. Not only in the pineal gland, melatonin is produced in many other organs as the retina, gastrointestinal tract, skin, lymphocytes, and bone marrow [72]. It is widely distributed throughout the human body [77] working as the internal timekeeping system. It was first isolated and identified in 1958 [54]. In melatonin biosynthesis, the precursor is tryptophan; after it is taken up from the circulation, it is converted into serotonin, and serotonin is then converted into N-acetylserotonin which finally metabolizes into melatonin [5]. After melatonin is formed, it is released into the capillaries and into the cerebrospinal fluid [107] and then rapidly distributes to body tissues [7]. When melatonin is administered intravenously, it has a half-life of 2 min and a second metabolic half-life of 20 min [9]. The liver is the organ where the circulating melatonin is metabolized; there it is hydroxylated by cytochrome P450 monooxygenases and then conjugated with sulfate and forms 6-sulfatoxymelatonin [95]. Also melatonin is a metabolized into kynuramine derivatives through oxidative pyrrole ring cleavage [34]. Melatonin is an indoleamine having two functional groups. These groups are sites for receptor binding and also provide the molecule amphiphilicity so the molecule can enter any cell compartment or body fluid easily; thus as it has solubility in lipids, melatonin can pass from the peripheral circulation to other fluids or cells by diffusion. In serum, 70 % of the melatonin is bound to albumin where 30 % diffuses to the surrounding tissues [30].

Melatonin displays a circadian rhythm in plasma with high levels at night and with low levels during the daytime. The plasma concentrations of melatonin peak between 02:00 am and 04:00 am in humans [109]. Melatonin levels change due to age, and in newborns, secretion is very little. Soon after birth, melatonin levels begin to increase and concentrations peak in humans between the ages 1 and 3 years. During puberty and adolescence, the secretory pattern turns into a circadian rhythm [109]. It acts in sleep commencement, adrenal function, antiexcitatory actions, vasomotor control, immunomodulation, and antioxidant actions and also influencing mitochondrial electron flux [31, 33]. Administration of melatonin hormone has been shown to have many beneficial effects in treating various diseases in humans. Yet, having a short half-life, much more stable melatonin analogs are suggested and required for the treatment of these diseases. Melatonin exhibits its actions through

P. Atukeren • H. Uzun, PhD (✉)
Department of Medical Biochemistry, Cerrahpasa Medical Faculty, Istanbul University, 34303, Cerrahpaşa, Istanbul, Turkey
e-mail: huzun59@hotmail.com

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antioxidative effects and binding to intracellular proteins, to nuclear receptors, and to its receptors localized in the plasma membrane [56], and these mechanisms can be receptor-mediated, protein-mediated, and nonreceptor-mediated effects. Receptor-mediated melatonin actions entail membrane and nuclear receptors [17]. Also, melatonin acts on the retinoic acid family of nuclear receptors and intervenes many of its actions [97].

Melatonin has two subtypes of receptors – MT1 (Mel 1a) and MT2 (Mel 1b) – both G protein coupled [14, 83], and the binding characteristics of these receptors are similar, and they both have a high affinity, and there is a third melatonin-binding site called the MT3 receptor which later was characterized as quinone reductase 2 (QR2) enzyme [66] which belongs to a group of reductases having a role in the protection against oxidative damage through prevention of electron transfer reactions of quinones. Also, another melatonin receptor has been shown in humans and in various other species, named as GPR50 [84]. Melatonin receptor expression also is said to display a circadian variation [73]. Melatonin can act dependently or independently from its receptors when activating or inhibiting signal transduction cascades, and the independent action is referred to its small and lipophilic structure [22, 61]. Deficiencies in melatonin production or in receptor expression and also receptor malfunction are related to various diseases [72].

The production of melatonin hormone decreases not only with age but also in various diseases such as Alzheimer's disease, cardiovascular diseases, and cancer and in older patient insomnia, and a high prevalence of cancer is the outcome [72]. Currently, research on the cytoprotective effects of melatonin has become crucial in various organs compromising a convenient treatment.

13.2 The Antioxidant Properties of Melatonin

Recently melatonin, the secretory product of the pineal gland, has been suggested having properties against free radical damage in tissues such as the liver [106], also having an anti-inflammatory action [70]. It protects nuclear DNA and the lipids in cellular membranes from oxidative damage through upregulating antioxidant enzymes' activities [80]; thereby, it improves total antioxidant status of the cells. Melatonin has a unique property being an amphiphilic molecule which differs it from other antioxidants that are either hydrophilic or lipophilic, so melatonin can cross cellular membranes as barriers and fights against oxidative damage in both the lipid and aqueous environments [81]. There are many studies which imply that melatonin upregulates antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) [28, 31]. After administration of melatonin orally, the circulating levels of this molecule are capable to increase both mRNA levels and the activities of these antioxidant enzymes [86]. The mechanisms emphasizing the antioxidant activity of this hormone are as follows: first the oxidation of this hormone which causes the generation of a melatonin cation radical and second this radical's binding to the nucleophilic centers in proteins just like in histidine residues.

Among the free radical species which melatonin scavenges, there is the carbonate radical which is presumed having a role in mitochondrial damage [31, 32]. The effects of melatonin in mitochondria are another aspect of melatonin's genius. Because mitochondria have a special pathophysiological role in many diseases, this organelle is mentioned as the powerhouse of diseases [48]. In various studies, melatonin was shown to decrease the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and protects against oxidative and nitrosative damage of the proteins in electron transport chain in the inner membrane [3, 31]. It was shown that melatonin exhibits the free radical scavenging activity through a nonenzymatic process of electron donation [78]. These observations support the possible mechanism of melatonin acting as a free radical itself being known as the indolyl or melatonyl cation radical [78] which are far less potent than the free radicals it neutralizes in vivo. Melatonin also reacts with other metabolites forming new products which cannot be recycled back to melatonin;

thus this hormone is also called as a "suicidal antioxidant" [96]. Melatonin is referred as a broad-spectrum antioxidant because it can also activate cytoprotective enzymes [86] which is much more powerful than glutathione in scavenging free radicals and can protect cell membranes from oxidative damage more effectively than vitamin E [79], and when oxidized, melatonin turns into different antioxidant compounds such as cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, and N1-acetyl-5-methoxykynuramine.

Melatonin hormone also exhibits immunological and anti-inflammatory effects. Melatonin shows its antioxidant effects via G protein-dependent receptors, and this leads to the activation of the synthesis of antioxidant enzymes [2]. Also it acts via nonreceptor-mediated mechanisms serving as a scavenger for ROS and RNS [26]. Among the reactive species it scavenges are hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), and nitric oxide (NO) [25].

The scavenger ability of melatonin against these free radical species was demonstrated through surgical removal of the pineal gland, and this eventuated with the increase of nuclear DNA damage [105]. This finding implies that melatonin not only is an important antioxidant when administered orally but also the physiological concentrations of this hormone seem sufficient to decrease the damage to DNA molecule by free radicals [105]. Consequently it can be said that melatonin helps to suppress the development of carcinogenic processes and helps to prevent carcinogenesis. It has been suggested that, both endogenously and exogenously, melatonin exhibits its antioxidant effects through inhibiting the cytochrome P450 which is a multifunctional oxidase [105].

13.3 Hepatoprotective Efficacy of Melatonin

The liver, also considered as a gland, is a vital organ in the body, functioning to detoxify some toxic and harmful substances such as alcohol and drugs, to produce bile for digestion of fats in the intestine; to produce cholesterol; to store iron, vitamins, and some chemicals and glucose; to break down macromolecules in case of energy need so as to manipulate blood levels of fats, amino acids, and glucose; to fight with infections such as caused by bacteria; to make some proteins and enzymes or break down some molecules such as hemoglobin and some hormones like insulin and sex hormones; and to convert ammonia to urea in metabolism which are all vital functions.

The liver is prone to some common diseases arising because of some viral infections, misuse of alcohol, accumulation of excess fat and iron and copper, damage because of some toxic substances, and cancer. Hepatitis, nonalcoholic fatty liver disease, cancer, cirrhosis, hemochromatosis, autoimmune liver disorders, cancer, and some inherited diseases are the most seen liver diseases which result in a progressive decline in the function of the organ and end up in liver failure.

As what we discussed in the previous section, ROS are highly reactive molecules that are generated naturally in metabolic reactions and could give harm to cellular macromolecules such as lipids, proteins, carbohydrates, or DNA, but only they can also affect intracellular signal transduction and gene regulation, and this results in the production of cytokines which is needed for the inflammatory processes; however, undoubtedly oxidative stress also plays a considerable role in many liver diseases contributing both to the progression and pathology.

The pathophysiology of hepatic oxidative stress damage consists of several metabolic pathways and the oscillation of cytokines that induce hepatic injury [53] and takes place in two steps: in the first step, Kupffer cell-induced oxidative stress is seen [37], and in the second step, the accumulation of the neutrophils happens which is related to many other widely distributed injuries such as sustained from toxins, obstruction of the bile duct, excess alcohol intake, liver ischemia, and viral infections [98]. Also, the liver is vulnerable to oxidative stress under hypoxia. In hypoxic animals, melatonin administration decreased lipid peroxidation levels and helped recovery [17].

Many antioxidant compounds exhibit hepatoprotective effects evidenced in many studies. Melatonin has been implied to be potentially effective in both preventing and improving many oxidative stress-induced diseases exerting a hepatic protective effect as an antioxidant. Due to the previous research, in the human liver, melatonin was detected in a concentration which was 15 times higher when compared with blood levels [62], and in the bile, the concentration of melatonin is in between 2000 and 11,000 pg/ml, which is sufficient enough to prevent ROS damage [103]. Melatonin has been shown to induce the activities of antioxidant molecules and enzymes such as SOD, GPx, and glutathione reductase (GR) [82] and also the activity of glutamylcysteine synthetase and thus incite glutathione (GSH) production which is also an important intracellular antioxidant [112]. In liver cells when melatonin occurs in high levels, it is suggested to provide antioxidative protection both in cells and in epithelium. Also its function as a modulator of the immune system has also been widely reported [59].

Alcohol consumption is one of the etiologic factors in chronic liver diseases ending up with fatty liver, alcoholic hepatitis, cirrhosis, or carcinoma of the liver [111], and recently both ROS and inflammatory responses are accepted as a reason in the pathogenesis of alcohol liver damage [108]. Due to these mechanisms, Kupffer cells, which are intrahepatic macrophages, are activated, and then proinflammatory cytokines, chemokines, and ROS are released followed by the interactivity of alcohol metabolism and the immune system [12, 65]. Alcohol intake increases hepatic endotoxin levels, and then Kupffer cells are activated generating ROS, NO, and proinflammatory cytokines and chemokines [tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), IL-8, monocyte chemoattractant protein-1 (MCP-1)] [115]. These cytokines and chemokines lead to the intrahepatic convalescence and activation of granulocytes which are found in acute severe alcoholic hepatic inflammation [108]. TNF-α is one of the most important cytokines taking place in alcoholic liver injury pathogenesis [38, 115]. Melatonin is suggested to have a protective role in alcoholic liver injury by attenuating oxidative stress, inflammatory response, and apoptosis as it prevents ROS and TNF-α production from Kupffer cells. These cells and neutrophils are the source of ROS during alcohol consumption [4]. In a study, when alcohol-feeding mice were treated with melatonin, a decrease was seen in oxidative stress markers such as MDA levels and an increase in antioxidant enzymes such as SOD and GPx activity [35]. Melatonin has shown an anti-inflammatory effect on Kupffer cells and thus inhibits ROS and inflammatory cytokine production and neutrophil infiltration also by decreasing protein and messenger RNA expression of these cytokines [35]. Therapy with melatonin didn't show any effects on hepatic expression of lipogenic genes induced by alcohol, and no significant effect on lipolytic enzymes' activities was seen. In a study, in female Balb/C mice, the mechanism of matrix metalloproteinase-9 (MMP-9) regulation in alcohol-induced acute liver injury and the therapeutic effect of melatonin were investigated [63]. When ethanol was administered orally, it raised serum alanine aminotransferase (ALT) levels and also increased hepatic lipid peroxidation and protein oxidation, yet melatonin administration decreased ALT activity and oxidative stress markers' levels and also downregulated MMP-9 expression in hepatic cells. Alcohol induces the translocation of NF-κB which has an important role in inducing inflammatory genes during oxidative stress in hepatocytes. On the other hand, ethanol-induced NF-κB translocation into the nucleus was inhibited by melatonin administration.

Melatonin seems to protect hepatic cells from oxidative and nitrosative stress by altering Nrf2 and NF-κB pathways [40, 41]. Not only it increases the mRNA transcription of the antioxidant enzymes via Nrf2 but also reduces TNF-α, IL-1β, IL-6, and IL-8 levels and nitric oxide synthase (iNOS) activity by inhibiting the nuclear binding capacity of NF-κB [11, 41, 58].

Nonalcoholic fatty liver disease, so-called steatohepatitis, is one of the reasons of hepatic diseases encountered frequently [55] which may progress with the accumulation of fat and inflammation or cell damage and cirrhosis [117]. In alcoholic steatohepatitis patients when compared

with healthy subjects, circulating levels of TNF-α in circulating blood samples were seen to be higher [46]. Hence, TNF-α appears which should be taken into consideration as a target for therapy. In a study, the antioxidant, antiapoptotic, and anti-inflammatory effects of melatonin were investigated on methionine- and choline-deficient diet-induced steatohepatitis experimentally, and it was seen that melatonin decreased oxidative stress, proinflammatory cytokines, and hepatocyte apoptosis and melatonin was suggested as a therapeutic option for nonalcoholic fatty liver disease [101]. These results were in correlation with the previous studies where methionine- and choline-deficient diet was shown to induce nonalcoholic steatohepatitis in rats, and the liver histology improved after melatonin treatment. Similarly, melatonin healed histopathologic abnormalities in the liver in another experimental study [71]. Also, melatonin in combination with other drugs such as pioglitazone showed a significant decrease in liver tissue malondialdehyde (MDA) levels and increase in GSH levels in steatohepatitis-induced rats [118] and a decrease in aspartate transaminase (AST) and ALT activities accompanied. These results agreed with the previous studies [13, 116].

Cirrhosis results from induction of oxidative stress, mitochondrial dysfunction, and depletion of antioxidant status [44] and might be called as the advanced stage of fibrosis. Meanwhile, liver cirrhosis is a critical stage of chronic liver diseases that can produce liver failure. It may be due to viral infection, toxic agents, or alcohol. Hepatic fibrosis can lead to irreversible cirrhosis and consists of many cellular and molecular events which result in accumulation of collagen and extracellular matrix protein in hepatic cells which reduces hepatic functions [24, 75]. Oxidative stress intensifies liver fibrosis with hepatic cell activation [74], and lipid peroxidation stimulates the transcription of the collagen gene in fibroblast cell culture and hepatic stellate cells [51]. In a study, the effects of melatonin on dimethylnitrosamine (DMN)-induced liver damage, which was a fibrosis model, were studied in rats, and melatonin suppressed fibrotic changes caused by DMN and increased GSH and SOD activities [100]. Melatonin was suggested to be a potent fibrosuppressant as well as being an antioxidant. A similar study done supported these findings [102] and suggested melatonin as therapeutic in liver fibrosis. As it is well known, melatonin production and release are suppressed or synchronized via light schedule; thereby, it can be determined by measuring in plasma or saliva as melatonin itself or in urine as sulfatoxymelatonin which is a hepatic metabolite. The liver is the main site for melatonin metabolism where almost 90 % of circulating melatonin is cleared. After being hydroxylated, it is excreted in urine as sulfate and glucuronide conjugates [23]. About 1 % of melatonin remains in the urine as 3-hydroxymelatonin, and this can also be measured in urine. In patients with liver cirrhosis, melatonin clearance seems to be decreased accompanied with delayed rise in plasma levels [36]. Melatonin can also be metabolized in the hepatic endoplasmic reticulum and where millimolar concentrations of melatonin were shown to be needed to prevent oxidative damage to microsomal lipids and thiol groups even more effectively than GSH, vitamin E, and mannitol in protecting microsomal lipids [29, 76].

Cirrhosis is also one of the leading causes of death in diabetics. Several studies have reported that the hepatocytes of streptozotocin (STZ)-induced diabetic mice showed cytoplasmic alterations similar to those observed in so-called oncocytic cells [49, 52]. Decreased nitrite levels with increased lipid peroxidation in the liver tissues of diabetic rats were shown to be restored after treating with melatonin [57]. In another study investigating the effects of melatonin on STZ-induced diabetic liver injury, melatonin was shown to improve the morphological and histopathological changes in the liver tissue and suggested to be a nutritional supplement as a therapeutic option for diabetic patients [45].

With aging the protective capacity of the liver reduces and the liver shows an increased susceptibility to ischemia reperfusion injury [10, 50]. Melatonin restores the oxidative status in aged liver cells [27], and the beneficial effects of melatonin were exhibited in hepatic ischemia reperfusion injury. It was reported that melatonin administration lowers TNF-α levels and inhibits

iNOS expression and NO production in ischemia reperfusion injury [87]. In patients undergoing major liver resections, treatment with melatonin helped to decrease postoperative ALT and AST levels [92]. Also the expressions of proinflammatory cytokines and NF-κB and iNOS were decreased in an experimental study where the liver tissues of rats were submitted to ischemia reperfusion [42]. In an experimental study, older rats were compared with younger rats submitted to hepatic ischemia reperfusion, and it was seen that in older rats serum ALT and AST levels were significantly higher than in young animals, and with melatonin treatment, the liver functions were improved [43]. Also, ischemia reperfusion is one of the critical problems in liver transplantation. This improvement may be due to a protection of hepatic mitochondrial redox status.

The protective effect of melatonin against toxic liver damage inflicted from environmental toxins and chemotherapeutics has been implied recently in many studies [18, 19]. Melatonin was shown to reduce liver enlargement and decrease the elevated serum AST and ALT levels in experimental thioacetamide (known as a fungicide)-induced acute and chronic hepatic injury model [88], and it seemed to be much more effective in chronic hepatic injury when compared with acute injury. In alpha-naphthylisothiocyanate-induced liver injury in rats, its neutrophil infiltration-inhibiting effects were shown [6]. Melatonin administration after ochratoxin A-induced oxidative stress helped to increase antioxidant enzyme activities in hepatic cells [60].

Melatonin also protects against oxidative damage to liver cell membranes and decreases lipid peroxidation levels in carbon tetrachloride-treated rats, dose dependently [68, 69]. The protective effect of melatonin is due to its antioxidant property and its inhibiting NF-kB expression and the production of proinflammatory cytokines [110]. The protective effect of melatonin against heavy metals in liver injury is also known. In lead-induced toxicity model, its maintaining liver parameters seem to be due to its both lipophilic and hydrophilic structure [16]. In this study, melatonin decreased lipid peroxidation levels and also stimulated SOD activity and GSH content. It is also well known that with cadmium intoxication the redox status of the liver changes; lipid peroxidation content increases where SOD activity and GSH content in hepatic cells decrease. Melatonin administration in cadmium intoxication was shown to decrease lipid peroxidation and increase the antioxidant status in the liver, thus suggested as a drug in protection against cadmium pollution [20]. Similar results were seen in the course of benzo(a)pyrene intoxication; melatonin was shown to increase SOD and CAT activity in liver tissue [64]. In benzene-induced liver injury model, melatonin administration for 1 month had beneficial effects on ALT and alkaline phosphatase (ALP) levels [93]. The liver is also highly susceptible to the oxidative events caused by nicotine toxicity. In a study, melatonin was found to be very effective in reducing nicotine-induced oxidative damage in hepatic cells [18]. At that, melatonin has some protective effects against immunosuppressive drugs which induce hepatotoxicity. In an experimental study, melatonin restored oxidative damage to liver cells induced by cyclosporine A toxicity which is an immunosuppressive drug used frequently indeed [85].

Hepatocellular carcinoma is a primary liver cancer that originates from hepatocytes [1]. It is the most common tumor seen in the liver and also the fifth most common cancer [15]. In a research, it is that experimental treatment with melatonin typically helped to reduce hepatocellular necrosis [47]. When the effects of melatonin in the presence of other drugs were studied in several tissues [8], in hepatoma cancer, it was reported that a dose of 10−5 to 10−8 mol/L in combination with doxorubicin as a drug increases apoptosis and inhibits hepatoma cell growth [21].

Melatonin has oncostatic effects in tumors and has a regulatory influence on lipid homeostasis. Melatonin's oncostatic effect on hepatoma 7288CTC cell lines is thought to be via inhibiting fatty acid uptake through MT1 receptors and a G1 protein-coupled signal transduction pathway [72]. In a study with inoperable patients having advanced primary hepatocellular carcinoma, IL-2 levels increased in the patients treated in

combination with melatonin [113]. In several studies, the clinical benefits of melatonin against carcinogens in liver cells were shown. Lower dose of melatonin administration caused a decrease in DNA damage caused by safrole-induced hepatic injury where a higher dose of melatonin administration almost recovered the hepatic DNA damage caused by carcinogenicity of safrole [104]. In another study, the chemopreventive function of melatonin was implied; melatonin administration significantly hindered tumor development against N-nitrosodiethylamine injury which is a potent carcinogenic agent that induces liver cancer [99]. Melatonin exerted an anticancerous effect against 2-nitropropane-induced hepatocellular carcinoma through its antiproliferative and antiangiogenic effects [1]. Melatonin slows cell cycle progression by increasing the number of cells in G0 and G1 phases, and it seems that melatonin prevents mitochondria-mediated apoptosis [39]. Melatonin acts as an efficient inducer of apoptosis also in hepatoma cells [67, 114].

New drug delivery systems were recently developed for melatonin [91] relevant to its encapsulation in polymeric nanocapsules or nanospheres [90]. This increases the efficacy of melatonin, for example, melatonin-loaded polysorbate 80-coated Eudragit S100 nanoparticles were shown to provide an important increase in the in vitro effect of melatonin against oxidative stress when compared with the effect in aqueous solution [89]. Aqueous drug solutions were almost ineffective. Therapy with melatonin solutions has been shown to protect against lipid peroxidation in the liver [94].

As it is well known, melatonin production and release are suppressed or synchronized via light schedule; thereby, it can be determined by measuring in plasma or saliva as melatonin itself or in urine as sulfatoxymelatonin which is a hepatic metabolite. The liver is the main site for melatonin metabolism where almost 90 % of circulating melatonin is cleared. After being hydroxylated, it is excreted in urine as sulfate and glucuronide conjugates [23]. About 1 % of melatonin remains in the urine as 3-hydroxymelatonin, and this can also be measured in urine. In patients with liver cirrhosis, melatonin clearance seems to be decreased accompanied with delayed rise in plasma levels [36].

Conclusion

This orchestrating hormone, melatonin, being an important component of the body's internal timekeeping system, has important therapeutic effects and represents a novel therapeutic molecule in hepatic injury presently, via inhibiting oxidative stress and inflammation, yet the appraised potential therapeutic benefits of melatonin need to be fully stated in future studies. In the same time, targeting the improvement of the efficacy against ROS damage should be regarded. Recent research is ongoing to enlighten the structural insights and the novel mechanisms as an antioxidant in liver diseases in order to enhance life quality, yet melatonin consumption via diet should be taken into consideration as it might have some synergistic effects with other antioxidant molecules too. Else, melatonin may be used in combination with other traditional therapies and/or drugs which commits a research field in pharmacology to recover hepatic injuries.

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Melatonin as a Novel Therapeutic Agent Against Chemical Warfare Agents

14

René Pita, Eva Ramos, José Luis Marco-Contelles, and Alejandro Romero

Abbreviations

AChE	Acetylcholinesterase
BBB	Blood-brain barrier
CNS	Central nervous system
CWAs	Chemical warfare agents
GSH	Glutathione
HAT	Hydrogen atom transfer
HOCl	Hypochlorous acid
iNOS	Inducible isoform of nitric oxide synthase
$NO^{\bullet}$	Nitric oxide anion
$ONOO^{\bullet}$	Anion peroxynitrite
OPs	Organophosphates
PARP	Poly(adenosine diphosphate ribose) polymerase
RONS	Reactive oxygen and nitrogen species
ROS	Reactive oxygen species

R. Pita
Chemical Defence Department, CBRN Defence School, 28240, Hoyo de Manzanares, Madrid, Spain

E. Ramos • A. Romero, PhD (✉)
Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Complutense University of Madrid, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain
e-mail: manarome@ucm.es

J.L. Marco-Contelles
Medicinal Chemistry Laboratory, Institute of General Organic Chemistry (CSIC), Juan de la Cierva, 3, 28006 Madrid, Spain

14.1 Introduction

The North Atlantic Treaty Organization (NATO) defines chemical warfare agents (CWAs) as substances intended for use in military operations to kill, seriously injure, or incapacitate people because of its pathophysiological effects [1]. The first CWAs were chemicals widely used in the chemical industry, such as chlorine and phosgene, two lung-damaging agents. Afterward, other CWAs, with no industrial use, were developed. This is the case of sulfur mustard [bis(2-chloroethyl)sulfide] (Fig. 14.1), a blister agent used in World War I, or nerve agents, discovered just before the start and developed during World War II.

Sulfur mustard has been the most widely used CWA. As opposed to chlorine (Cl_2) and phosgene ($COCl_2$, carbonyl dichloride) (Fig. 14.1), which were gases at room temperature, sulfur mustard is a persistent agent that remains in the attacked area and affects not only the respiratory tract but the whole body surface. The blisters that appeared in affected troops in World War I are the reason why sulfur mustard and similar agents are called blister agents. Lewisites [dichloro(2-chlorovinyl) arsine] and nitrogen mustards (Fig. 14.1) were developed afterward; however, these blister agents did not provide any special advantages to sulfur mustard [2].

The 1994 and 1995 sarin (Fig. 14.1) terrorist attacks in Japan showed that CWAs should not

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only be considered in military scenarios but also in terrorist attacks. The confirmed use of sarin in 2013 in the Syrian military conflict and the clear intentions and attempts to acquire CWAs by jihadist terrorism, including the Islamic State, make the chemical threat a main concern subject in terrorism studies and toxicological sciences.

Nowadays, total immediate decontamination after exposure is difficult to achieve, and there are not completely effective antidotes and treatments against all CWAs; therefore, more research is necessary in this field. This fact suggests us to study novel pharmacological approaches able to reduce CWA-induced damage.

Melatonin (*N*-acetyl-5-methoxytryptamine), well known as a potent indirect antioxidant and as a direct free radical scavenger [3–5], possesses antiexcitatory actions and regulates immune function and energy metabolism, including anti-inflammatory properties [6, 7]. In mammals, three major melatonergic membrane receptors have been identified. Among them, MT1 and MT2 are considered to represent the melatonin receptor system, and their physiological functions and pharmacological properties are well documented [8]. MT3 receptor has also been described and identified as a quinone reductase 2 [9]. However, nuclear binding sites/receptors also exist for this indole derivative and have been identified as a further class of melatonin receptors [10]. The distribution of receptors and other binding sites indicates the remarkable pleiotropy of melatonin, which may potentially affect most of the cells, in addition to other receptor-independent mechanisms that may contribute to explain the different and tissue-specific functions of melatonin [11–13]. Melatonin is highly lipophilic and consequently crosses easily cell membranes, including the blood-brain barrier (BBB) [14], allowing it to be administered either orally or intravenously and to reach all subcellular compartments. The antioxidant capacity of melatonin is even more important owing to its ability to cross all morphophysiological barriers [15]. The subcellular distribution of melatonin allows it to interact with toxic molecules in the entire cell, reducing oxidative damage both in lipid and in aqueous cell environments. Moreover, its intermediate size is optimum for transportation across cellular membranes. However, rather little is known concerning the intracellular concentrations of melatonin.

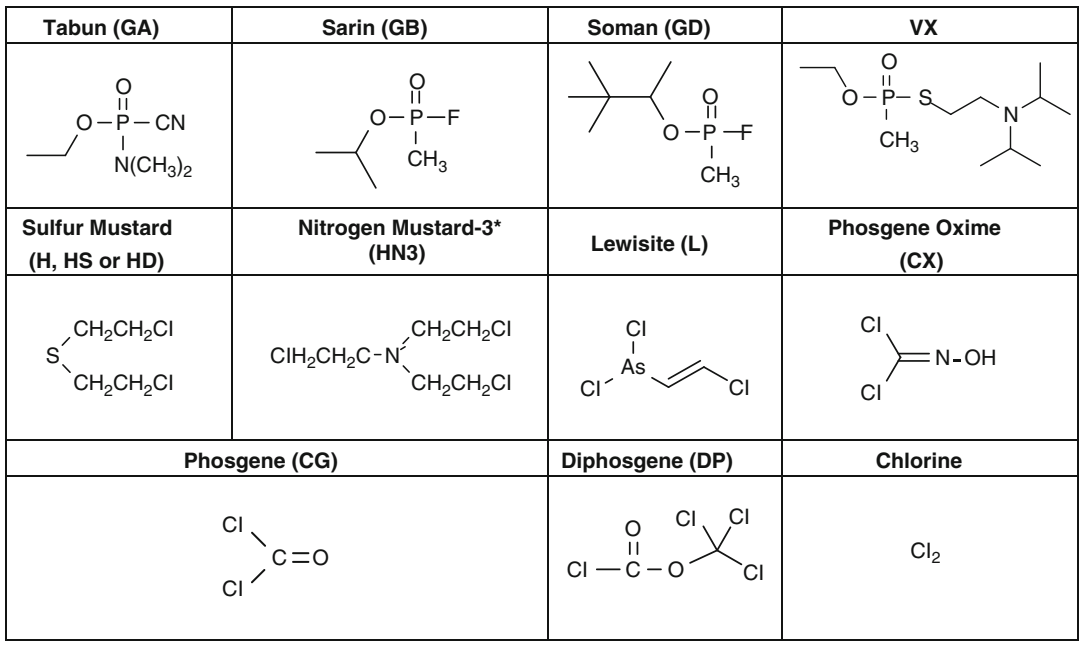

Fig. 14.1 Chemical structures of nerve agents, blister agents, and lung-damaging agents (*HN-3: Tris(2-chloroethyl) amine is the most important nitrogen mustard, produced for military purposes)

Furthermore, in several comparative studies, melatonin has been shown greater protective effect than other antioxidants [16–19]. Taking into account melatonin's low toxicity and that patients treated with high doses of melatonin do not experience any harmful side effects [20], its potential spectrum for improving human health against CWAs seems to be wide.

Herein we summarize and hypothesize the possible protective mechanism of melatonin against several CWAs.

14.2 Blister Agents (Vesicants) and Protection by Melatonin

Blister agents include sulfur mustards (H, HS, or HD), nitrogen mustards (HN), lewisites (L), and phosgene oxime (CX) (Fig. 14.1). The most important blister agent is sulfur mustard, popularly known as "mustard gas." Although it is referred as a "gas," sulfur and nitrogen mustards and lewisites are liquids at room temperature. CX, in contrast, is a highly volatile crystalline solid. The information available on the clinical effects of vesicants pertains mainly to sulfur mustard and is derived largely from experience during World War I and the Iran-Iraq War. After exposure to mustard gas, there is an asymptomatic latency period before the first signs and symptoms of poisoning appear. The latency period varies between 2 and 48 h according to dose (often expressed as the product of concentration and time or Ct), temperature, humidity, and area of the body exposed. The most sensitive areas are the thinnest and moistest: the respiratory tract and eyes and, on the skin, the armpits, neck, elbow creases, groin, genitals, and perineum [21, 22]. Thus, local effects usually involve the skin, respiratory tract, and eyes, although ingestion of the agent, for example, by eating contaminated food, can also cause gastrointestinal lesions directly.

Sulfur mustard is absorbed through the skin and eyes, in the lungs when inhaled, and even through the gastrointestinal tract when ingested [23, 24]. The skin is a good point of entry for mustard gas in both liquid and vapor forms, given the highly lipophilic nature of the agent. Once absorbed, the systemic effects occur mainly in the bone marrow, the gastrointestinal tract, and the central nervous system (CNS). It has also been observed that, after absorption, mustard gas accumulates in fatty tissues and the CNS [25].

Nitrogen mustards have similar effects to sulfur mustards, but their effects on the CNS are more severe and in vivo animal studies have shown convulsions after intravenous administration [26]. Lewisite is also well absorbed by the skin and mucosal tissues [1]. The main difference from mustards is that there is no latency period for the effects of lewisite, as pain and irritation occur immediately. Although lewisite's effects are less widely studied than those of the mustards, in vivo studies in animals and cases of accidental exposure in humans have shown very similar effects [24, 27].

Although sulfur mustard was used for the first time in 1917, its precise mechanism of action is still unknown. However, different hypotheses have been proposed.

Sulfur and nitrogen mustards undergo intramolecular cyclization reactions giving rise to sulfonium or immonium ions, respectively [28]. These intramolecular reactions are favored by the presence of water and high temperatures [29]. For this reason, the moist body areas are the most susceptible. Sulfonium ions are potent alkylating agents of molecules such as DNA, RNA, glutathione (GSH), and proteins. DNA alkylation produces cross-linking and breakage of strands, and polymerases such as poly(adenosine-ribose diphosphate) polymerase (PARP) are activated, leading to depletion of the substrate nicotinamide adenine dinucleotide (NAD^+) and inhibition of adenosine triphosphate (ATP) synthesis, leading to apoptosis [30–32]. The fastest-dividing cells are therefore the ones most affected by mustards [29]. Sulfur mustard appears to have a special affinity for nitrogen at position seven of guanine [32, 33].

Sulfonium and immonium ions also alkylate nucleophile molecules such as enzymes which contain sulfhydryl groups, responsible for

regulating Ca^{+2} homeostasis in cells [34]. Such processes would cause an increase in the intracellular calcium concentration, thereby disrupting the microfilaments responsible for cell integrity, with activation of endonucleases, proteases, and phospholipases, which finally induce apoptosis [29]. Moreover, mustards interact with GSH and increase the concentration of free radicals, which, through peroxidation of membrane lipids, affect the integrity and function of the membrane [29, 35].

Oxidative stress is the first and key element in the pathogenesis of sulfur mustard toxicity. Some studies have suggested that oxidative stress due to ROS plays an important role in the toxic mechanism of action of mustard gas [36–38]. However, the powerful nitrosating agent anion peroxynitrite ($ONOO^{\bullet}$) is involved in initial detrimental effects of all mustards [39]. $ONOO^{\bullet}$ interacts with and covalently modifies all major types of biomolecules including membrane lipids, thiols, proteins, and DNA [40]. $ONOO^{\bullet}$ activates metalloproteinase (MMP) enhancing NF-κB activation and promotes pro-inflammatory responses [41]. Furthermore, at higher concentrations, $ONOO^{\bullet}$ is associated with necrosis rather than with apoptosis through activation of the DNA repair enzyme poly(ADP ribose) polymerase-1 (PARP-1) [41]. The activities of these enzymes are implicated in DNA repair process, the maintenance of genomic stability, the regulation of gene expression, and DNA replication. Experimental evidence has established that the PARP-1 pathway of cell death plays a critical role in tissue damage and organ dysfunction in mustard-induced acute toxicity [42, 43]. Multiple therapies by a combination of cell membrane receptor blockers, antioxidants, NOS inhibitors, peroxynitrite scavengers, and PARP inhibitors may be a promising approach to effectively control mustard-induced acute toxicity [44].

The tripeptide GSH, a vital constituent of cells, playing an important redox buffer, and cofactor role for signal transduction, antioxidant defense, and electrophile defense, especially in the brain, is involved in the defense against metal cations, oxyradicals, xenobiotics, and others. Thus, dysregulation of GSH homeostasis and deactivation of GSH-dependent enzymes are believed to contribute to initiation and progression of neurodegenerative diseases.

Mustard depletion of GSH leads to oxidizing compounds, which finally produce cytotoxicity and cell injury. Animal model experiments have shown that antioxidants protect the liver and lung from oxidative mustard-induced damage [45].

Nowadays, there are many experimental evidences that show the excellent capacity of melatonin as major scavenger of both oxygen- and nitrogen-based radicals, including $ONOO^{\bullet}$ [13, 46, 47]. In this line, both melatonin and its metabolites have important advantages with respect to "classical antioxidants" including iNOS inhibition and $ONOO^{\bullet}$ scavenging properties against mustard-induced acute toxicity [48–50]. This is thought to be a primary event triggering the inflammatory cascade and tissue injury [39]. Because pathological inflammation has been related to a number of different diseases produced by chemical agents, it is important to identify new pharmacological approaches to reduce the inflammatory response after nitrogen and sulfur mustard exposure. Melatonin exerts anti-inflammatory effects through the regulation of different molecular pathways [51]. In this line, it has been recently reported that melatonin reduced oxidative stress, inflammation, and kidney toxicity induced by the bifunctional alkylating agent mechlorethamine (Fig. 14.1) [52]. Besides, Macit et al. [53] found that intraperitoneal administration of melatonin (100 mg/kg) in rats 30 min after application of mechlorethamine (MEC), a nitrogen mustard synthesized for military purposes, was more efficient than the selective iNOS inhibitor, S-methylisothiourea, to counteract almost all pathological manifestations induced by MEC.

It has been described that sulfur mustard leads to the production of different cytokines such as IL-8, IL-6, and TNF-α, among others, and, as a consequence, enhances intracellular oxidative stress, antioxidant defense mechanism [54], and markers of oxidative stress, including malondialdehyde, 8-hydroxydeoxyguanosine,

4-hydroxynonenal, and heme oxygenase-1 [55]. Thus, decreased levels of GSH and markers of pulmonary inflammation after sulfur mustard exposure have been found [56, 57].

In a recent study, it has been reported that oxidative stress production in vivo accompanied by increasing oxidative stress markers after mustard gas exposure was counteracted by melatonin administration [58]. Melatonin was also able to ameliorate lung injury and oxidative stress induced by nitrogen mustard in rodents, suggesting a potential therapeutic target for treating mustard gas poisoning [48].

In an interesting review article, Korkmaz et al. [59] proposed that the mechanism of delayed sulfur mustard toxicity may be due to epigenetic disruption that occurs in cells in which the genome has genetic and/or epigenetic modifications. As a multifunctional indole, melatonin could counteract delayed sulfur mustard toxicity modulating the expression of mRNA levels for antioxidant enzymes both under physiological conditions and during increased oxidative stress [60].

14.3 Proposed Reaction Chemical Mechanisms of Melatonin with Sulfur Mustard

As described above, melatonin is an excellent and broad-spectrum antioxidant agent, widely distributed in the body, and easily transported across the BBB, and has very low toxicity. Consequently, melatonin has proved to be effective in the prevention of sulfur mustard-mediated damage [58, 59, 61, 62]. However, the mechanisms involved in this activity still remain to be investigated and clarified. In this section, we will update the most recent and accepted chemical mechanisms of the reaction of melatonin with sulfur mustard. Based on experimental data and theoretical calculations, a hydrogen atom transfer (HAT) process has been identified as one of the most plausible mechanisms underlying the free radical-scavenging properties of melatonin, but other mechanisms, such as the radical adduct formation or single electron transfer, cannot be neglected and must be taken into consideration [63].

In Fig. 14.2, we have shown a possible HAT mechanism for the reaction of melatonin with sulfur mustard based on a N1 and N2 starting radicals. As depicted, hydrogen abstraction by a generic ROS should lead to a nitrogen-centered radical at N1 that after reaction with the chlorine atom on the sulfur mustard initiates a fragmentation reaction that results in the loss of ethylene and forms a new reactive radical species (**I**) that in a intramolecular substitution radical reaction displaces the second chlorine radical and forms thiirane, a neutral, volatile molecule. The same analysis could also be operating at N2, in a similar free radical series of events.

The melatonin metal-chelating properties [64] could explain the potential protective role of melatonin against lewisite-induced cell death, without discarding the radical-scavenging feature and its anti-inflammatory activity. Furthermore, knowing its low toxicity and high capacity to cross-cellular barriers, melatonin could be a candidate molecule to prevent after vesicant-induced tissue damage.

14.4 Toxicity of Nerve Agents and Protective Role of Melatonin

Nerve agents are organophosphate (OP) compounds that were initially synthesized by German scientists prior to World War II and are one of the deadliest compounds known to man [65]. Furthermore, these agents present higher mammalian toxicity than their OP biocide homologues. The four major OP nerve agents are tabun, sarin, soman, and VX (Fig. 14.1). They differ from one another in their potency and volatility as well as in their ability to cross the BBB and to exert CNS toxicity. Soman is the most potent of the four [66]. Nerve agents have both chemical names and two-letter NATO codes. There are two main series: “G” agents (“G” stands for “German”) and “V” agents (“V” stands for “venomous”). “G” agents include tabun

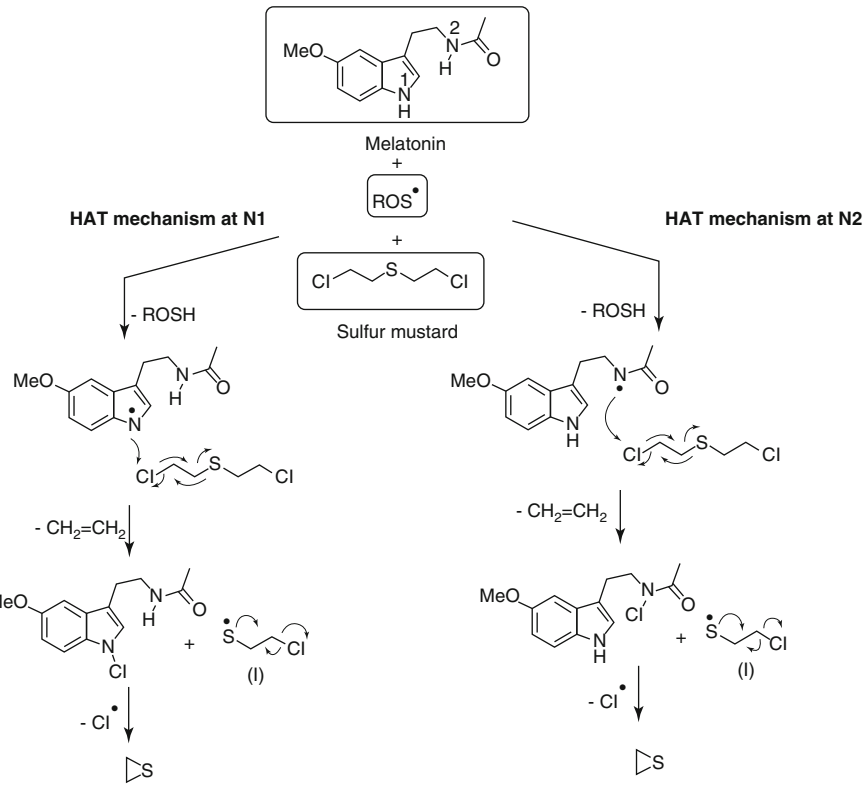

Fig. 14.2 Postulated chemical reaction of melatonin, in the presence of free radicals, to neutralize the toxic effects induced by sulfur mustard

(*O*-ethyl *N*, *N*-dimethyl phosphoramidocyanidate, GA), sarin (*O*-isopropyl methylphosphonofluoridate, GB), and soman (*O*-pinacolyl methylphosphonofluoridate, GD), among others (Fig. 14.1). The most important "V" nerve agent is *O*-ethyl *S*-2-diisopropylaminoethyl methyl phosphonothiolate, known as VX. "G" and "V" agents are both liquids at room temperature, but "G" agents are more volatile than "V" agents; thus, "G" agents are nonpersistent CWAs, while "V" agents are considered persistent agents [67].

The different OPs are absorbed nearly through all body surfaces, but mainly through the skin, eyes, and respiratory tract [68–70]. Due to their high toxicity, once absorbed, they produce systemic effects more rapidly than their biocide homologues. Their main toxicological mechanism of action is the powerful irreversible inhibition of peripheral and brain cholinesterases, which produce a cholinergic syndrome that may lead to death by asphyxia caused by airway obstruction, respiratory muscle paralysis, and respiratory depression at CNS level [71, 72]. If untreated, the cholinergic overstimulation triggers peripheral or central muscarinic and nicotinic signs. In addition, increased acetylcholine levels initiate seizures via the overactivation of muscarinic receptors [72–74] that modulate the release of glutamate [75] and γ-aminobutyric acid (GABA) [76]. Glutamatergic response induces a prompt activation of AMPA and NMDA receptors, causing a massive influx of Ca^{2+} which activates proteolytic enzymes and triggers the generation of large amounts of nitric oxide (NO) via activation of nitric oxide synthase (NOS) [77]. The

production of NO in intact neurons occurs in response to excitatory overstimulation that requires an intracellular Ca^{2+} ion influx (Kiss 2000). Nitric oxide anion ($NO^{\bullet}$) reacts with superoxide radical ($O^{2\bullet-}$), yielding highly reactive free radical species such as peroxynitrite ($ONOO^{\bullet}$) radical, and eventually hydroxyl radical ($HO^{\bullet}$), as well as DNA peroxidation [78]. Excitotoxicity, followed by intracellular Ca^{2+} overload and reactive oxygen and nitrogen species (RONS) production, which is known to damage the mitochondria [79, 80], is involved in the maintenance of epileptic seizure leading to induce a neural cell death process [71, 72].

The abovementioned data show that the toxic effects of OPs are not limited to acetylcholinesterase (AChE) inhibition. Oxidative stress is an important mechanism in the pathophysiology of OPs, and RONS are part of normal oxidative metabolism, but when produced in excess, they cause tissue injury. In both acute and chronic OP toxicity, changes in antioxidant enzymes occur, and lipid peroxidation increases in many organs, especially in the brain. At the neuromuscular junction level, it could also be possible that the increased cholinergic activity produces ROS due to muscle hyperactivity, leading to myopathies [81]. Thus, it has been suggested that free radical reactions induced by soman might be a primary cause of neural degeneration [82]. In another study, sarin proved to be an initiator of oxidative stress, and as a marker of DNA oxidative degradation after intramuscular administration of the toxin into rats [83], significant alterations in the oxidative homeostasis simultaneously with AChE inhibition have been shown [84].

Interestingly, melatonin prevents neuronal cell death in a large number of neurodegeneration models [85–88]. Furthermore, melatonin seems to play an important role in the regulation of the neuronal loss induced by excitotoxicity [89]. Recent studies have shown that melatonin inhibits the activity of iNOS, besides its $NO^{\bullet}$ and $ONOO^{\bullet}$ scavenging activity, and maintenance of Ca^{+2} homeostasis [90]. Inhibition of $NO^{\bullet}$ production may be another pathway whereby melatonin can reduce oxidative damage induced by nerve agents that seems to play an important role in various pathophysiological conditions. It has also been shown that melatonin was able to prevent ROS production, Ca^{2+} influx, and cell death induced by oxidative stress, glutamate excitotoxicity, and TNF-α toxicity in VSC4.1 motoneurons [91], strongly suggesting the involvement of multiple mechanisms, including melatonin receptor, in providing melatonin-mediated neuroprotection. Thus, recent findings indicate that the G protein-coupled melatonin receptors are expressed in brain neurons in mammals, including humans [92], and that these receptors mediate the physiological actions of melatonin [92–94].

Furthermore, an important neuroinflammatory process occurs after OP exposure [95–97]. Pro-inflammatory cytokines are known to mediate cellular communication and play a significant role in the pathological processes involved in various brain diseases, such as *status epilepticus* [98, 99]. In vitro studies have shown that pro-inflammatory cytokines play an important role in cell death process, firstly, through an excitotoxicity mechanism, inhibiting glial cells from taking up the excess of extracellular glutamate [100] and, secondly, by likely contribution to nerve agent toxicity on the BBB function [101].

Additionally, analysis of gene expression indicates that signaling pathways related to inflammatory processes are highly modified after sarin and soman exposure [102, 103]. Following soman poisoning, a massive activation of microglia and astroglia was observed [104–106]. Recently, cytokine expression induced by nerve agent exposure in rodents has been explored. Pro-inflammatory cytokines secreted by microglial and astroglial cells, interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) are overexpressed on injured rat brain after soman poisoning [107, 108]. Sarin exposure in animal models has also shown alteration of immune and endocrine activities that produce alterations in the cytokine levels [109]. All these

events indicate the presence of an important neuroinflammatory role in toxicity induced by nerve agent exposure.

Recent insights and new perspectives about melatonin's anti-inflammatory properties and molecular aspects have been recently reviewed [51]. A number of different targets of melatonin have been described, including iNOS and cytokine production. Melatonin's anti-inflammatory effects are modulated through transcriptional pathways and different kinases both under in vitro and in vivo conditions. In this complex scenario, we think that a multipotent agent such as melatonin may constitute an interesting modulator agent in the treatment of inflammatory pathophysiological processes after nerve agent exposure.

However, it would be important to determine if the neuroprotective efficacy of melatonin against OPs should be prophylactic or as post-treatment, because some drugs exert higher protective activity when given under one set of conditions versus the other. The absence of side effects makes this compound an attractive and suitable candidate to translate into the clinic as one agent in the armamentarium against nerve agents.

14.5 Lung-Damaging Agents and Beneficial Use of Melatonin

Chlorine and phosgene are the main lung-damaging agents, also known as choking agents (Fig. 14.1). Both are gases at room temperature and were used as CWAs in World War I from cylinders in trench warfare. Nowadays both agents are widely used in the chemical industry.

During World War I, Germans also introduced diphosgene (trichloromethyl chloroformate) (Fig. 14.1), which is liquid at room temperature and could be used to charge shells. While cylinders were useful in trench war, shells were needed to use in "movement" warfare.

Chlorine (Fig. 14.1) is a very reactive and soluble substance that affects the respiratory tract when inhaled, producing irritation. With water, chlorine produces hydrochloric and hypochlorous acid (HOCl) and nascent oxygen. Among other damage mechanisms, these compounds interact with the respiratory tract mucosa releasing pro-inflammatory cytokines that produce systemic inflammatory response as well as endothelins that produce vasoconstriction and pulmonary hypertension [2]. Furthermore, HOCl has a high capacity to inactivate the enzymes involved in the antioxidant defense system, inducing a disruption of redox homeostasis.

Melatonin is amphiphilic and reaches all subcellular compartments to scavenge the reactants generated during the oxidative stress. The ability of one molecule of melatonin to apparently interact with more than one HOCl molecule is consistent with observations made by Tan et al. [110], who found that melatonin also neutralizes more than one hydroxyl radical. In another study, it has been shown that human respiratory tract epithelial cells exposed to HOCl induce a DNA double-strand breaks and the formation of modified nucleotides; this suggests that HOCl diffuses into the nucleus and attacks DNA [111]. An interesting study, using the Chinese hamster B14 cell line, provided further evidence that HOCl-induced toxicity was counteracted by melatonin. Melatonin coadministration significantly protected cells during HOCl exposure, diminishing its cytotoxic effects and reducing protein carbonyl generation [112]. Therefore, melatonin may be an attractive therapeutic option to prevent inflammatory diseases where HOCl levels are known to be elevated.

Phosgene (Fig. 14.1), extensively used as an industrial chemical, acylates the sulfhydryl, amine, and hydroxyl groups of proteins that regulate the permeability of the alveolar membranes [113] and in severe cases may produce pulmonary edema. Phosgene also produces oxidative stress through GSH redox cycle disruption [2, 114, 115].

There are no antidotes for lung-damaging agent poisonings, and medical treatment is based

on ABC resuscitation, trying to prevent bronchospasm, hypoxia, and pulmonary edema. The use of steroids has also been considered. Favoring the replacement of damaged airway epithelium may be able to avoid long-term effects.

Many antioxidants have been used to prevent oxidative stress induced in phosgene-induced acute lung injury. Recently, Zhang and coworkers [116] showed that melatonin afforded a protective effect in phosgene-induced lung injury, and they postulated that its protective mechanism may be associated with scavenging free radicals and inhibiting expression of iNOS and NF-κB. However, melatonin has been used to attenuate acute lung injury in several models [117–119]. Its versatility as antioxidant and anti-inflammatory molecule could be used as treatment against phosgene exposure.

14.6 Cyanides and Therapeutic Action of Melatonin

Sometimes wrongly called "blood agents" (something that may cause confusion, leading one to think that cyanides affect the oxygen transportation in the blood), cyanides like hydrogen cyanide (HCN) and cyanogen chloride (ClCN) gases were used during World War I. However, its low persistence and low thermal stability when used in munitions made them bad CWAs. Because cyanide salts are widely used in the chemical industry, these agents are also of special concern due to their possible use in terrorist attacks.

The mechanism of action of cyanides is due to the interaction with cytochrome C oxidase, causing mitochondrial asphyxia; despite the presence of oxygen in blood, cells cannot use it to produce adenosine triphosphate (ATP). Recently, Bhattacharya and Flora [120] described a pathway for the ROS generation and cyanide-induced oxidative stress due to an increase in cellular calcium and an inhibition of antioxidant defense enzyme system in the CNS. The calcium influx is produced by three main actions: (1) activation of voltage-sensitive calcium channels, (2) enhancement of NMDA receptors function, and (3) glutamate release from intracellular stores.

Subcutaneous injection of potassium cyanide administration to rats (6, 8, or 9 mg/kg) elevated levels of products of lipid peroxidation in the brain. This observation suggests that the interaction of cyanide with oxidative phosphorylation generates increased numbers of free radicals, likely via elevating electron leakage from the respiratory complexes. Yamamoto and coworkers found that melatonin not only reduced cyanide-induced lipid breakdown in the brain but also lowered the death rate of animals that followed their exposure to the lethal agent [121, 122]. In a combination of in vitro and in vivo studies, this group also found that melatonin limited cyanide-mediated damage to brain mitochondrial DNA [123]. In another study, potassium cyanide-induced cell death in the *substantia nigra* of mice was attenuated by giving melatonin [124].

Recent evidences showed that 6-hydroxymelatonin, the major urinary melatonin metabolite, also exhibits significant antioxidant capacity. Indeed, 6-hydroxymelatonin was found to reduce lipid peroxidation in the liver, muscle, and brain of rats induced by oxidative stress [125]. Additionally, 6-hydroxymelatonin prevents neuronal toxicity caused by cyanide [126]. The protective mechanism of 6-hydroxymelatonin on tissue damage is attributed to its direct free radical-scavenging and antioxidant capacities [127].

14.7 Concluding Remarks

CWAs were designed to produce rapid incapacitation or death at exceedingly low doses. Nowadays, total immediate decontamination after exposure is difficult to achieve, and antidotes, if available for a specific CWA, would be effective if timely protective action is taken and exposed persons are treated immediately; thus, the mortality and morbidity can be considerably reduced. In this complex scenario, we think that a broad-spectrum multipotent molecule as melatonin could provide a good strategy to

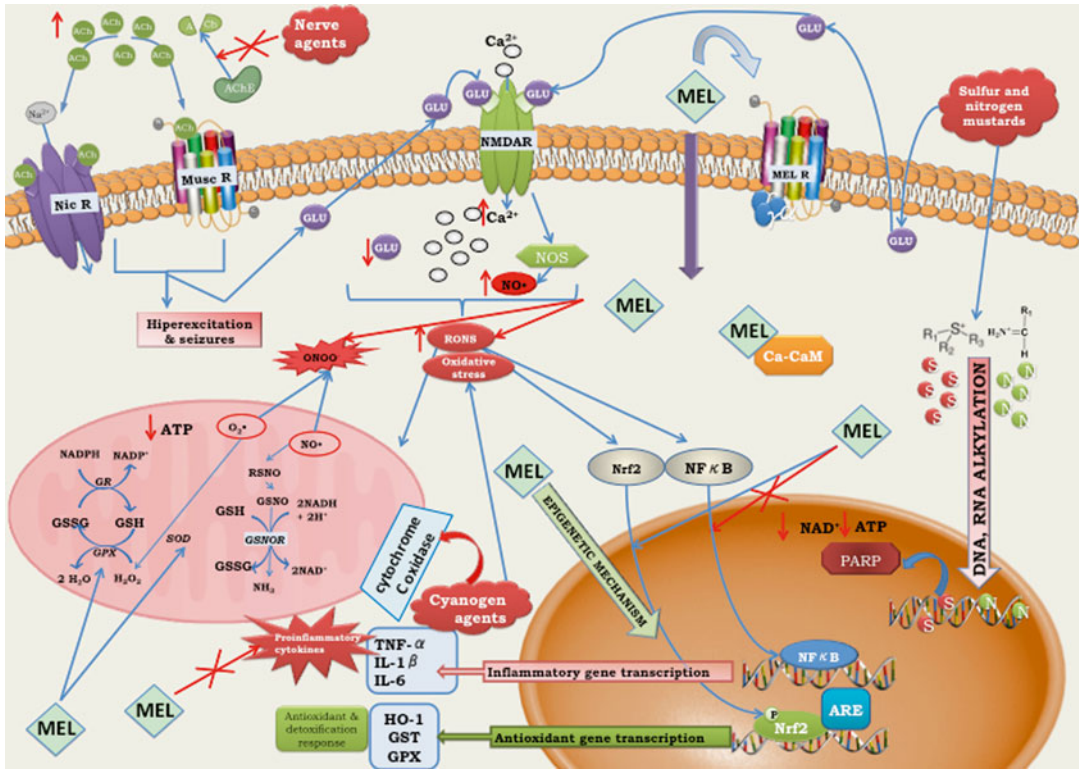

Fig. 14.3 Schematic diagram illustrating the protective mechanisms of melatonin against CWAs. Nerve agents inhibit acetylcholinesterase (AChE) activity increasing acetylcholine (ACh) levels in the extracellular medium. ACh action through nicotinic (NicR) and muscarinic (MuscR) receptors induces a hyperexcitation and seizures as well as modulates the release of glutamate (GLU). GLU binds to NMDA receptor (NMDAR) increasing intracellular concentration of Ca^{2+} and NO• by activating nitric oxide synthase (NOS). High levels of Ca^{2+} and NO• lead to a RONS flux overload within the cell. Cyanides cause oxidative stress and inhibit cytochrome C oxidase leading to mitochondrial injury. At this point, melatonin acts both as a direct radical scavenger, regulating RONS overload, and as an indirect agent stimulating antioxidant enzymes such as glutathione peroxidase (GPX) and superoxide dismutase (SOD). In this way, melatonin regulates mitochondrial homeostasis and consequently improves cellular energetic efficiency. Besides, in response to oxidative stress, melatonin induces Nrf2 gene expression and suppresses NF-κB through epigenetic processes. Within the cell, sulfur and nitrogen mustards transform into sulfonium or immonium ions, respectively, which are potent alkylating agents. Herein we postulate that melatonin may have a neutralizing effect for these agents (Fig. 14.2). In addition, melatonin activates MT1/MT2 receptor mediating redox control, by direct binding to Ca^{2+}-calmodulin (Ca-CaM) complex

counteract CWA-induced injury, due to its wide range of actions, such as a direct free radical scavenger activity, indirect antioxidant properties, and anti-inflammatory and neuromodulatory effects (Fig. 14.3). Furthermore, considering melatonin's low toxicity and high efficacy in reducing oxidative damage and its marked potential for improving human health, it should be earnestly considered as a worthy candidate against the most widely used CWAs.

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New Galenic Formulations of Melatonin

15

Pilar García-García, Francisco López-Muñoz, and Cecilio Álamo

15.1 Introduction

Melatonin is an indole amide neurohormone produced by the pineal gland and stimulated by adrenergic receptors. It is regulated by the circadian pacemaker located in the suprachiasmatic nucleus [7] and secreted into the blood in a circadian rhythm [25]. Serum melatonin has a circadian rhythm, with low production during the day, higher levels in the evening, and maximum levels at night. Melatonin has a very short elimination half-life and, when given orally, has a high first-pass hepatic metabolism. Melatonin has multiple actions in the human organism [21], but studies have involved widely varying dosages [16]. The main studies have focused on sleep disorders (e.g., jet lag, shift worker problems, insomnia); also, because of its antioxidant and immunomodulatory effects, melatonin could be used in the treatment of various cancers and neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease but it is necessary to carry our more studies [7, 25].

Melatonin promotes sleep in humans by inhibiting the circadian mechanisms responsible for vigilance. Furthermore, melatonin affects the activity of brain networks involved in the induction of sleep. Exogenous melatonin may regulate the circadian rhythm of sleep and may advance or delay sleep phases depending on the time of administration [38, 58].

Different administration routes have been studied for improving the absorption of drugs; for example, the intranasal pathway has been proposed as a non-invasive alternative route to deliver therapeutics to the brain. This route would bypass the blood-brain barrier and limit systemic side effects. Formulations have been developed to further enhance this nose-to-brain transport, mainly with the use of nanoparticles [55]. Melatonin is a compound that is easily absorbed across the mucosa, but it is sensitive to oxidation [25], and to achieve adequate dosages and to obtain the best galenic formulas for administration in different pathologies it is necessary to carry out randomized controlled studies. Melatonin has been studied in different galenic formulations, such as liquid, solid, and immediate- or prolonged-release preparations that modify its pharmacokinetic characteristics (Table 15.1) [45, 47–49, 52].

P. García-García • C. Álamo
Department of Biomedical Sciences (Pharmacology Area), Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain

F. López-Muñoz (✉)
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal
e-mail: francisco.lopez.munoz@gmail.com; flopez@ucjc.edu

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Table 15.1 Melatonin administration routes and galenic formulations

Administration route	Release system	Galenic formulation
Oral	Immediate release	Tablet/liquid
	Prolonged release	Tablet
Transdermal	Prolonged release	Patch
Intranasal	Immediate release	Liquid
Intravenous	Prolonged release	Liquid

Melatonin is obtained from different sources (natural or synthetic) (Table 15.2) [27, 45]. It was first identified in bovine pineal tissue [53]. Natural sources are found in fruits (pineapple, banana, orange, and grape-related foodstuffs). The levels and activity of melatonin have been investigated in different foodstuffs to evaluate their nutraceutical properties [42, 29]).

The dosage of melatonin has a wide range and varies depending on the study or the study goal [24, 56]. A dose of 0.5–1 mg is indicated for the induction of sleep—usually corresponding to the dose of nutritional supplements, 2 mg is used for prolonged release (drug), and megadoses are related to the antioxidant role of melatonin.

15.2 Physiology and Pharmacology of Melatonin

Endogenous melatonin is synthesized from tryptophan via 5-hydroxytryptamine (Fig. 15.1). Melatonin is produced by the pineal gland and is also present in essentially all biological fluids, including cerebrospinal fluid, saliva, bile, synovial fluid, amniotic fluid, and breast milk [1, 17]. In the absence of light the rhythm of melatonin production is persistently elevated. Dim-light melatonin (DLMO) is an approximation of the onset of synthesis and secretion, and it is considered to be a phase marker of melatonin rhythm [7].

Table 15.2 Sources of melatonin

Synthetics
Fruits
Algae
Fungi
Plants
Insects
Vertebrates (humans)

The 24-h rhythm of the primary urinary melatonin metabolite 6-sulfatoxy-melatonin is shown in Fig. 15.2 [3, 7]. This metabolite has no biological activity [16]. However, this melatonin metabolite is a marker of circulating melatonin in the body [29].

Melatonin has a very short plasma half-life ($t_{1/2}$ <30 min), variable oral absorption, and undergoes an extensive first- pass effect, making the oral mode of melatonin administration less preferable; for this reason other formulations have been developed, such as prolonged action systems, and formulations for intranasal and transdermal routes [17].

15.3 Melatonin for Sleep

Sleep is a vital process, modulated by the brain, responsible for restoring the body and mind. Insomnia (DSM-IV and ICD-10) is a disorder characterized by difficulty in falling or staying asleep. Unrefreshing insomnia or poor quality sleep that occurs for at least a month is associated with clinically significant disorders during the day, such as daytime fatigue; feelings of personal distress; and significant impairment in social, occupational, or other important areas of personal activity. The disorder has been classified as "primary insomnia" when there is no physical, mental or environmental good cause [64, 37].

New updated classifications of insomnia (DSM-V and ICD-11) have modified the previous criteria (Table 15.3). In this sense, the objective was to adapt definitions for better clinical utility, especially for mental health clinicians and general physicians. For this reason the term "pri-

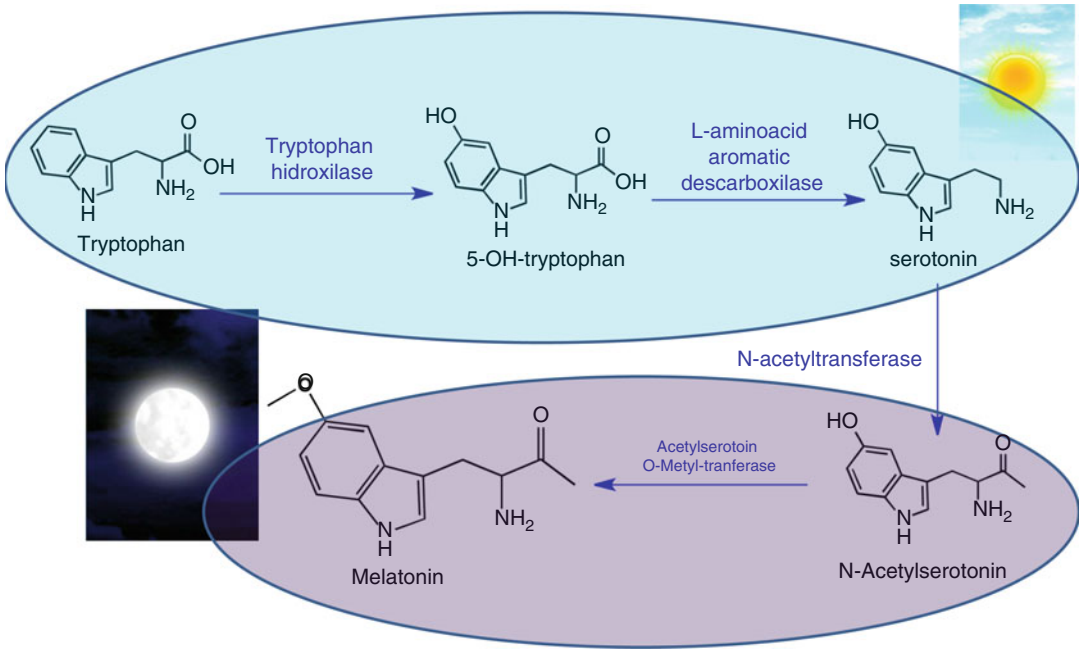

Fig. 15.1 Melatonin synthesis

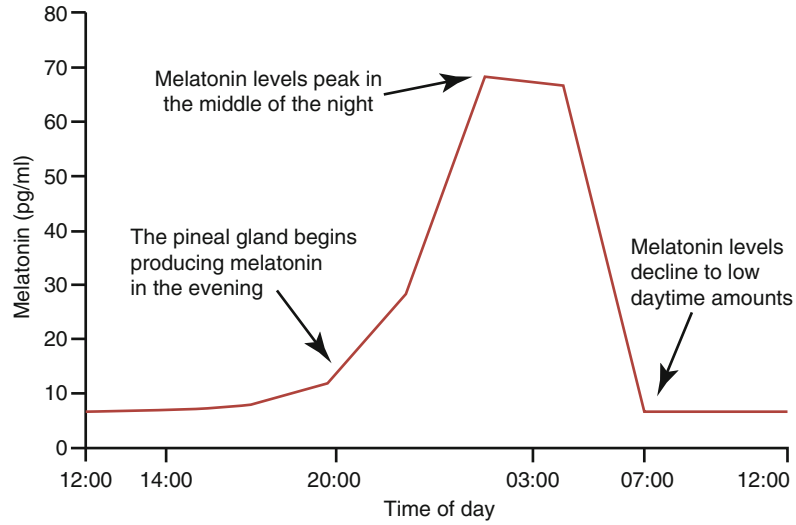

Fig. 15.2 Melatonin levels in a 24-h period

mary insomnia" has been replaced with the diagnosis of insomnia disorder, with the aim being to avoid the primary or secondary designation when this disorder co-occurs with other conditions and to reflect changes throughout the classification. The major diagnostic criteria for a diagnosis of insomnia are shown in next Table 15.3. [2, 51].

Clinical symptoms of insomnia are usually associated with irritability; fatigue; memory impairment; poor concentration; loss of energy, motivation and initiative; drowsiness; depressed mood; and anxiety. In addition there may be deterioration in occupational, social, or other spheres of life [43, 46, 60].

Table 15.3 DSM-V major criteria for a diagnosis of insomnia

Dissatisfaction with sleep quality or quantity, with 1 or more of the following symptoms:
Difficulty initiating sleep
Difficulty maintaining sleep
Early-morning awakening
The sleep disturbances cause significant distress or impairment in social, occupational, educational, academic, behavioral, or other important areas of functioning
The sleep difficulty occurs at least 3 nights per week, is present for at least 3 months, and occurs despite adequate opportunity for sleep
The insomnia does not co-occur with another sleep disorder
The insomnia is not explained by coexisting mental disorders or medical conditions

Insomnia is associated with impaired functioning and significant distress during the day, which impairs psychosocial, physical and occupational functioning. Commonly, insomnia is accompanied by tiredness, fatigue, lethargy, mood disturbances, cognitive decline, motor impairments, social malaise, and non-specific physical complaints. In addition, insomnia is associated with a significantly increased risk of various health problems such as depression, anxiety, and immune, metabolic, and cardiovascular diseases [15, 34, 54].

In subjects with sleep problems melatonin secretion was decreased compared with that in age-matched elderly subjects without sleep problems [31, 35]. Moreover, the deterioration of the biological clock with age [12] deprives the brain of a better sleep regulator. This deterioration is accompanied by an increase in complaints about poor sleep quality in the elderly population, revealed not only at night but also during the daytime [8, 37]. Such is the importance of the biological clock and melatonin in sleep regulation that it is considered that there may be abnormalities in melatonin levels in most cases of insomnia. Exogenous melatonin replacement thus may improve sleep quality and simultaneously restore the biological clock [9], and may advance or delay sleep phases depending on the time of administration [38, 58].

15.4 Galenic Formulations of Melatonin

15.4.1 Melatonin Immediate Release

Most melatonin formulations have a short half-life because they undergo intense first-pass hepatic metabolism. Therefore, they are usually considered to be immediate-release systems. One problem in comparing different formulations is that there are many melatonin products from different sources. In this sense, Ham et al. [26] evaluated and compared the quality of different melatonin products according to the American-USP (United States Pharmacopeial Convention). Measured by the United States Pharmacopeial Convention (USP) General Tests and Assays for Nutritional Supplements (other than Microbial Limits). For this aim they employed five immediate- release, two sublingual, and two controlled-release products. One controlled-release product released 90% melatonin in four hours in the dissolution test, and another product showed 90% release in 12 h. These authors concluded that some products showed evidence of poor formulation and poor quality control.

In order to maintain effective serum concentrations of melatonin throughout the night high doses or repeated administration of low doses are required [62, 63] (Fig. 15.3).

15.4.2 Sublingual/Transbuccal

Sublingual forms of melatonin are absorbed directly into the bloodstream, via the blood vessels under the tongue and in the cheeks, bypassing the liver and allowing for quick entry into the system.

Dawson et al. [13, 50] studied a prolonged transbuccal system for sleep treatment. Twelve elderly people more than 55 years old with sleep maintenance insomnia were treated with 0.5 mg transbuccal melatonin or placebo for two sessions of four consecutive nights, at least 3 days apart. Objective parameters such as polysomnography (PSG) were used. Transbuccal melatonin administration significantly increased mean nocturnal 6-sulfatoxy-melatonin compared with placebo. Moreover, there was a significant reduction

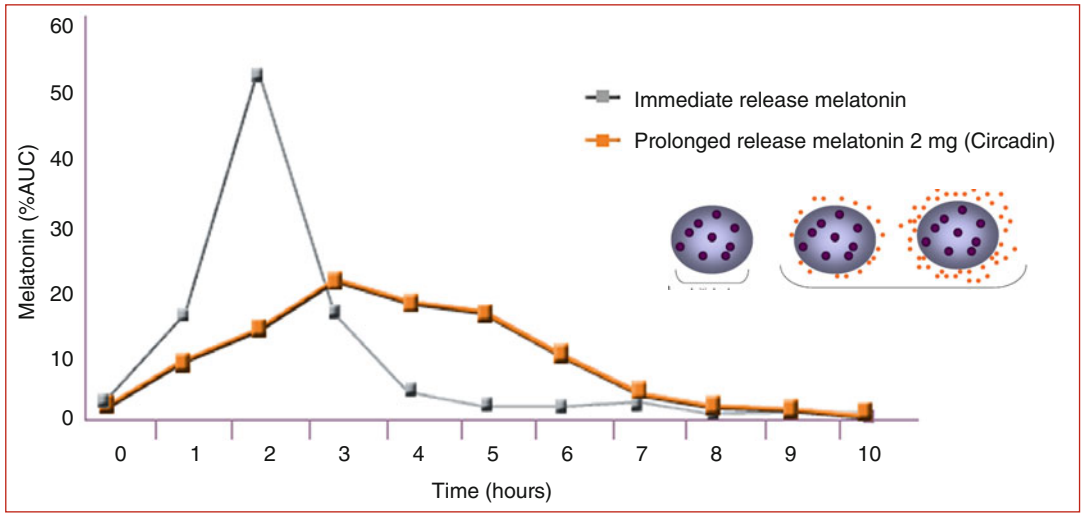

Fig. 15.3 Immediate and prolonged release of melatonin (Adapted from Zisapel [63])

in core body temperature. However, no positive effect on any PSG measure of sleep was shown. These authors concluded that the sustained nocturnal administration of melatonin, in the low pharmacological range, might be of limited clinical benefit in this population.

A new food-grade liquid emulsion (melatonin in emulsion spray by Medestea SpA) has been formulated in spray form, with an additional increase of the contact surface and the possibility of efficient mucosal absorption. Five milligrams (5 sprays of the food-grade liquid emulsion directly under the tongue) of melatonin versus 5-mg tablets were studied. The AUC values obtained following a single dose of the two preparations showed significant differences. The amount of melatonin in the systemic circulation after administration of the melatonin oral spray was significantly higher than that of the oral tablet. The melatonin spray was well tolerated without any adverse reactions [4].

15.4.3 Melatonin Prolonged Release

Melatonin prolonged release (Circadin®; RAD Neurim Pharmaceuticals EEC Limited, UK) is an authorized drug that has been developed as a dosage form for sustained release over a period of 8–10 h (Fig. 15.3). This is a unique drug approved by the European Medicines Agency (EMA) in 2007 [18, 19]. This prolonged release drug has a major advantage, as although immediate-release melatonin is rapidly absorbed, it undergoes the hepatic first-pass phenomenon so that more than 80 % is metabolized and excreted in the urine, as 6-sulfatoxymelatonin [3, 61]. Bioavailability is very low, about 15 %, with the immediate-release melatonin having a half-life of only 40–50 min in humans [59]. The maximum concentration (C_{max}) is reached between 20 and 240 min after ingestion, causing a decrease in levels, depending on dose, from 120 min [50]. Accordingly, the pharmacokinetics of immediate-release melatonin are not the most appropriate for about 8 h covering physiological sleep. To achieve maintenance of effective concentrations of melatonin at night requires high doses, with the risk of phase changes and receptor desensitization, which is an unnecessary burden on liver function.

The pharmacokinetic profile of melatonin prolonged-release 2 mg was studied in three specific studies in healthy volunteers, in one of which a possible interaction with food was studied. With melatonin prolonged release 2 mg physiological levels are achieved throughout the night, since it is an extended release formulation of melatonin, which prevents the rapid hepatic metabolism of the hormone through the intestinal release thereof over an extended period of

time, thus mimicking the physiological patterns of melatonin secretion (EMA 2012). The time (T_{max}) to reach maximum concentration (C_{max}) was 0.75 h fasting and increased to 3 h with food intake. Thus, the peak plasma concentration (C_{max}) appears at 2.6 h after ingestion and persists for about an additional 3.5 h (terminal half-life ($t_{½}$) is 3.5–4 h), covering the night period [37, 64].

Melatonin prolonged-release 2 mg approval by the EMA was based on three randomized, double-blind placebo trials, with similar designs, to demonstrate its efficacy in primary insomnia in patients aged over 55 years [36, 37, 39, 57]. An objective placebo-controlled, double-blind study was performed in 24 men and 16 women over age 55 with insomnia [39]. Sleep was assessed objectively by PSG and spectral analysis of the electroencephalogram (EEG) overnight. Wakefulness and arousal were assessed by the Leeds psychomotor test battery. The effects at 1 day and at 3 weeks after treatment withdrawal were also assessed. With melatonin prolonged-release 2 mg, latency to sleep onset, determined by PSG, decreased by 50% compared with pretreatment, and was 9 min lower than placebo ($p=0.011$). The duration of the activation time before sleep onset was reduced by 50% ($p=0.011$), without adversely affecting the structure or architecture of sleep or change in the duration of REM sleep. No differences between the groups regarding the efficiency and duration of sleep were observed. After treatment, the group treated with melatonin prolonged-release 2 mg showed significant improvement ($p=0.008$) in daytime alertness (Critical Flicker Fusion test). Half of the patients reported a significant improvement in sleep quality at home with melatonin prolonged-release 2 mg compared with only 15% of those treated with placebo ($p=0.018$). According to the variables recorded on day 1 or 3 weeks after treatment completion, no rebound or withdrawal effects were detected [39].

Subjective clinical assessment of sleep quality was based on three measures: the Leeds Sleep Evaluation Questionnaire (LSEQ), the Pittsburgh Sleep Quality Index (PSQI), and the daily reports of patients. The LSEQ consists of ten visual analogue scales measuring four domains of sleep: sleep onset, quality of sleep (QOS), hangover, and awakening and mental wellbeing the next morning. Clinical response was defined as an improvement of 10 mm or more on visual analogue scales, which is considered of clinical importance and relevance. The PSQI consists of nine questions regarding the sleep habits of patients during 2 and 4 weeks prior to treatment. The PSQI is an algorithm used to calculate the scores of seven components, and these are added to give an overall score.

In the study by Lemoine et al. [36], 189 outpatients of both sexes were included. Melatonin prolonged-release 2 mg was associated with a statistically significant improvement in sleep quality, as measured by the LSEQ, and quality of night (QON), as measured by a five-point categorical rating scale, compared with placebo (QOS: $p=0.047$; QON: $p=0.003$). A statistically significant improvement was also observed in morning alertness, as measured by the behavioral domain integrity in the morning (BFW) of the LSEQ, in the melatonin prolonged-release 2 mg group compared with the placebo group ($p=0.002$ BFW). Improved sleep quality was significantly correlated with improved morning alertness (BFW $p<0.01$) in the melatonin prolonged-release 2 mg group, indicative of an induced sleep restorative effect of melatonin prolonged-release 2 mg [36].

In the study by [57], the primary endpoint was predefined as "responders", who improved at least 10 mm in the visual scales of QOS and the morning behavioral integrity (BFW) scale of the LSEQ. The analysis of responders in the two scales showed a significant benefit ($p=0.014$) of melatonin prolonged- release 2 mg (26% responders) over placebo (15%). Melatonin prolonged-release 2 mg statistical superiority was also demonstrated in each of the variables studied in isolation, including sleep quality, assessed by the LSEQ ($p=0.014$) and PSQI ($p=0.036$) and sleep latency, as assessed by the LSEQ ($p=0.013$) and PSQI (−24.3 min vs −12.9 min melatonin prolonged-release 2 mg compared with placebo, $p=0.028$) and return the next morning, determined by scoring the LSEQ BFW ($p=0.038$). Furthermore, this formulation also significantly improved ($p=0.034$) quality of life as measured by the WHO-5

questionnaire [57]. In this study a favorable significant difference was obtained with melatonin prolonged-release 2 mg, both in improving the quality of sleep and in morning alertness. Also, the latency to sleep onset was shortened and the use of hypnotics was reduced among patients receiving melatonin prolonged-release 2 mg compared with placebo. Similarly, the quality of life improved significantly. Therefore, in this study melatonin prolonged-release 2 mg was proven to be effective and safe for the short term (3 weeks) in adults 55 years of age and older with primary insomnia (in whom the decline of melatonin had altered the examined sleep parameters), producing a significant clinical improvement in latency to sleep onset and sleep quality, and in morning alertness. Furthermore, melatonin prolonged-release 2 mg showed efficacy and a favorable safety profile, along with a lack of detrimental effects on memory and driving, supporting its use in primary insomnia in patients aged over 55 years [57].

Circadin® is considered by the British Pharmacological Association (BPA) to be a first-line agent for the treatment of insomnia, parasomnias and circadian rhythm disorders in patients over age 55 years [33].

15.4.4 Melatonin Topical Preparations for Skin and Body

Melatonin shows antioxidant and immunological effects and thus might play a role as a topical drug for use against oxidative damage in the skin and body [21]. Melatonin is absorbed by the skin and appears at detectable concentrations in the plasma.

The penetration properties of melatonin have been studied in alcoholic solutions and creams [21] and in various vesicular approaches such as elastic liposomes and ethosomes [17]. Melatonin penetrates the skin at concentrations of 0.01 and 0.03 %, and accumulation in the stratum corneum has been described. Fischer et al. [21] showed that alcoholic solution was better than cream formulation for melatonin delivery. Also, undecanoic, lauric, and oleic acids have been suggested to enhance melatonin skin permeation [44]. Melatonin topical application could be used against UV-induced oxidative damage [20, 22].

15.4.5 Transdermal Delivery

Transdermal delivery could be a good system for melatonin administration, given the variability of absorption, short biological half-life, and extensive first-pass metabolism of melatonin when administered orally. In this sense, transdermal melatonin avoids extensive hepatic clearance and low bioavailability and may have the potential to more closely mimic the normal endogenous plasma melatonin profile [17, 30, 32]. In spite of several advantages offered by the transdermal route, however, only a few molecules are successfully administered transdermally because of the extraordinary barrier nature of the stratum corneum [17, 23].

One the most relevant aspect for transdermal systems is the choice of a suitable vehicle, and in this sense it is necessary to consider the solubility of the drug in the vehicle, the release of the drug, and the capacity for subcutaneous penetration [32]. Another advantage is a favorable octanol-water partition coefficient (log P) of 1–2 [6, 40].

Alcoholic solutions of melatonin have been studied for their penetrative properties [21]. In this sense, ethanol is suitable as a solvent for the transdermal delivery of melatonin. There is a theory about the possible deposition of melatonin in the skin that is supported by the finding that when transdermal delivery of melatonin is compared with transmucosal and oral administration, melatonin blood levels show a significant delay with the transdermal route [6].

15.5 Other Galenic Formulations

15.5.1 Intranasal

Intranasal administration of melatonin has been examined with the goal of improving the characteristics of alternative formulations, because oral administration leads to significant degradation in the gastrointestinal tract or metabolization to a high degree via the first-pass effect of the liver [41, 55].

Intranasal melatonin (starch microspheres)[a]						
Particle size	% Bioavailability (F)	T_{max}	$T_{1/2}$	C_{max} (ng/ml)	AUC (ng min/ml)	MRT (min)[b]
30–60 μm	84.07 %	7.8 min	12.3	32.16	1,852	150.58

[a]Studies in rabbits
[b]Mean retention time (intravenous: 45.41; intranasal: 64.91)

Different formulations, such as intranasal solutions, sprays, microspheres, gel, and liposomes have been developed. The main advantage is their ease of use; however, due to their short residence time in the nasal mucosa, they have poor bioavailability [41, 10, 14]. Mao et al. [41] have developed melatonin starch microspheres for intranasal administration, prepared by an emulsification crosslinking technique, and having a rapid onset and controlled release without toxicity. The mean particle size was 30–60 μm, which is in the ideal range for favorable nasal absorption (40–60 μm), and the entrapment ratio of melatonin in the microspheres was 11.0 %. Two hours after administration >80 % starch microspheres could be detected in the nasal tissue. This microsphere swells, and compared with gelatin microspheres shows a much longer deposition time in the nose [11, 41].

Bechgaard et al. [5] studied the bioavailability of melatonin in rabbits after the nasal administration of two formulations. A total of 1.5 mg melatonin in 50 μl was administered and compared with the same quantity for i.v. administration. They used different test solutions (polyethylene glycholate, sodium glycolate, and another solution without sodium glycolate). The results showed that sodium glycolate had enhancer effects. But the higher bioavailability without glycolate showed that it might not be necessary to use an enhancer. The absorption of both formulations was very fast (T_{max} = 5 min).

15.5.2 Intravenous Injections

Mallo et al. [40] showed that during melatonin infusion (n = 4 bolus i.v. injection of 5 or 10 μg/person and after a 5-h infusion of 20 μg per person in six healthy subjects), the plasma hormone level reached a steady-state after 60 and 120 min, when it was equal to the nocturnal level. The infusion regime may be valuable in replacing blunted hormonal secretion in disease states.

Two intravenous formulations of melatonin at a strength of 5 mg/ml have been studied in Wistar rats, one formulation with hydroxypropyl-B-cyclodextrin and propylene glycol to increase solubility and stability, and the other with an antioxidant and chelating agent to reduce oxidation and hydrolysis. This study provides evidence of suitable intravenous formulations of melatonin [28].

Conclusions

Melatonin has many actions in the human organism; one of its main roles is for insomnia treatment. There are different formulations for administering this hormone, but at present the most studied is the oral formulation. Also, there are different dosages of melatonin. Because of the pharmacokinetic characteristics of melatonin it necessary to maintain its concentration for a long time to imitate the physiological release of this molecule, especially for insomnia treatment. In this sense, prolonged-release formulations of melatonin have been developed that will cover the entire night and improve sleep disorders.

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Melatonergic Drug Ramelteon in Neurotherapeutics

16

Venkataramanujam Srinivasan†, Rahimah Zakaria, Domenico de Berardis, Francisco López-Muñoz, Mohd Jamil Yaacob, Zahiruddin Othman, and Amnon Brzezinski

16.1 Introduction

Ramelteon is a melatonergic chronohypnotic drug (Rozerem, Takeda Pharmaceuticals, Japan) and has the chemical formula of (S)-N-[2-(1,6,7,8-tetrahydro-2H-indeno-[5.4-b]furnan-8-yl)-ethylpropionamide (Fig. 16.1). The drug received FDA approval in 2005. It is a selective agonist for melatonin MT_1 and MT_2 receptors. The selectivity of ramelteon for MT_1 receptors is much greater than that of MT_2 receptors, and this selectivity accounts for its specific effect in initiating sleep onset than melatonin itself [1]. Ramelteon has no affinity for benzodiazepine (BZP), dopamine, opiate, or serotonin receptors [2]. Ramelteon's efficiency in treating patients with chronic insomnia has been proved in a number of clinical studies undertaken since

†Author was deceased at the time of publication.

V. Srinivasan, PhD, MAMS
Sri Sathya Sai Medical Educational and Research Foundation, International Medical Sciences Research Study Center Prasanthi Nilayam, 40-Kovai Thirunagar, Goldwins, Kovai Coimbatore 641014, Tamilnadu, India

R. Zakaria
Department of Physiology School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian 16150, Kelantan, Malaysia

D. de Berardis
NHS, Department of Mental Health, Psychiatry Service of Diagnosis and Treatment Hospital "G.Mazzini", ASL-4, Teramo, Italy

Department of Neurosciences and Imaging and Clinical Sciences, University "G.D Annunzio", Chiety, Italy

F. López-Muñoz (✉)
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain
Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal
e-mail: flopez@ucjc.edu; francisco.lopez.munoz@gmail.com

M.J. Yaacob
Department of Psychiatry, Faculty of Medicine, Asian Institute of Medicine, Science and Technology, Bedong 08100, Kedah Darul Aman, Malaysia

Z. Othman
Department of Psychiatry, School of Medical Sciences Universiti Sains Malaysia, Kubang Kerian 16150, Kelantan, Malaysia

A. Brzezinski
Department of Obstetrics and Gynecology, Haddasah Medical Center, The Hebrew University, Jerusalem 91120, Israel

F. López-Muñoz et al. (eds.), *Melatonin, Neuroprotective Agents and Antidepressant Therapy*,
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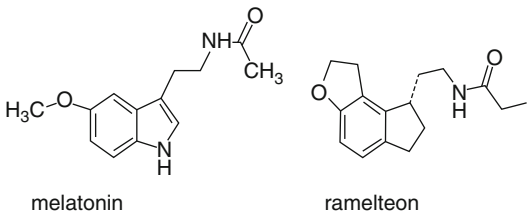

Fig. 16.1 Structure of ramelteon and melatonin

2005, and the drug's efficacy and safety have been proved in multicenter clinical studies undertaken in Europe, Japan, and theUSA, and this has been reviewed in a number of studies [3–5]. Ramelteon has been used in treating patients with REM sleep behavior disorder (RBD) and is found to be effective in increasing sleep efficiency and reducing the intensity of behavioral symptoms of this disorder [6]. In addition to sleep disorders, ramelteon has been successfully applied for treating patients with neurobehavioral disorders, and clinical studies show a promising future in this direction. Both in autistic disorder [7] and in patients with delirium [8], ramelteon satisfactorily improved the clinical states of the patients. The findings of ramelteon increasing the BDNF levels in the in vitro studies undertaken on mouse cerebellar granule cells indicate its potential for treating the neurobehavioral symptoms in neurodegenerative diseases like Alzheimer's disease [9].

16.2 Ramelteon, the Chronohypnotic Melatonergic Agonist

As has been stated in the introduction, ramelteon is a melatonin agonist that has selective affinity for MT_1 and MT_2 melatonin receptors. When administered orally, it is rapidly absorbed from the gastrointestinal tract [10], and the half-life of circulating ramelteon ranges from 1 to 2 h. The drug is metabolized mainly in the liver. The hydroxyl and carbonyl groups of the drug are oxidized and then conjugated with glucuronide [11]. Cytochrome P_{450} 1A2 (CYP1A2) is identified as the major hepatic enzyme that metabolizes ramelteon giving rise to four principal metabolites, namely, M-1, M-II, M-III, and M-IV; and M-II occurs in higher concentration in the blood with systemic levels 20–100 times higher than ramelteon itself [11]. As the active metabolite, M-II has a longer half-life with greater systemic exposure than ramelteon, and it is suggested to contribute for the therapeutic actions on ramelteon itself [11]. The binding affinity of M-II with MT_1 and MT_2 melatonin receptors is low when compared with ramelteon. However, because of its higher systemic levels, it accounts for a major share of ramelteon's clinical effects [12].

16.3 Ramelteon as Therapeutic Drug in Insomnia

Insomnia is a common disorder seen in 30–35 % of the adult population, and of this 10 % becomes very chronic in nature [13]. The sequelae of insomnia like fatigue, reduced alertness, irritability, and impaired concentration have greater negative impact on the quality of life of these individuals [14]. In addition, the economic burden to the country due to insomnia is also very high accounting to billions of dollars [15]. Hence, effective treatment of insomnia benefits not only the individual suffering from insomnia but to the whole nation as such. Pharmacotherapy of insomnia involves sedative-hypnotic drugs like benzodiazepine and non-benzodiazepine drugs that act mainly through gamma-aminobutyric acid (GABA) receptors. Needless to say that GABAergic neurons in the brain have an active role in sleep induction and maintenance [16]. The non-benzodiazepine drugs like zolpidem, zaleplon, and zopiclone are also used for treating insomnia, but all these drugs only have moderate efficacy in increasing sleep efficiency [17] but exhibit number of adverse effects [18]. Because of their adverse effects, they pose a big challenge for long-term use as hypnotics. Nonpharmacologic interventions like adhering to sleep hygiene, relaxation, cognitive and behavioral therapies, and lifestyle modifications

are all considered as other effective methods for treating insomnia [19]. With the search for identification of a natural agent involved in sleep induction and regulation, interest has been focused on melatonin, the hormone secreted mainly by the pineal gland, and this has resulted in fruitful results [20]. Reports of significant correlations between melatonin production and insomnia point out melatonin deficiency as the major cause for insomnia [21, 22]. With melatonin deficiency identified as one of the major causes for insomnia, melatonin replacement therapy has been suggested for treating insomnia especially those of the elderly. Because melatonin is a natural substance with low toxicity and limited side effects (no significant adverse effects), the drug is considered as more suitable for treating insomniacs. Indeed melatonin administration significantly improved sleep onset, total sleep time (TST), and sleep quality, and this has been proved in a number of clinical studies. However reduction in endogenous melatonin production is considered as essential for exogenously administered melatonin to exert its therapeutic hypnotic effects [23]. A meta-analysis on the therapeutic effects of melatonin in sleep disturbances of all age groups (young adults and elderly individuals with presumably normal levels) failed to document significant and clinically meaningful effects of exogenous melatonin on sleep latency, quality, and efficiency [24]. But another meta-analytical study found melatonin as effective in increasing sleep efficiency (SE) and reducing sleep-onset latency (SOL) in elderly insomniacs [25]. Since melatonin has short half-life, melatonin prolonged-release (2 mg) was introduced, and this was found effective in improving the sleep quality, reducing SOL and morning alertness in elderly insomniacs >55 years, and the drug was well tolerated in clinical trials with a tolerability profile similar to that of the placebo [26]. Ramelteon's efficiency in improving sleep efficiency has been proved in a number of placebo-controlled clinical studies conducted in Europe, the USA, and Japan. The results of these studies are presented in Table 16.1 with references [27–39].

16.4 Ramelteon in REM Sleep Behavior Disorder [RBD]

RBD is subcategorized under parasomnias usually associated with REM sleep. It is characterized by loss of atonia with prominent motor activity and dreaming [40]. Nocturnal disturbances and sleep arousals are specific to the RBD. The components of the circadian system that mediates the onset and timing of melatonin secretion combined with clinical pathological findings are considered as essential for defining the neurochemical correlates of RBD [41]. Melatonin was found effective in treating 14 patients with secondary RBD [42], and they supported the use of melatonin either alone or as add-on therapy in patients with RBD. Recently, ramelteon was tried in two patients with secondary RBD, and ramelteon's efficacy was evaluated both by subjective and objective evaluations in these two patients. Ramelteon's efficacy was proved in these patients [6]. The total sleep time was improved very much in both these cases after taking ramelteon (8 mg/day). The percentage of wake time after sleep onset was also very much decreased. The periodic limb movement as assessed by periodic limb movement (PLM) index per hour was reduced in both these cases after ramelteon. When the severity of RBD was evaluated in one case before and after 2 years of ramelteon administration, the score by using REM sleep behavior disorder screening questionnaire (RBDSQ) improved from 41 in 2009 to 14 in 2012. In another case with ramelteon intake (8 mg/day), the RBDSQ score improved from 70 in 2009 to 17 in 2012. Although clonazepam is a drug of choice for treating RBD, it has not been effective in alleviating RBD symptoms. As melatonin has been found effective in protecting L-dopa from auto-oxidation in the striatum, melatonin is considered as displaying neuroprotective effects [43]. It is well known that RBD is one of the prodromal symptoms of neurodegenerative diseases [44]. With improvement of secondary RBD symptoms and being beneficial to patients with neurodegenerative diseases, ramelteon can be ranked as the neuroprotective agent.

Table 16.1 List of clinical trials and ramelteon's beneficial effects in primary insomnia

Dosage (mg/day)	Duration of administration	Number of insomnia patients	Sleep-onset latency	Sleep efficacy and quality	Total sleep time	References
16 and 64	Single dose 30 min before bedtime	375 healthy adults with chronic insomnia (aged 35–60 years)	Reduced	–	Increased	[27]
4 and 8	5 weeks	829 older adults with chronic insomnia (mean age, 72.4 years)	Reduced	Increased	Increased at the end of first week, third week, and fifth week	[28]
4, 8, 16, and 32	2 days	107 patients with chronic insomnia (mean age, 37.7 years)	Reduced	Increased	Increased	[29]
4	5 weeks	100 older adults with chronic insomnia	Reduced	Increased	Increased	[30]
8 and 16	5 weeks	371 patients with chronic insomnia	Reduced	Increased	Increased at all doses	[31]
8	5 weeks	270 patients with chronic insomnia	63 % reduction in week 1 and 3, 65.9 % reduction in week 5	–	–	[32]
	6 weeks	20 healthy peri- and postmenopausal women	Reduced	Increased	Increased	[33]
8	6 months	451 adults with chronic insomnia	Reduced latency to persistent sleep consistently	–	–	[34]
8	4 weeks	21 older adults with obstructive sleep apnea	Reduced	–	–	[35]
4 and 8	2 weeks	1,130 adults with chronic insomnia	Reduced with 8 mg only	Increased in the first week	Increased	[36]
4, 8, and 16	24 weeks	190 patients with chronic insomnia	Reduced	Increased	Increased up to 20 weeks, and then it was maintained	[37]
4 and 8	2 nights	65 patients with insomnia	Reduced	Increased sleep quality	Increased	[38]
8	3 weeks	552 adults with insomnia (mean age 43.2 years)	Reduced	–	–	[39]

16.5 Ramelteon as a Chronobiotic Agent: Human Studies

Ramelteon's sleep-promoting effect in patients with primary insomnia or in secondary insomnia's does not exhibit dose response [28, 29]. Ramelteon exhibits circadian phase-shifting effects at much lower doses (1, 2 and 4 mg) which produces much larger phase shifts than the higher doses (8 mg) [45]. In a study conducted on 75

healthy adults aged 18–45 years, oral ramelteon was given in the dose of 1, 2, 4, and 8 mg daily for 4 days, 30 min before bedtime. The primary end point was assessed at the time at which salivary melatonin levels declined below 3 pg/ml after morning awakening (dim-light melatonin offset; DLMoff). After administration of 1, 2, and 4 mg of ramelteon daily for 4 days, the participants exhibited statistically significant phase shifts in DLMoff 88.0 ± 16.6, 80.5 ± 14.5, and 90.5 ± 15.2 min, respectively (statistical significance; $P = 0.002$, $P = 0.003$, and $P = 0.001$, respectively, for 1, 2, and 4 mg doses). From this study, the investigators concluded that ramelteon at 1, 2, and 4 mg intake at night can facilitate re-entrainment of circadian rhythms after 5-h phase advance in healthy adults. With 8 mg of ramelteon, change in DLMoff was 27.9 ± 16.4 min which was statistically significant [45]. Since such phase advance is seen during eastward jet travel across five time zones, ramelteon was suggested as the drug of choice for treating jet lag.

16.6 Ramelteon for Treatment of Jet Lag

Jet lag is characterized by symptoms like sleep disturbances, excessive sleepiness, fatigue, gastrointestinal disturbances, and daytime functional impairment which persist for several days [46]. Therapies for jet lag disorder require drugs that can accelerate circadian alignment as well as improve sleep disturbances and daytime functional impairment. Number of drugs like sedative hypnotics, bright light, caffeine, modafinil, and melatonin are being used for alleviating the symptoms of jet lag. Of these melatonin displays, both sleep inducing and circadian phase-shifting effects through its actions on both MT_1 and MT_2 melatonin receptors present in the suprachiasmatic nucleus (SCN) of the hypothalamus [47]. As discussed in the previous paragraphs, ramelteon also displays both sleep-promoting and circadian phase-shifting effects. Based on this property, ramelteon was tried for treatment of jet lag. In a randomized double-blind placebo-controlled parallel group study involving 110 subjects with a history of jet lag disturbances, the ability of 1, 4, and 8 mg of ramelteon was evaluated with comparison to placebo to alleviate the sleep-onset difficulties associated with jet lag following eastward jet travel across five time zones. Participants flew across five time zones from Hawaii to East Coast of the USA overnight with one stopover either at Los Angeles, California, or Atlanta, Georgia. All participants had to depart from Hawaii in the afternoon (e.g., 1:00 pm or 4:30 pm, Hawaii-Aleutian Standard Time) and arrive at East Coast destination airport 12–13 h later (7:45 am or 10: 30 am, Eastern Standard Time). All participants were instructed not to sleep on the plane. On arrival, participants were taken directly to the sleep laboratory and were given ramelteon 1, 4, and 8 mg or placebo in a randomized manner. Some (35 subjects) were kept in dim-light conditions with sunglasses on (for evaluating melatonin phase), while others spend their time in natural setting. On day 6, all participants were flown back to Hawaii. Next morning residual effects, daytime sleepiness and functioning, level of alertness, ability to concentrate, quality of sleep, and ease of awakening in the morning were all assessed using post-sleep questionnaire. Actigraphy watches were worn throughout the study. Results of the study showed significant reduction in the mean latency to persistent sleep (LPS), for nights 2–4 in participants taking ramelteon 1 mg ($n = 27$) compared with those taking placebo. In subjects who took ramelteon 4 mg ($n = 27$) and 8 mg ($n = 27$), there was a trend toward reduction in mean LPS, but these groups did not reach statistical significance when compared to placebo. Significant reduction in LPS was noted at night 2 in ramelteon 1 mg group ($P = 0.044$) and at night 4 for the ramelteon 8 mg group ($P = 0.025$). A trend toward reduction in LPS was noted for the 1 mg group at night 3 and 4 ($P = 0.069$ and $P = 0.057$, respectively) and for ramelteon 4 mg at nights 2 and 3 ($P = 0.059$ and $P = 0.100$, respectively) [48]. With regard to daytime ability, significant improvement was noted on day 4 and day 5 in subjects treated with 4 mg ramelteon. The ability to concentrate on day 4 and day 5 was improved very much with 4 mg of ramelteon. This study shows that ramelteon has the potential of being used for treating jet lag symptoms. However, more number

of field studies is required to prove the efficacy of ramelteon in treating jet lag symptoms.

16.7 Mechanism of Chronohypnotic Effects of Ramelteon

The presence of high density of MT_1 and MT_2 melatonin receptors in SCN of the hypothalamus suggests that they may be involved in regulating circadian rhythms including sleep-wake rhythm. As ramelteon's affinity to these melatonin receptors is rather high, it indicates that it has a definitive role in sleep induction and phase shifting of circadian rhythms. SCN plays an active role both in promoting sleep and wakefulness, and this depends upon a complex neuronal network involving a number of neurotransmitters like GABA, glutamate, arginine vasopressin, and somatostatin [49]. By influencing the hypothalamic sleep switch and by inhibiting SCN electrical activity, the circadian wake signal is inhibited by ramelteon which in turn promotes sleep onset [50]. Each subtype of melatonin receptors MT_1 and MT_2 present in the SCN region of the hypothalamus regulates differently the vigilance states with MT_2 receptors regulating NREM sleep and MT_1 receptors regulating REM sleep. Evidences for this have been obtained by elegant studies carried by administering melatonin in MT_1 and MT_2 receptor knockout mice [51]. Until recently, it was hypothesized that MT_1 receptors are involved in the control of sleep, whereas MT_2 receptors are involved in time-shift by their actions on the neural activity of the SCN [52]. But recent studies point out that MT_2 receptors modulate sleep especially NREM sleep [53]. MT_2 receptors present in the reticular thalamus have been shown to be activated by MT_2 partial agonist, UCM 765, belonging to the N-substituted aminoethylamides that display a different degree of selectivity and intrinsic activity toward MT_1 and MT_2 receptors [54]. IIK7 is a full MT_2 receptor agonist of the chemical nature 6H-isoindolo[2,1-α]indole [55]. UCM 765 is found to have 100-fold higher affinity for MT_2 receptors than MT_1 receptors, whereas IIK7 displayed 90-fold higher activity for MT_2 receptors than MT_1 receptors. Both UCM 765 and IIK7 reduced the latency of NREM sleep and increased the amount of NREM sleep [53]. As microinfusion of UCM 765 into the reticular thalamus increased the firing and burst activities of reticular thalamic neurons, and this effect was blocked by prior infusion of 4P-PDOT (selective MT_2 receptor antagonist), it was concluded that MT_2 receptor agonists promote sleep through activation of reticular thalamic neurons [53]. As ramelteon is both MT_1 and MT_2 receptor agonist, its hypnotic action can be attributed to its activation of MT_2 receptors present in the reticular thalamus in addition to its activation of MT_1 and MT_2 receptors present in the SCN of the hypothalamus (Fig. 16.2).

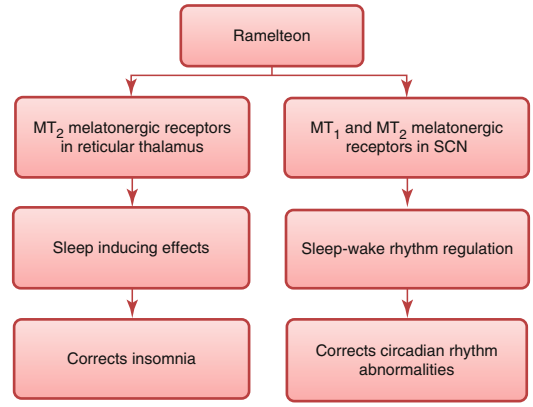

Fig. 16.2 Mechanism of ramelteon's sleep-promoting and chronobiotic action

16.8 Melatonin Agonist Ramelteon's Beneficial Actions in Delirium

Delirium is a serious acute neuropsychiatric syndrome characterized by inattention and global cognitive dysfunction and is associated with longer hospitalization and higher morbidity [56]. With increasing age, the percentage of its occurrence is on the rise. The risk of developing delirium is high in patients with dementia [57], and in one recent study involving 145 patients with delirium, it was found that the vulnerability of

patients with dementia developing delirium is 15 times higher than any other patients [58]. Research studies on the basic mechanisms of delirium reveal that it is a multifactorial disease. A neurotransmitter imbalance like cholinergic deficiency or neuroinflammation is suggested as a possible contributory factor in some studies [59–61]. The generation of acute oxidative stress is also implicated in the pathogenesis of delirium [59]. Impairment of melatonin rhythm [62] and consequent disruption of sleep-wake cycle via MT_1 and MT_2 receptors are suggested as more important contributory factors for the development of delirium [63] than neurotransmitter imbalance as has been suggested in the earlier study [64]. Findings of the study on urinary levels of melatonin metabolites in patients without delirium and patients with delirium reveal that the levels are normal in patients without delirium, high in those with hypoactive delirium, and low in those with hyperactive delirium [65]. Pineal hormone, melatonin, is implicated in the etiology of delirium either by its direct actions on the symptoms of delirium or through its chronobiotic action of regulating sleep-wake rhythm [66]. In the first randomized, double-blinded placebo-controlled study involving 145 patients of the Internal Medicine Tertiary Care Unit aged 65 and above, 72 patients were randomly assigned to the melatonin group (to receive 0.5 mg of melatonin) and 73 patients to the placebo group [58]. Results of this study showed that significantly smaller proportions of patients experienced delirium on melatonin as compared to placebo (12% vs. 31.0%, $P=0.014$). In this first double-blinded controlled clinical study, low-dose (0.5 mg) melatonin administered at nighttime was found to be effective in decreasing the risk of delirium. Melatonin was well tolerated in all these patients. In the earlier clinical trials conducted on delirium, patients received neuroleptics [67] or cholinesterase inhibitors [68]. But the use of these agents did not decrease delirium duration or severity. The low melatonin dosage used in the melatonin study [58] was designed in such a way that it approximated the physiological dose presumably with the aim to restore the normal melatonin levels without producing any undesirable side effects. As melatonin administration was associated with a lower risk of delirium, the authors of this first clinical study with melatonin (as a possible protective agent against delirium) concluded that low dosage of melatonin is a potential protective agent against delirium [58]. As ramelteon's half-life is longer and its affinity to MT_1 and MT_2 receptors is very high (as has been discussed in the earlier paragraphs on insomnia), physicians and neurologists started using ramelteon for treating delirium. In the first study of the use of ramelteon in delirium, the drug was administered at a dose of 8 mg at 21.00 h in three patients for 7 days. Memorial Delirium Assessment Scale (MDAS) was administered before commencement of ramelteon administration and 7 days after ramelteon treatment. In all cases, MDAS score improved significantly from the baseline to day 7 indicating the efficacy of ramelteon in delirium [69]. No adverse effects like over sedation or changes in laboratory findings were reported. The authors of the study attribute ramelteon's beneficial effects in delirium to its antioxidant and anti-inflammatory activity and to the regulation of sleep-wake cycle [69]. In the other study on the use of ramelteon for delirium in five cases, marked improvement of delirium was noted within 1 day. The marked improvements of symptoms were not only related the sleep-wake cycle but also to the improvement of attention and cognitive functions [63]. The effectiveness of ramelteon in this study included delirium cases of hyperactive type, and the authors concluded that ramelteon can be a viable treatment option for the management of neurobehavioral symptoms of delirium [63]. In the study conducted on 35 patients with insomnia and delirium, 7 patients (mean age 77 years) received ramelteon and 21 patients received brotizolam, zopiclone, or haloperidol. In the ramelteon group, all patients manifested beneficial effects within 7 days as seen in the Richmond Agitation-Sedation Scale (RASS). The RASS scale included a number of items like combative (+4), very agitated (+3), agitated (+2), restless (+1), alert and calm (0), and other items like drowsy (−1), light sedation (−2), moderate sedation (−3), deep sedation (−4), and unarousable (−5). Ramelteon was

superior in inducing sleep than any other drug that was administered and did not worsen agitation or oversedation of these patients. Moreover, ramelteon was found superior than melatonin in regulating sleep-wake cycle [70]. All patients treated with ramelteon displayed significant improvement within a week. None of the patients experienced oversedation, neurologic deterioration, or any other worsening effect associated with ramelteon, and the study showed ramelteon as effective for treatment of delirium in elderly patients with acute stroke [70]. The importance of using ramelteon as a therapeutic agent in preventing delirium was assessed in a multicenter, rater-blinded, randomized placebo-controlled trial involving 67 patients admitted in intensive care units and regular acute care wards. Of this, 33 were assigned to receive ramelteon, while 34 received placebo. Baseline characteristics of randomized patients were much the same in both groups. Ramelteon use was associated with lower risk of delirium (3 % vs. 32 %; $P = 0.003$, with a relative risk of 0.09) than those receiving placebo. As this finding exceeds the previous report with melatonin (12 % for melatonin vs. 31 % for placebo) [58], ramelteon (8 mg) is found superior in reducing the risk of delirium. The reduced frequency of the delirium seen in this study was suggested to be due to higher affinities of the drug to MT_1 and MT_2 melatonin receptors supporting the possible role of melatonin in the pathogenesis of delirium [8]. Besides its efficacy, ramelteon was found to be well tolerated by acutely ill patients of this study. More number of clinical trials of this nature will help to substantiate the efficacy of ramelteon in preventing the occurrence of delirium.

16.9 Melatonin and Melatonin Agonist Ramelteon in Autism Spectrum Disorders (ASD)

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders that are characterized by delayed or abnormal development and use of language, poor reciprocal social interactions, repetitive behaviors, and restricted interests [71]. Marked impairment in the use of multiple nonverbal behaviors such as eye to eye contact; facial expression; lack of spontaneous seeking to share enjoyment; preoccupation with one or more stereotyped, restricted patterns of interest; and repetitive motor mannerism are a few of the symptoms listed in the DSM IV-TR diagnostic criteria for autistic disorder [72]. Although the etiology of ASD is still not known, the core symptomatology has focused on the brain [73]. Neurological activation and presence of inflammatory cytokines in the brain of autistic patients have been demonstrated [74]. Results of a number of studies show propionic acid (PPA) induces behaviors characteristic of autism like repetitive dystonic behaviors, seizures, as well as social avoidance, and also other molecular features of ASD like neuroinflammation, reactive astrogliosis, activated microglia, increased protein and lipid oxidation, and reduction of glutathione (indicators of increased oxidative stress) [75, 76]. Genomic studies reveal that differently expressed genes in autistic versus normal nonautistic siblings are said to be responsible for the autistic phenotype. Gene expression study of lymphoblast cell lines (LCL) from 20 sib pairs (in which one sibling had a diagnosis of autism, and the other was not affected) reveal that alteration of genes involved in both metabolic and signaling pathways in ASD plays an important role in the pathogenesis of ASD involving inflammation, disturbances in axon guidance, and neuronal survival and differentiation [77]. Heterogeneity of behavioral, functional, physiological, and metabolic manifestations contributes to the clinical complexity of autism [77]. In another study, 15 genes that regulate circadian rhythms, with their multiple effects on neurological as well as metabolic functions are found to be differently expressed in patients with ASD. Dysregulation of circadian genes can have a systemic impact on affected individuals causing many of the symptoms that are associated with ASD. Hence, it was proposed that

interventions aimed at normalizing the circadian clock may ameliorate some of the symptoms associated with ASD [78]. Genetic analysis shows that genes ITGAM, NFKB1, RHOA, SLIT-2, and MBD 2 are affected in ASD phenotypes, and these genes are responsible for synaptic plasticity, learning, memory, inflammation, and cytokine production [77]. Reduced expression of the gene encoding arylalkylamine-N-acetyltransferase(AA-NAT, the rate-limiting enzyme for melatonin synthesis) has been noted in ASD individuals with severe language impairment [78]. An earlier genetic analytical study showed two polymorphisms in the promoter gene for acetylserotonin O-methyltransferase (ASMT), the last enzyme in the biosynthesis of melatonin in ASD [79]. The PAR1 of the sex chromosome located at the tip of the short arms has been found deleted in a number of subjects with ASD [79]. Study of 12 PAR1 genes showed that the gene ASMT is the one responsible for susceptibility to ASD, as it encodes the enzyme for acetylserotonin methyltransferase [80]. Variations in two melatonin pathway genes acetylserotonin-O-methyltransferase (ASMT) and CYP1A2 are recently reported in a clinically unique ASD subgroup consisting of children with comorbid expression of sleep-onset delay. In these subjects, higher frequencies for variants with substantial decrease of ASMT expression and related decrease in CYP1A2 enzyme activity ($P < 0.0007$) than reported earlier. From this study, the defective expression of melatonin pathway genes was responsible for sleep-onset delay seen in this subgroup of ASD patients [81]. Inconsistent with the identification of the defective gene contributing for the enzymatic deficit of the melatonin biosynthetic pathway, abnormal levels of melatonin have been detected in ASD patients. Abnormal melatonin levels in ASD patients have been reported in a number of studies [78, 82–85], but in the study undertaken by Dr Melke et al. 2008, abnormal melatonin levels were detected not only in children with autism but also in parents of autistic children suggesting a genetic origin of this disease [85]. Abnormal melatonin concentrations are suggested to have an impact on human behavior as this has been proved in Smith-Magnes syndrome who display inverted melatonin rhythm [86]. Moreover, they might impair the development of communication as well as socialization, the two domains of autistic disorder.

16.10 Sleep Problems in Autistic Spectrum Disorders (ASD)

Autistic children suffer from several sleep problems like irregular sleep-wake pattern, early morning awakenings, and poor quality of sleep [87, 88]. ASD children often display a preference to unusual bedtime routines which may be detrimental for adapting themselves to have a good time [89]. Irregularities in circadian sleep-wake cycle have been found in individuals with ASD [90–92]. Melatonin treatment has been effective in suppressing the free-running pattern of sleep-wake pattern [93]. Sleep problems in ASD children are more severe in nature and occur at a significantly higher rate than other children of normal type [94]. In a comparative study of the evaluation of sleep quality by using sleep questionnaire in 50 children with Asperger's disorder (a subgroup of ASD) and 43 controls, it was found that children of ASD had longer sleep latency and a shorter sleep duration [95]. Treatment of sleep disorders in autistic children may not only improve sleep but their daytime behavior and autism symptoms as well [95]. As difficulty in initiating and maintaining sleep is the major symptom in children with autistic disorder, therapy should be directed toward treating the sleep problems of ASD [96].

16.11 Melatonin and Melatonin Agonist Ramelteon Therapy for Treating ASD

A number of clinical trials have been conducted using melatonin for treating children with ASD. Reductions in sleep latency, improvement

in TST, and sleep efficiency have all been noted with supplementation of melatonin in children with neurodevelopmental disorders [97–99]. In a large sample of children of over 100 with ASD, melatonin treatment improved sleep in 85% of the subjects without producing any adverse effects [100]. The use of melatonin in an open-label study on children with ASD (3 mg of immediate-release melatonin) 30 min before bedtime for 2 weeks significantly decreased sleep latency [101]. In another large sample study involving 128 children (4–10 year) with ASD suffering from sleep-onset insomnia and impaired maintenance, patients were randomized to melatonin group (3-mg controlled-release Mel group), cognitive behavioral therapy (CBT) group, and combined therapy (number of subjects in each group; Mel group, 34; CBT group, 33; combined therapy, 35; placebo group, 32). Of these groups, melatonin therapy alone was found more effective than CBT in improving sleep-onset delay, night waking, and sleep durations [102]. In an open-label dose-escalation study of 24 children with ASD conducted over a period of 14 weeks, melatonin improved symptoms after 1 week of supplementation, and the beneficial effects were maintained for several months. It alleviated both sleep and behavioral problems and was well tolerated by subjects [103]. A therapeutic trial studying the melatonin-dose effect in 32 male children with autistic disorder is on progress, and the main objective is to prove the efficacy of melatonin effect on the severity of behavioral autistic impairments [88].

As ramelteon the melatonin agonist has greater affinity toward MT_1 and MT_2 receptors and proved to be more efficient in treating insomnias, the drug was tried in treating ASD. In the first study on ramelteon's use in youth with autistic disorder (aged 7 years and 18 years), ramelteon was given in 4–8 mg/day over the duration of 16–18 weeks. The target symptoms of delayed sleep onset and frequent nocturnal awakening improved significantly with ramelteon, as determined by Clinical Global Impressions-Improvement (CGI-I) scale. Ramelteon was also well tolerated [104]. In the recent study on three cases of autistic disorder, the effectiveness of ramelteon was demonstrated in 2 mg/day dosage, and in one case, ramelteon had been tried with higher dose (8 mg/day). In the first case, patient (9-year-old boy) exhibited interfering behaviors consisting of hyperactivity, restless activities, and also insomnia. The patient did not have regular sleep hours or regular sleep-wake rhythm. Ramelteon was started orally (2 mg/day) at 9:00 pm. Four weeks after starting ramelteon, the patient began to sleep before 11 pm and was able to maintain sleep throughout the night. His behavioral symptoms were also reduced. The frequency of panic attacks had been reduced two to three times weekly. In another patient diagnosed with autistic disorder (11-year-old boy) who was found to be hyperactive and impulsive and with irregular sleep-wake rhythm, administration of 2 mg/day ramelteon phase advanced the sleep-wake rhythm and reduced his hyperactivity. This beneficial action of ramelteon was maintained for 50 weeks. No adverse effects were reported during treatment with ramelteon. In the third case (6-year-old girl), ramelteon was administered orally in 4 mg/day and then it was raised to 8 mg/day. One week later, the patients were able to sleep from 10:00 pm to 6:00 am and overeating at midnight disappeared. Ramelteon improved patient's overnight sleep and reduced bedtime resistance. In this study, the drug alleviated both the sleep-disturbance and autistic behavior [7]. More number of clinical trials should be undertaken to prove the efficacy of ramelteon in alleviating the sleep and behavioral problems of ASD patients. New therapeutic perspectives should focus on restoring the basic physiological rhythms such as circadian rhythms, and in this aspect, melatonin and especially ramelteon will be more useful than any other drug currently employed for treating ASD. Ramelteon's use in insomnias and neurobehavioral conditions is shown in Fig. 16.3.

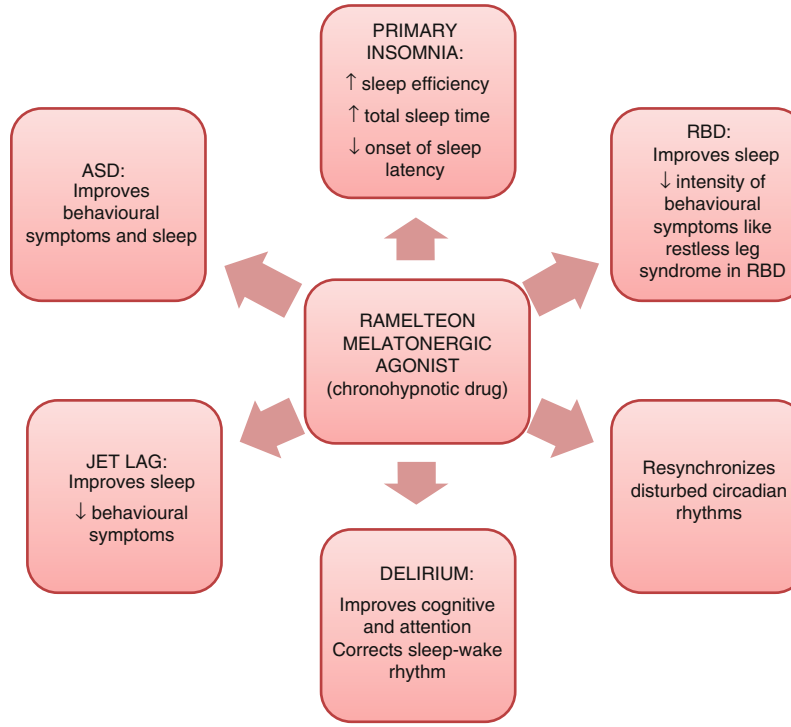

Fig. 16.3 Ramelteon's beneficial effects on sleep and behavioral symptoms in insomnia, REM sleep disorder, and neurobehavioral and neurodevelopmental disorders

Conclusion

With melatonin deficiency identified as the one of the major causes, melatonin replacement therapy has been tried to treat primary insomnias and insomnias associated with neurodegenerative diseases and neurodevelopmental disorders seen in children. Meta-analytical studies undertaken on the therapeutic efficacy of melatonin showed mixed results. As melatonin is a short-lived molecule, melatonergic agonist ramelteon with a high affinity on MT_1 and MT_2 melatonin receptors (FDA-approved drug) has been tried in treating primary insomnia and insomnias associated with neurological and psychiatric disorders. Ramelteon's efficacy has been proved in a number of clinical trials conducted from 2005 till today. Moreover, administration of this even for a longer time (more than 2 years) did not result in any adverse effects. When used as a therapeutic agent in RBD, ramelteon not only ameliorated sleep problems but also reduced the severity of RBD neurobehavioral symptoms. More clinical studies are required for establishing the superiority of ramelteon over other conventional drugs in treating RBD. As ramelteon has chronobiotic properties, the drug has been tried in treating symptoms of jet lag, and it improved both daytime alertness and nighttime sleep after traveling across five time zones. As melatonin is implicated in the pathogenesis of delirium, melatonin has been tried in a number of clinical studies involving patients with delirium and was found to be effective in reducing the development of delirium in patients with stroke or admitted in the intensity care units. Ramelteon caused marked improvement not only on the sleep problems associated with delirium but also attention and cognitive functions of patients; thereby, it can be used for management of neurobehavioral symptoms seen in delirium. Melatonin has been implicated in the pathogenesis of ASD, and abnormal

melatonin levels have been documented in children with autistic disorder. As abnormal melatonin levels are suggested to be one of the causes for the impairment of communication and socialization (the cardinal symptoms of ASD), melatonin has been tried in treating children with autistic disorder. Melatonin therapy improved sleep efficiency and behavioral problems. As ramelteon has higher affinity for MT_1 and MT_2 melatonin receptors, the drug has been tried in treating children with autism and found effective, although ramelteon can be useful in treating patients suffering from RBD, delirium, and neurodevelopmental disorders like autism where the drug has been found effective in treating sleep disorders as well as behavioral manifestations associated with these disorders. However, more clinical studies are required to substantiate the efficacy of these drugs in these behavioral disorders where sleep disturbances also are predominant.

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17 Melatonergic Antidepressant Agomelatine and Its Efficacy in Depressive Disorders

Venkataramanujam Srinivasan†, Domenico de Berardis, Michele Fornaro, Francisco Lopez-Muñoz, Rahimah Zakaria, Mohd Jamil Yaacob, and Zahiruddin Othman

17.1 Introduction

The World Health Organization (WHO) report indicates that depression affects nearly 121 million people across the world [1]. Depression is listed among the top 10 causes of morbidity and mortality [1] and ranked second only to cardiac ischemia [2]. The average age of onset of major depressive disorder (MDD) was found to be around 25 years [3]. Because of this reason, depression has become the most burdensome of all diseases, and in the European Union, the economic cost of MDD was estimated to be 92 billion euros in 2010 and has been predicted to be on the rise [4]. Hence, there is an urgent need for focusing on novel pharmacotherapies that can give rapid remission of depressive symptoms. Pharmacotherapies for treatment of depressive disorders have been employed since early 1950, and indeed antidepressants

†Author was deceased at the time of publication.

V. Srinivasan, PhD, MAMS
Sri Sathya Sai Medical Educational and Research Foundation, International Medical Sciences Research Study Center "Prasanthi Nilayam", 40-Kovai Thirunagar, Goldwins, Kovai Coimbatore 641014, Tamilnadu, India

D. de Berardis
NHS, Department of Mental Health, Psychiatry Service of Diagnosis and Treatment, Hospital "G. Mazzini", ASL-4, Teramo, Italy

Department of Neurosciences and Imaging and Clinical Sciences, University "G. D'Annunzio", Chieti, Italy

M. Fornaro
Polyedra Clinical Group, Teramo, Italy

Department of Formative Sciences, University of Catania, Catania, Italy

F. Lopez-Muñoz (✉)
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal
e-mail: flopez@ucjc.edu; francisco.lopez.munoz@gmail.com

R. Zakaria
Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian 16150, Kelantan, Malaysia

M.J. Yaacob
Department of Psychiatry, Faculty of Medicine, Asian Institute of Medicine, Science and Technology, Bedong 08100, Kedah Darul Aman, Malaysia

Z. Othman
Department of Psychiatry, School of Medical Sciences Universiti Sains Malaysia, Kubang Kerian 16150, Kelantan, Malaysia

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constitute the third most widely prescribed therapeutic agents worldwide with selective serotonin reuptake inhibitors (SSRIs) accounting for 80 % of the total market share [5]. Other antidepressants that are being used include tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and serotonin-norepinephrine reuptake inhibitors (SNRIs). Although the introduction of SNRIs and SSRIs has changed the strategies of clinical improvements in the treatment of MDD, they also pose a number of adverse side effects which often threaten the life of the patient himself. Moreover, depression is not a single disease but a heterogeneous syndrome characterized by a collection of physiological, neuroendocrine, behavioral, and psychological symptoms [6]. Among many signs and symptoms manifested by patients with depressive disorders, sleep and circadian rhythm disturbances constitute the major symptoms that need immediate attention for clinical remission. All major symptoms seen in depressive patients like decreased energy and alertness and fatigue seen during daytime, early morning wakefulness, delayed sleep onset, and decreased sleep efficiency all attributed to malfunctioning of the circadian clocks and the circadian timekeeping system [7]. Most of the antidepressants that are in clinical use today aggravate sleep disturbances. Hence, the therapeutic focus of the antidepressant treatment should not only to cause clinical remission of depressive signs and symptoms but to improve the sleep and circadian rhythm functioning of the depressive patient [8]. Disturbances of circadian rhythms seen in mood disorders are linked invariably to the malfunctioning of the suprachiasmatic nucleus (SCN)-pineal melatonin link. As a rhythm-regulating factor and a hormone involved regulation of sleep-wake rhythm, pineal hormone melatonin has an important role in the pathogenesis of mood disorders [9]. Patients with depressive disorders manifest abnormalities in the timing of REM/non-REM (NREM) cycle, and it has been suggested to be due to disruptions in sleep-wake regulatory mechanisms [10]. Depressive patients experience difficulty in falling asleep, early morning awakenings, decreased sleep efficiency and total sleep time, and increased sleep onset latency [11]. Classical antidepressants can influence the sleep of depressed patients. However, the effects are diverse and vary among compounds. Some of them which are in clinical use improve sleep of depressed patients in about 3–4 weeks of time, but their greatest effects are on REM sleep with less consistent effects on NREM sleep. However, the majority of available drugs generally produce unwanted negative effects on sleep [11]. An ideal antidepressant should be able to act much earlier (earlier onset of action) and should be able to mitigate the symptoms of depression with improvement of sleep quality and efficiency [12]. A new novel antidepressant agomelatine (Valdoxan) with a chemical structure N-[2-(7-methoxynaphth-1-yl)ethyl]acetamide known as compound S-20098 was developed by Servier and Novartis, USA. In February 2009, Valdoxan was approved by the EU-EMEA for treatment of MDD patients. Agomelatine was found to be unique as it acts synergistically on MT_1 and MT_2 melatonergic receptors and also on 5-HT_{2c} receptors which play an important role in resetting the desynchronized circadian rhythms, disturbed sleep-wake cycles, and poor sleep quality that are considered as the primary symptoms of depressive patients [13] (Figs. 17.1 and 17.2).

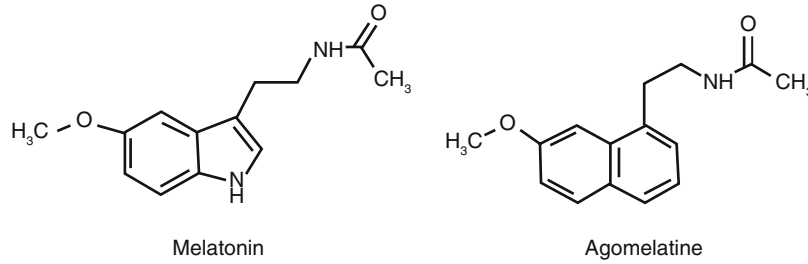

Fig. 17.1 Structure of agomelatine and melatonin

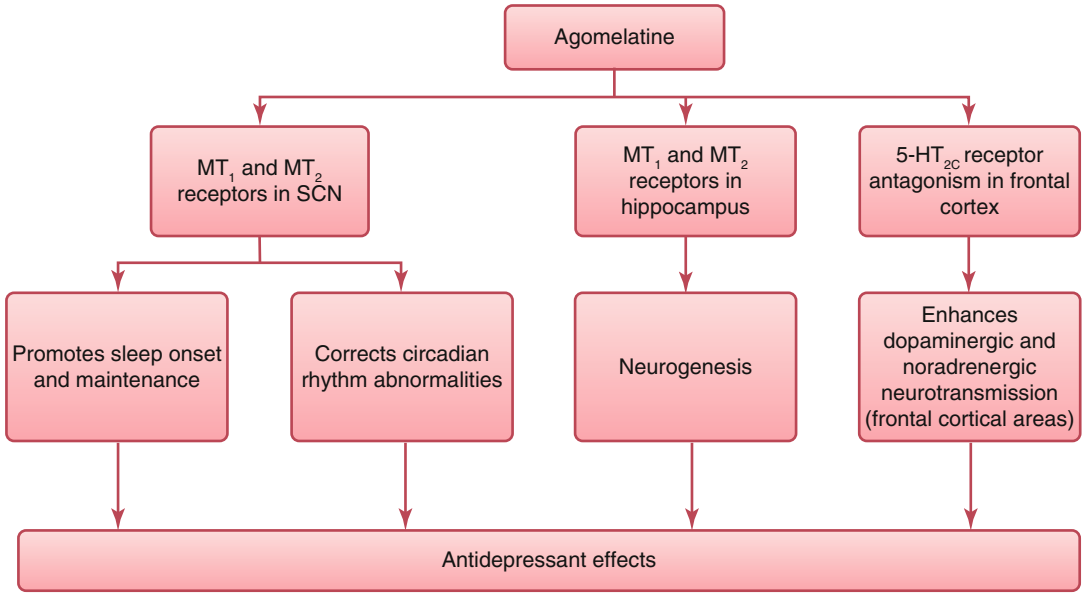

Fig. 17.2 Mechanism of antidepressant actions of agomelatine

17.2 Agomelatine

Agomelatine's overall selectivity for MT_1 and MT_2 melatonin receptors is over >100-fold, and its half-life in human is much longer than that of melatonin (T_{max} ranging from 0.5 to 4 h) and is metabolized mainly in the liver by three CYP isoenzymes, CYPA 1, CYPA2, and CYP2C9 [14]. As agomelatine is absorbed rapidly (80 %), its bioavailability becomes low (<5 % at the therapeutic oral dose) due to its high first-pass metabolism [15]. At therapeutic doses, agomelatine blood concentration increases proportionately with dose. About 90 % of agomelatine is metabolized by CYP1A2 and about 10 % by CYP2C9 [16]. Metabolites are conjugated with glucuronic acid and then sulfonated. Nearly 80 % of the drug is eliminated through urinary excretion of the metabolites (61–81 % of dose in human), whereas a small amount of the metabolites undergoes fecal excretion [17].

17.3 Efficacy of Agomelatine in Clinical Studies

The availability of antidepressants with efficacy in severely depressed patients is very important from clinical point of view, because this group is relatively resistant to current antidepressant therapy that relies heavily on SNRIs and SSRIs.

Following the successful testing of agomelatine in various animal models of depression, the drug was introduced in human clinical studies in a number of centers in Europe. The first such study was undertaken by Dr Loo and his associates in 2002. The study was designed in such a way that it involved multinational, multicenter, double-blind, placebo-controlled investigations with 711 patients drawn from Belgium, the UK, and France. Among these 711 patients, 67.1 % met the Diagnostic and Statistical Manual of Mental disorders (DSM-IV) criteria for recurrent MDD with 33.5 % of patients displaying an episode of severe intensity. The mean baseline score on the 17-item Hamilton Rating Scale for Depression (HAMD) was 27.4. Patients received either agomelatine (25 mg/day) or paroxetine (20 mg/day) for the study period of 8 weeks [18]. On completion of treatment, remission analysis noted that both agomelatine and paroxetine brought out significant remission with agomelatine displaying 30.4 % and paroxetine 25.7 %. Responder analysis (defined as 50 % or more reduction of baseline HAMD score) showed agomelatine to be superior (61.5 %) to placebo (46.3 %), whereas paroxetine's score did not much differ from placebo

score (56.3 %) [18]. In severely depressed patients among these 711, with HAMD score of more than 25 (586 patients), agomelatine caused significant clinical effect ($P<0.05$) as compared to placebo, whereas paroxetine did not cause much difference from that of placebo [18]. Following this successful clinical study, a second multicenter, multinational study involving 21 centers across countries like Finland, Canada, and South Africa involving 212 patients was undertaken to evaluate the clinical efficacy of agomelatine in patients with MDD. In this double-blind placebo-controlled trial, agomelatine was given in 25–50 mg/day (the basal HAMD score was above 22) for a period of 6 weeks. In this group, a subgroup of 106 intent-to-treat patients improved significantly with agomelatine in around 6 weeks of time ($P=0.045$). The HAMD score was significantly reduced with agomelatine at 6 weeks of study [19]. In a study of little longer duration covering 12 weeks on 277 patients with MDD, patients were randomized to receive agomelatine 50 mg/day, or venlafaxine XR (extended release) received at two different doses (75 mg/day for the first 2 weeks and 150 mg/day for the rest of the duration). The rates of clinical remission were found to be 73 % for agomelatine- and 67 % for venlafaxine XR-treated patients [20]. This study was followed by another similar study in which 60 patients were randomly assigned to receive either agomelatine (25–50 mg/day) or venlafaxine (75–150 mg/day) in the immediate release form. Significant reduction of HAMD scores was reported in both agomelatine and venlafaxine groups with agomelatine causing reduction of HAMD score from 25.9 to 9.0 on clinical remission. Venlafaxine reduced the HAMD score from 26.0 to 8.9 on clinical remission [21]. Whereas the above studies evaluated only the clinical response of agomelatine or other antidepressant used, the effects of agomelatine and venlafaxine were assessed on both sleep quality, and antidepressant response was studied in a group of 332 patients with MDD for a period of 6 weeks. Agomelatine was administered at a dose of 25–50 mg/day, and venlafaxine was given at a dose of 75–150 mg/day. The antidepressant efficacy of both agomelatine and venlafaxine were found to be the same, but the improvement of sleep quality as assessed by Leeds Sleep Evaluation Questionnaire was found better with agomelatine than with venlafaxine [22]. The efficacy and tolerability of agomelatine in patients with MDD were tested in a 6-week double-blind parallel group study on 238 patients. For the first 2 weeks, agomelatine was given at a dose of 25 mg/day, and subsequently it was raised to 50 mg/day for patients who did not respond properly. Agomelatine was found to be significantly superior to placebo ($P<0.0001$) with a difference of 3.44. The response rate to agomelatine was 54.3 % and 35.5 % for placebo. The drug was well tolerated and safe [23]. In a short open-label study on 30 MDD patients, administration of agomelatine in flexible doses ranging from 25 to 50 mg/day for the duration of 8 weeks improved the clinical status in about 80 % of the patients. The improvement of anhedonia with agomelatine was noted for the first time in this study [24]. In a longer duration of study of 32 weeks, agomelatine's efficacy in improving the clinical status of MDD patients with prevention of relapses was evaluated in 165 patients. At the end of 8–10 weeks after administration of agomelatine, the patients were randomly assigned to receive either agomelatine 25–50 mg/day ($n=165$) or placebo ($n=174$) for the treatment period of 24 weeks. In this study, the incident of relapse was significantly low in patients who continued their treatment with agomelatine than those who switched over to placebo ($P=0.0001$). The cumulative relapse for agomelatine was 21.7 %, and for placebo it was 46.6 % [25]. In a recent study conducted on a large scale of patients with depression ($n=5781$), the efficacy of agomelatine was evaluated on completion of 8 weeks of treatment. All patients received agomelatine 25 mg/day and 4,993 (79.6 %) continued on this dose throughout therapy. The HAMD-17 total score decreased from 22.5 to 4.7 at 8 weeks of treatment ($P=0.0001$). The proportion of patients showing response to treatment (HAMD-17 total score decreased by 50 % from baseline) increased slightly between baseline and the second week of treatment and rapidly thereafter reaching 90.1 % by week 8. The proportion of patients showing remission from depression increased progressively throughout

the treatment period reaching 25.3 % at week 3 and 79.1 % at week 8. Evaluation of single HAMD-17 item score during treatment with agomelatine showed that every item score decreased significantly during treatment period ($P = 0.0001$) [26]. The efficacy of agomelatine was also high in the subgroup of more severely depressed patients ($n = 3{,}478$) with HAMD-17 baseline score being 21. The onset of antidepressant efficacy was also rapid in this subgroup. The most interesting finding of this study is that changes in HAMD score relative to baseline were evident at week 1 itself and also in each subsequent visit in all cases. The efficacy results from the present CHRONOS study testifies agomelatine as a very good novel antidepressant.

17.4 Agomelatine for Treatment of Bipolar Depression

As adjunctive treatment along with either lithium or valpromide, agomelatine was tried on bipolar-I patients in an open-label study. Agomelatine was administered for a minimum period 6 weeks followed by an optimal extension up to an additional 46 weeks. Using intent-to-treat data, it was found that 81 % of the patients met the criteria for marked improvement. Patients belonging to severe category of depression with HAMD score over 25.2 (47.6 % of the total number of patients) responded to treatment as early as in first week of treatment. In this study, 19 patients entered the optimal extension of 211 days (mean) and of these 11 patients completed 1 year extension of treatment. The efficacy of agomelatine was attested in this study [27]. In a recent study of evaluating the efficacy and safety of adjunctive agomelatine pharmacotherapy in the treatment of acute major depression in bipolar II disorder, 28 subjects with a mean HAMD score of 25.9 were enrolled. 17 patients taking valproate (60.7 %) and 11 (39.3 %) taking lithium as primary mood stabilizer were enrolled [28]. A fixed dose of agomelatine (25 mg/day) was administered during the entire period of study (six consecutive weeks as an adjunct to treatment with lithium or valproate followed by an optional treatment extension of 30 weeks). Using intent-to-treat analysis, 12 of the 17 valproate-treated (70.6 %) and 6 of 11 (54.5 %) lithium-treated subjects demonstrated clinical response (defined as >50 % decrease in severity from the baseline HAMD score) to agomelatine augmentation at the sixth week of primary study endpoint. Six valproate-treated and one lithium-treated patient (25 % of the total) responded as early as second week of treatment. At the 36-week end point, 14 of the 17 (82.4 %) valproate-treated and 10 of the 11 (90.9 %) lithium-treated subjects had a clinical response [28]. During both the short- and long-term periods of the trial, agomelatine treatment was associated with improvement in depression and sleep quality and was found to be well tolerated. From this study, it was concluded that adjunctive agomelatine treatment may be useful in the treatment of acute bipolar depression with rate of switch over to mania/hypomania much lower than the expected as reported in earlier studies with other antidepressants added to mood stabilizers in the treatment of bipolar disorders.

17.5 Agomelatine in the Treatment of Seasonal Affective Disorder/Winter Depression

In an open-label study on depressed patients suffering from seasonal affective disorder (SAD), the efficacy of agomelatine was evaluated (25 mg/day) for a period of 14 weeks using various psychometric scales including Structural Interview Guide for the Hamilton Depression Rating Scale (SAD version, SIGH-SAD), the Clinical Global Impression of Improvement (CGI-I). Agomelatine used in these patients caused a progressive and significant decrease in SIGH-SAD, CGI-S, and CGI-I scores from second week of treatment [29]. Circascreen, a self-rating scale for the assessment of sleep and circadian rhythm, was used to evaluate these parameters. Circascreen improved substantially after treatment with agomelatine. Treatment with agomelatine for 14 weeks yielded a response rate of 75.7 % (defined as the SIGH-

Table 17.1 Antidepressant effects of agomelatine: clinical studies

Dosage (mg/day)	Type of study	Duration of study	Number of patients	Type of patient	Antidepressant response	Reference
25, 50	Double blind, placebo controlled	8 weeks	711	MDD	More effective than placebo in severely depressed	[18]
25, 50	Placebo controlled	6 weeks	212	MDD	More effective in depressed and severely depressed	[19]
50	Double blind	12 weeks	137	MDD	Antidepressant efficacy was superior	[20]
25, 50	Placebo controlled	6 weeks	332	MDD	Effective antidepressant response and improved sleep quality	[22]
25	Double blind, placebo controlled	6 weeks	238	MDD	Both depressive and sleep symptoms improved	[23]
25–50	Open-label study	8 weeks	30	MDD	Significant response	[24]
25, 50	Placebo-controlled, double-blind study	8 weeks plus 24 weeks	339	MDD	Very effective antidepressant effect	[25]
25, 50	Observational CHRONOS study	8 weeks	6,276	MDD	Effective and well-tolerated antidepressant	[26]
25	Open-label study with lithium or valpromide	6 weeks	21 lithium ($n=14$); valpromide ($n=7$)	Depressed bipolar I	Improved depression	[27]
25	Open study	14 weeks	37	SAD	Remission was sustained	[29]

SAD score <50 % of the baseline value) and a remission rate (SIGH-SAD, 8) of 70.3 % in the intent-to-treat sample. The efficacy and tolerability of agomelatine were well demonstrated [29] (Table 17.1).

17.6 Agomelatine's Mechanism(s) of Antidepressant Actions

Agomelatine acts synergistically on MT_1 and MT_2 melatonergic receptors and with antagonism to 5-HT_{2c} receptors expressed in SCN, and its therapeutic efficacy in depressive disorders is attributed to this action [30]. 5-HT_{2c} receptors are concentrated in the frontal cortex, amygdala, hippocampus, and cortico-limbic structures, and all these structures are involved in the regulation of mood and cognition [31]. Antidepressants that are in clinical use today exert their therapeutic effects by antagonizing 5-HT_{2c} receptors [30]. Blockade of 5-HT_{2c} receptors will result in release of both norepinephrine (NE) and dopamine (DA) at the frontocortical dopaminergic and noradrenergic pathways [30, 32]. It is the dopaminergic and adrenergic mechanisms in the frontal cortex that are involved in the regulation of mood and cognitive functions. Agomelatine's therapeutic effects are attributed mainly to its actions in improving sleep quality at night and improving daytime alertness. Synergistic activation of both melatonergic receptors MT_1 and MT_2 and blockade of 5-HT_{2c} receptors are essential for this action [33]. It is testified in patients with MDD that agomelatine improves all aspects of the sleep-wake cycle starting from as early as 1 week of treatment [33, 34]. Agomelatine's superiority over other antidepressants is attributed to this fact. Antidepressants like SSRIs cause profound sleep disturbances and they aggravate insomnia. Hence, while treating patients with depressive disorders with SSRIs, sleep medications have also to be used concomitantly. Some antidepressants like MAOIs, TCAs, and SSRIs interact with 5-HT_{21A} receptors and exert their antidepressant effects. But interaction with these receptors also results in gastrointestinal problems,

sleep disturbances, and sexual dysfunctioning [35]. Agomelatine's affinity for 5-HT_{2A} receptors and h5-HT_1 receptors is very low. However, its bending with h5-HT_{2B} and h5-HT_{2c} is very similar [30]. Hence, its actions on melatonergic receptors present in SCN and 5-HT_{2c} receptors blockade are suggested as ideal actions for ameliorating the symptoms of depressive illness. Another possible mechanism through which agomelatine could exert its antidepressant effects has been inferred from studies carried out on animal models of depression. In animals as well as in human beings, the ventral hippocampal region is implicated in the regulation of mood and anxiety behavior, and the dorsal hippocampus is concerned with spatial memory [36, 37]. Effects of agomelatine or fluoxetine were tested on dendritic maturation of both dorsal and ventral hippocampal regions of corticosterone-treated mice (an animal model of depression). Although both drugs modified the maturation index, the number of doublecortin (DCX)-expressing cells with tertiary dendrites was increased with agomelatine (10–40 mg/kg/day) only in the ventral hippocampal region of corticosterone-treated rats [38]. Agomelatine induced an early acceleration of cell maturation at eighth day of development [39]. This is in contrast to the earlier report published in which antidepressant fluoxetine induced hippocampal acceleration at 21st day [40]. Because agomelatine caused dendritic maturation at the earliest interval of time in the animal model of depression, it is considered as more effective than fluoxetine. The ventral hippocampus is endowed with both 5-HT_{2c} as well as MT_1 and MT_2 melatonergic receptors; agomelatine's antidepressant effects through the ventral hippocampus are attributed to its actions on both these receptors [39]. As the ventral hippocampus projects to the prefrontal cortex and amygdala, agomelatine is suggested to target this region for exerting its antidepressant effects. Based upon its diversified actions on various parameters like (a) improving sleep efficiency, (b) resynchronization of disrupted circadian systems, and (c) enhancing hippocampal dendritic maturation, agomelatine stands as a unique choice in antidepressant therapy.

17.7 Safety and Tolerability of Agomelatine

In all the clinical studies that have been undertaken so far, agomelatine has exhibited a good tolerability and safety. The frequency of adverse effects reported (with both 25 and 50 mg/day) such as cardiovascular profile [41], headache, anxiety, abdominal pain, and diarrhea [18] is similar to that reported for placebo. The specific side effects such as increase in body weight or sexual dysfunction which are common with some other antidepressants are not seen with agomelatine [42, 43]. Impaired sexual function that often occurs with other antidepressants is a major cause of noncompliance [44]. In an analysis of sexual symptoms associated with antidepressants intake (either agomelatine or venlafaxine), it was shown that 7.3 % of agomelatine-treated patients and 15.7 % of venlafaxine-treated patients reported deterioration of sexual function [20].

With reference to discontinuation symptoms, only fewer patients discontinued treatment with agomelatine versus fluoxetine (11.9 % vs. 18.6 %), agomelatine versus venlafaxine (2.2 % vs. 8.6 %), and agomelatine versus sertraline (13.6 % vs. 18.9 %) [20, 45, 46]. A double-blind placebo-controlled study on 192 patients who were randomized to receive either agomelatine 25 mg/day or paroxetine 20 mg/day for 12 weeks followed by an abrupt discontinuation of treatment for 2 weeks during which they were randomized to placebo or their initial antidepressant. No discontinuation symptoms were noted in patients who discontinued agomelatine compared to paroxetine [47].

With regard to safety parameters, mild elevations in serum aminotransferases were reported in 1.1 % of the patients treated with agomelatine, and these increases were isolated and reversible and occurred without any clinical signs of liver damage [42]. In other recent studies, aminotransferase elevations were noted in 2.4 % of the patients in one study and 4.5 % of the patients treated with agomelatine 50 mg/day [45]. These elevations in aminotransferases seen with 50 mg/day of agomelatine are not

accompanied by any clinical signs of liver damage but only reflected the higher prevalence of hepatobiliary disorders at baseline, according to medical history, in the agomelatine 50 mg/day group than in agomelatine 25 mg or placebo group (both at 0.6 %).

Conclusion

Agomelatine is a novel melatonergic antidepressant which acts on both MT_1 and MT_2 melatonergic receptors of SCN and antagonize $5\text{-}HT_{2c}$ receptors in the frontal cortex, amygdala, hippocampus, and cortico-limbic structures. Its regulation of mood and cognition has been shown to be beneficial in treatment of MDD, SAD, and bipolar depression. Its 5-HT2c antagonism also enhances fronto-cortical dopaminergic and noradrenergic transmission which is essential for modulation of mood and antidepressant effect [32, 48]. In addition to its therapeutic efficacy, agomelatine does not manifest any of the adverse effects like sexual dysfunction, sleep disturbances, and discontinuation effects commonly seen with the use of other antidepressants. Its clinical efficacy in MDD combined with its early onset of action and its good tolerability and safety has been supported by numerous studies [41, 42, 49–55].

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Agomelatine, Melatonin and Depression

18

Trevor R. Norman

18.1 Introduction

Recent developments in the treatment of major depressive disorder (MDD) have revived interest in an old aetiological hypothesis, namely, that depression might arise as a dysfunction of circadian rhythms. While physiological evidence for abnormalities of rhythm in major depression is modest, the symptomatology of the disorder suggests at least some relationship to circadian abnormalities. MDD has been associated with a wide range of alterations in sleep, while circadian abnormalities have been documented in the disorder (see Table 18.1). So-called 'melancholic' forms of the disorder are usually associated with early morning wakening and marked diurnal mood variation [48]. A characteristically phase advanced pattern of circadian rhythm in melancholia is noted, for example, in daily variations of motor activity. Decreased appetite and weight loss characteristically occur in these severe forms of depression. 'Atypical' depressions are more likely to be associated with later sleep onset and offset, prolonged sleep times, daytime tiredness and fatigue. Diminished motor activity in the mornings and overeating, weight gain and increased risk of metabolic dysfunction may occur in this depressive subtype. Alterations in the patterns of symptoms and behaviour displayed by such patients suggest an underlying phase delay of the circadian system [48].

Evidence for circadian disturbances of physiological functions is often associated with MDD. Normal circadian patterns of the release of ACTH (and consequential cortisol release) and thyroid-stimulating hormone (TSH), which regulates thyroid hormone release, may be disturbed. Altered patterns of cortisol release are consistent with a general phase advance of circadian rhythm or at least a loss of synchrony with other daily rhythms. The changes in the patterns of cortisol release are correlated with daytime fatigue and feelings of lack of energy. Similarly, a pattern of phase advance is suggested by changes in the amplitude and timing of the TSH release pattern [105].

It is not the purpose here to review the circadian hypothesis of MDD, which has been explored in detail in various previous publications [29, 38, 40, 47, 78, 115, 126]. The focus of this review is to explore the extent to which agents affecting the circadian system might be clinically useful as antidepressants. In this context, a number of compounds which act by affecting the melatonin system, and indeed melatonin itself, are considered. The focus on melatonin and melatonin agonists is justified since it is well recognised that melatonin is intimately associated with circadian rhythms. Further, abnormalities of

T.R. Norman
Department of Psychiatry, University of Melbourne, Austin Hospital, Heidelberg 3084, Victoria, Australia
e-mail: trevorrn@unimelb.edu.au

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Table 18.1 Findings suggestive of a circadian disturbance in major depression

Subjective sleep-wake complaints[a]	Difficulty falling asleep, staying asleep or early morning awakening
	Disturbing dreams
	Unrefreshing shallow sleep
	Daytime fatigue and sleepiness
Sleep architecture	Abnormal sleep duration
	Prolonged sleep-onset latency
	Shortened REM latency and increased rapid eye movements
	Increased sleep fragmentation
	Decreased SWS and increased REM sleep and (especially in the first sleep cycle)
	Reduced slow wave activity and number of slow waves
	High co-morbidity with sleep-related breathing disorders
Biological rhythms	Abnormal sleep phase
	Reduced melatonin secretion[b]
	Increased 24-h levels and variability of cortisol secretion
	Reduced circadian amplitude and increased night-time body temperature
	Reduced heart rate circadian amplitude
	Depressive symptoms associated with increased nocturnal blood pressure in males
	Abnormal cytokines, neurotransmitters and endocrine circadian rhythms
	Abnormal circadian mood variations
	Possible seasonal variations

[a]May occur before the onset or recurrence of episodes
[b]Not found in all studies. See Table 18.2 and text for further discussion

melatonin secretory patterns can result in, or be a consequence of, disturbances of the 24-h cycle.

The nocturnal peak of pineal melatonin forms the basis for the major chronobiological effects of the hormone [42]. Melatonin is the signal for 'darkness' and participates in resetting mechanisms which synchronise the hypothalamic circadian pacemaker, the suprachiasmatic nucleus (SCN), with the external 24-h rhythm [94]. The SCN receives its information about the photo phase via the retinohypothalamic tract mainly from light-sensitive, melanopsin-containing retinal ganglion cells [33]. Pineal melatonin biosynthesis largely depends on the interaction of noradrenaline with β-receptors located on pinealocytes, although other receptor subtypes have been shown to alter melatonin production [94]. Synchrony of sleep and wake rhythms as well as core body temperature is dependent on the circadian rhythm of melatonin [25, 59]. The effects of melatonin are mediated by the presence of specific receptors for the hormone located in the SCN [37]. Melatonin can affect both the phase and the amplitude of the circadian oscillation by its action at the melatonin G-protein-coupled membrane receptors [87]. Preferential effects on phase shifting are exerted by MT2 receptors [28]. Interaction with the MT1 receptor affects neuronal firing rates [51]. Melatonin has been demonstrated to improve sleep in some studies, a controlled release formulation being approved for sleep disturbance on the basis of efficacy demonstrated in patients aged 55 years or more [62, 117–119]. Melatonin administration has been demonstrated to be capable of phase resetting which may be related to its sleep-regulating mechanism [2]. Thus, melatonin has been successfully used for treatment of sleep problems related to circadian rhythm alterations such as shift work, jet lag or delayed sleep phase syndrome (DSPS) [3, 64].

18.2 Melatonin and Depression

As circadian abnormalities have been suggested as of aetiological significance in MDD, the natural progression would seem to be the measurement of the principal circadian pacemaker melatonin concentrations in patients with the disorder. Further, as the monoamine hypothesis suggests decreased noradrenergic function in depression, a neurotransmitter intimately involved in the synthesis of melatonin, it might be anticipated that melatonin concentrations would be diminished in MDD. A summary of studies examining melatonin secretion in MDD

Table 18.2 Melatonin secretion in major depression

Diagnostic system	N of subjects; M/F	Melatonin measurement	Results	Authors
RDC	1M, 5F; depression; 4M, 2F HC	Urinary MT; bioassay	No difference in patients and controls	Jimerson et al. [50]
Clinical	1F depressed and recovered	Serum MT (RIA)	Lower MT in depressed state	Wetterberg et al. [123]
Clinical	4M, 2F MDD; 10M HC	Plasma MT (RIA)	Reduced MT in MDD	Nair et al. [77]
DSM-III	10F, 1M MDD; 8M HC	Plasma MT	Blunted MT secretion in MDD	Claustrat et al. [19]
DSM-III	7M MDD; 5M HC 19F melancholia; 9F without melancholia; 7M HC	Serum MT; RIA	Lower MT in melancholic patients compared to HC or non-melancholic patients	Brown et al. [13]
DSM-III	7M, MDD; 5M, HC 5M, 14F melancholia; 9F non-melancholia; 7M HC	Serum MT; RIA	Lower MT in melancholic patients; no difference in non-melancholic patients	Frazer et al. [35]
DSM-III	4M, 7F melancholia 4M, 14F HC	Plasma MT; RIA	Lower MT in depressed patients	McIntyre et al. [70]
RDC	4M, 7F; depression; 4M, 7F HC	Serum MT (RIA)	Higher MT profile in depressed patients	Thompson et al. [111]
DSM-III	38 MDD; 38HC (15M, 23F)	Serum MT	Elevated MT in premenopausal women only; no difference for males and postmenopausal women	Rubin et al. [97]
DSM-III	7M, 30F MDD; 3M, 11F HC	Serum MT (RIA)	Higher mesor MT in MDD	Rao et al. [92]
Interview	9M, 13F depression; 11M, 8F HC child-adolescent cohorts	Serum MT (RIA)	Higher MT profile in depressed patients	Shafii et al. [100]
DSM-III-R	6M, 3F melancholia; 6M, 3F HC	Serum MT (RIA)	No difference from controls	Volderholzer et al. [116]
DSM-IV	15M, 35F MDD;	Plasma MT, single sample	Blunted MT secretion in melancholia, cf atypical depression	Fountoulakis et al. [34]
DSM-IV	15M, 5F MDD; 8M, 6F HC	Serum MT	Higher MT in MDD	Szymanska et al. [108]
DSM-IV	12F; 199HC; postmenopausal women	Urinary aMT6S 24-h (RIA)	No differences between patients and controls	Tuunainen et al. [114]
DSM-IV	14MDD; 14HC (5M, 9F)	aMT6S 24-h urine; serum MT	No difference from controls; evidence of phase shift	Crasson et al. [20]
DSM-IV	32 MDD; 32 HC (23F, 9M) 15 MDD, 15 HC (10F, 5M)	aMT6S 24-h urine	No difference from controls	Carvalho et al. [17]
DSM-IV-TR	19F, MDD; 12F HC	Plasma MT (RIA)	Reduced MT in MDD	Meliska et al. [71]

is presented in Table 18.2. In essence, should a circadian hypothesis be supported, then a phase shift in the melatonin rhythm might have been expected. The majority of studies were not designed to examine phase changes specifically and certainly not under conditions of constant

routine where such changes, should they exist, might be expected to be unmasked. For the most part, studies have focused on total secretion of melatonin either by examining urinary output of the melatonin metabolite, 6-sulphatoxymelatonin (aMT6s), by measuring the area under the nocturnal melatonin plasma/serum concentration time curve or by comparing plasma or serum melatonin concentrations at a single time point during the night.

The first study to examine a potential role of melatonin in depression measured urinary output of the hormone in six patients with depression and six healthy controls [50]. The study failed to find any statistically significant differences between the two sets of subjects. A bioassay was used (the dermal melanophore response of larval anurans to melatonin in their bathing medium) to quantitate melatonin, a qualitative method which was claimed to provide equivalent results to a radioimmunoassay (RIA). Melatonin metabolites in urine do not appear to have been measured separately, if at all. Unchanged melatonin concentration in urine is relatively low, and without available sensitivity data for the assay, it is possible that small differences in output may have been obscured. Nevertheless, further studies utilising a specific RIA for the melatonin metabolite also showed no difference in the 24-h urinary excretion pattern between depressed patients and controls [17, 20, 114]. Urinary measurements potentially obscure phase changes so that while total secretion remains constant, alterations in the time to onset and offset of melatonin may be shifted. Indeed, one study suggested a phase delay of melatonin in MDD patients compared to age- and gender-matched controls [20]. This suggestion was based on the data from serial 8-h urine collections and the measurement of urinary aMT6s. Measurement of urinary sulphatoxymelatonin concentration in day- and night-time aliquots was conducted in eight endogenous depressed patients who were drug-free for 2 days [11]. There was no control group. Three of the patients had no increase in the metabolite at night.

Findings based on the measurement of serum or plasma melatonin concentrations also present an array of contradictory findings. Thus, increases in nocturnal melatonin plasma concentrations observed in healthy controls were absent in three of four depressed patients similarly examined [72]. The results supported the notion of altered biological rhythms in depressed patients, an abnormality that was not reversed by treatment, despite clinical improvement. Lowered nocturnal melatonin secretion (based on four time points from midnight to 6 AM) was also reported in a severely depressed woman compared to her euthymic state [123]. In this case, recovery seemed to have been associated with a restoration of the melatonin rhythm. These early reports and many of the subsequent reports are based on small numbers. Diminished nocturnal secretion of melatonin in MDD compared to healthy controls has been observed by several independent studies albeit utilising somewhat different methodologies [13, 19, 32, 34, 70, 71, 77]. For example, serial melatonin concentrations were compared in seven drug-free DSM-III male melancholic patients and seven healthy controls matched for sex and weight but not age, height or season of testing [13]. Plasma was sampled every one and a half hours. The melancholic group had lower melatonin concentrations than the controls. In another study, 19 melancholic and 9 non-melancholic patients were compared with 7 unmatched healthy controls. Melancholic patients had lower melatonin levels than either non-melancholic depressed patients or normal subjects [13].

The consistency of these findings led to a proposal that melatonin secretion may be considered a trait marker of depression. An investigation of pineal-pituitary-adrenal relationships in ten patients with major depressive illness showed that patients unresponsive to a dexamethasone test (failure to suppress plasma cortisol) also had the lowest plasma melatonin concentrations [8]. Further, the ratio of serum cortisol to melatonin at 2 AM was significantly higher in the group of patients having abnormal plasma cortisol suppression compared to controls. The temporal organisation of plasma melatonin and cortisol secretion was examined in 8 male healthy controls and in 11 depressed patients (10 female, 1 male) over a 24-h period.

Blood samples were collected at two-hourly intervals during the day and one-hourly at night. Controls showed low or undetectable (<5 pg/ml) diurnal plasma melatonin levels and a marked nocturnal rhythm. Depressed patients also showed a significant melatonin rhythm but with lower amplitude and mesor.[1] The rhythm was not significantly phase advanced with respect to the controls. In 9 of the 11 patients, nocturnal melatonin secretion was lower and associated with hypercortisolemia [19]. These sets of data indicate an impairment of pineal function coupled with a pituitary-adrenal disinhibition in patients with major depression. Thus, a proposed low melatonin depression was based on a further study in 32 acutely depressed in-patients and 33 healthy controls which measured maximum melatonin concentrations in relation to findings on the dexamethasone suppression test [6]. A low melatonin syndrome in depression was characterised by an abnormal dexamethasone suppression test and a disturbed 24-h rhythm of cortisol in addition to low maximum melatonin concentrations.

In contrast to the 'low melatonin' depression findings, a second group of studies have reported elevated nocturnal melatonin secretion in MDD patients compared to healthy controls [92, 97, 100, 108, 111]. The 24-h melatonin serum profile was compared in 38 patients (23 females and 15 males) with depression and a matching set of healthy controls. There was a trend for average nocturnal melatonin concentrations to be significantly elevated compared to controls [97]. This trend was accounted for by the 14 premenopausal women, whereas postmenopausal women and male depressive patients did not differ significantly from their respective controls. Average diurnal melatonin concentrations also tended to be higher in female and male depressed patients compared to controls. None of the melatonin measures were consistently related to any HPA-axis measures in these subjects. A similar trend was noted in nine patients with MDD compared to nine matched control subjects [111]. Following the addition of data from two bipolar depressed patients to the cohort, nocturnal melatonin secretion was significantly greater in the depressed phase compared to the euthymic phase. Higher nocturnal melatonin plasma concentrations were noted in 22 depressed patients compared to 14 healthy controls between midnight and 4 AM [108]. There was no evidence for a phase shift of the melatonin rhythm in this study. Nocturnal melatonin secretion between 6 PM and 7 AM was significantly greater than in a group of 22 depressed children and adolescents (8–17 years) than in a healthy control group [100]. In this study, the data were not analysed based on pubertal status, as nocturnal melatonin concentrations are known to be lower after the onset of puberty [106, 120].

In a study of nine patients with MDD and 9 age- and gender-matched healthy controls, no differences in serum melatonin concentrations were reported between patients and controls [116]. There was some evidence for a phase advance of the melatonin rhythm, but the small sample size precluded any firm conclusions. Nocturnal cortisol secretion was elevated in the patient group but was not statically significantly associated with melatonin secretion.

Multiple explanations have been advanced to account for these three sets of discrepant findings, mostly based on methodological differences. Some earlier melatonin studies utilised patients who were not drug-free [7, 124] or the washout periods from medication were relatively short [13, 19]. Low melatonin levels could be attributable to persistent receptor downregulation after antidepressant washout. Equally, high melatonin concentrations could be due to the effects of antidepressants. Nocturnal melatonin secretion can be altered through interference with melatonin metabolism (e.g. [44]) or by direct receptor-mediated effects (e.g. [84, 104]). Furthermore, many studies do not state if patients were receiving hypnotic agents. It is now well recognised that benzodiazepine and other hypnotic agents can suppress nocturnal melatonin secretion [69]. Discrepant findings might be due to differences in ages between patient and control

[1] Mesor is defined as the average of the rhythmic variable (melatonin concentration in this case) over a single cycle determined as the mean of a fitted cosine curve.

groups. In some studies, a control group was absent or not matched for age [13, 19, 72, 124]. Lower melatonin concentrations in depressed patients might be partly explained by the age-dependent decrease of melatonin secretion [77].

In summary, it would appear that nocturnal melatonin plasma concentrations do not appear to be a reliable biomarker of the disorder. The notion of a 'low melatonin syndrome' [122, 125] in at least some cases of MDD does not appear to be supported by studies which have adequately controlled for the factors that can affect nocturnal melatonin secretion. However, more recent studies have explored the relationship between the phase angle of cortisol and dim light melatonin onset (DLMO) as a potential biomarker [14]. This small study (six patients and six controls) argues for a high sensitivity and specificity of the phase angle between the cortisol acrophase[2] and DLMO in discriminating between MDD and controls. Intriguingly, mean nocturnal melatonin concentrations between 1600h and 1000h were decreased in patients compared to controls in this study. Phase angle differences have also been shown to correlate with the severity of depression [30, 45], while abnormal phase angles between sleep, melatonin and temperature have been observed in young adults with unipolar and bipolar disorders [96]. These small studies suggest that further exploration of the DLMO in patients with mood disorders might be useful for discriminating MDD subtypes. Their relationship to pharmacotherapy response has yet to be evaluated.

18.3 Melatonin as an Antidepressant?

While melatonin has been proposed as a marker of MDD or of circadian phase changes in depression, evidence for the antidepressant activity of the molecule itself remains controversial. There is a current vogue for the use of antioxidant molecules in the treatment of MDD, at least as adjunctive therapy [85]. These properties for melatonin are well documented, with counteractive effects on reactive nitrogen and oxygen species prominent [95].

Antidepressant effects of melatonin have been suggested in some preclinical models, but the data are far from unanimous. In BALB/c mice subjected to the chronic mild stress model, daily oral administration of melatonin (1 and 10 mg/kg) for 3 weeks counteracted the degradation of the animals' coat state, decreased grooming and increased serum corticosterone levels, effects regarded as indicative of antidepressant-like activity. This was also seen in animals treated with imipramine (20 mg/kg) over the same time frame, but not with placebo-treated animals [24]. In the same paradigm in C3H/He mice, melatonin administration was shown to prevent the decrease in sucrose consumption (a putative measure of anhedonia) [58]. However, the effectiveness of melatonin was reported to be less than that of fluoxetine (10 mg/kg). Night-time administration of melatonin (10 mg/kg) in chronically stressed C57Bl/6 mice was effective in significantly ameliorating stress-induced behavioural disturbances [43]. The stress-exposed mice showed a reduction in weight gain, hedonic deficit (decreased sucrose preference), cognitive deficits in the Novel Object Recognition Task and decreased mobility in the forced swim test.

Nocturnal, but not morning, administration of melatonin (10 and 50 mg/kg) for 5 weeks was effective in reversing the chronic mild stress-induced decrease in sucrose preference of rats [86]. The effect was blocked by a non-selective melatonin antagonist (see below). Melatonin has been demonstrated to produce an antidepressant-like effect in the mouse tail suspension test [67, 88], as well as in the rat forced swimming test [73]. In this latter test, the non-selective melatonin MT1/MT2 receptor antagonist, luzindole, reversed the antidepressant-like activity of melatonin [73]. The effects of citalopram, melatonin and their combination were investigated in the forced swim test in BalbC mice [91]. Both melatonin (0.5–10 mg/kg) and citalopram (1–40 mg/kg) alone decreased immobility behav-

[2] Acrophase: Time of maximum of a fitted cosine curve given as a delay from a given phase reference. The acrophase may also be specified with the phase reference being the point of an environmental cycle (e.g. mid-light time) or the acrophase of another rhythm in the same individual (e.g. mid-sleep time).

iour supporting an antidepressant-like effect after 14 days of treatment. The combination treatment was also effective in decreasing immobility compared to control mice.

Using these findings, the mechanism by which melatonin brings about its antidepressant-like effects in the models has been the subject of speculation. Clearly, actions at MT1 and MT2 receptors are prominent in speculation about this issue. The role of the MT1 receptor has been highlighted by studies showing that mice lacking this receptor display depression-like behaviours in the forced swim test [121]. The action of luzindole to block the effect of melatonin has already been mentioned. Contributions from other neurotransmitter receptor subtypes cannot be ignored. A putative hypo-function of the GABAergic system in depression suggests a role for GABA agonists as antidepressants in animals and humans [82]. In accord with this notion, melatonin increases GABA-A receptor number which may contribute to its antidepressant-like effects [98]. Furthermore, peripheral benzodiazepine receptors [89], central serotonergic neurotransmission [73], NMDA receptors and the L-arginine-nitric oxide pathway [67] have all been implicated in the antidepressant-like effects of melatonin based on rodent model studies.

In contrast to these positive findings, some preclinical studies do not find a strong antidepressant effect for melatonin when administered alone. Thus, in the olfactory bulbectomy model, melatonin was significantly less effective than either imipramine or agomelatine in producing an antidepressant-like effect [80]. Stress-mediated increases in prefrontal cortical glutamate release were not prevented by the chronic administration of melatonin but were by agomelatine [109]. These findings are discussed in greater detail in a subsequent section.

There are relatively few well-controlled clinical studies in which melatonin has been administered as monotherapy for depression. A randomised, double-blind, crossover study was conducted in six patients with moderate to severe depression [15]. Oral and intravenous melatonin was administered in variable doses to each patient for different lengths of time. Melatonin made little or no difference to the symptoms of depression in any of the patients studied. On the contrary, in four patients, there was a marked exacerbation of symptoms, while two had some minor deterioration in symptomatology. Melatonin doses were administered four times daily and reached a peak total daily dose of 150–1,600 mg/day. This administration of the compound, without consideration of the normal nocturnal rise in melatonin concentrations, may have introduced greater chaos in an already disturbed circadian rhythm. Thus, the detrimental effects on mood may possibly have arisen by exacerbating phase changes which were causal of the depressive disorder. These findings are in contrast to a study of melatonin in winter depression (seasonal affective disorder) [63]. In a parallel group design, five patients received either melatonin or placebo, and the effects on depressive symptomatology were evaluated over 3 weeks. Melatonin (0.125 mg) or placebo was administered at circadian times 8 and 12 estimated from each subjects' reported awakening time. All five melatonin-treated subjects reported significant improvement in symptoms (>39 % reduction in depression score) but only one placebo-treated patient. Responses to treatment were attributed to an advance in circadian phase, although this was not verified by any independent measures.

Melatonin has been used as an adjunctive treatment in patients receiving antidepressant regimens. A combination of 5–10 mg of melatonin with fluoxetine over 4 weeks did not significantly improve depressive symptoms [27]. On the other hand, the slow-release preparation of melatonin employed in the study improved the subjective symptoms of sleep quality by more than half. A similar finding was noted in patients with treatment-resistant depression, defined as a failure to respond to at least 8 weeks treatment with two or more antidepressants [22]. In this open evaluation, nine patients with DSM-IV MDD received 5–10 mg of sustained-release melatonin in addition to their ongoing antidepressant medication. There was an average 20 % decline in depression scores after 4-week combined treatment. On the other hand, the insomnia item of the Hamilton rating scale decreased by 36 % from baseline with four patients achieving

>50 % improvement on this scale. Slow-release melatonin (6 mg/day) was evaluated as an add-on to existing antidepressant medication to assess its ability to improve sleep in depressed patients in a double-blind placebo-controlled study [99]. Sleep was measured by dairies and rating scales as well as wrist actigraphy over the 4 weeks of treatment. Neither objective nor subjective measures of sleep parameters showed any statistically significant effects of melatonin compared to placebo. Similarly while depression improved slightly, there were no differences between melatonin and placebo. Given the relatively small number of subjects in each treatment arm, the study was probably underpowered to detect small differences.

From these studies, it can be concluded that melatonin is unlikely to have an antidepressant effect when administered alone. Alternatively, concomitant use of antidepressants and low doses of melatonin may benefit sleep disturbances in some patients. It would seem most likely that alterations in sleep phase, particularly delayed sleep phase, would respond best to melatonin addition at night. This hypothesis, which requires testing in a clinical population, is based on the well-recognised ability of melatonin to advance the circadian phase.

18.4 Agomelatine: A Melatonergic Antidepressant

Based on the putative disruptions of the circadian system in depression, the notion that the manipulation of rhythm might be antidepressant led to the development of the first melatonergic agent for this purpose [23]. A series of naphthalene derivatives were synthesised, and their activity in displacing the binding of [^{125}I]-melatonin to the pituitary gland was investigated [128]. Coupled with electrophysiological studies, which demonstrated agonist properties at melatonin receptors, one derivative S20098, later called agomelatine, was chosen for further development [127]. This agent was shown to be capable of the re-entrainment of disrupted circadian rhythms in a dose-dependent manner [93]. Detailed pharmacological and clinical investigations of the drug were undertaken which indicated that compound was an antidepressant [23]. Subsequently, there have been numerous reviews of the clinical efficacy of agomelatine for the treatment of MDD, but despite the success of this agent, no further melatonergic drugs for depression treatment have emerged to date. The reasons might lie with the details of the pharmacology of agomelatine.

18.4.1 Pharmacology of Agomelatine

18.4.1.1 Receptor Binding

Following the demonstration that agomelatine possessed agonist properties at melatonin-binding sites, the binding profile of the drug to various other receptor subtypes was investigated in vitro. Agomelatine demonstrated potent effects at human MT1 and MT2 receptors cloned in CHO cells, which were higher than that of melatonin (Table 18.3). In addition, antagonist activity was demonstrated at human 5HT2C receptors as agomelatine produced a dose-dependent blockade of the induction of [^{35}S] GTPγS binding induced by 100nM serotonin [74]. Similar antagonist effects were noted for the activity of agomelatine at the 5HT2B receptors where the effects were about equivalent to the action at 5HT2C receptors (Table 18.3). Interactions with more than 50 other receptors and receptor subtypes were considered pharmacologically non-significant [18]. The role of the 5HT2C receptor in the aetiology and treatment of depression is regarded as important. The receptor density is enriched in the prefrontal cortex, hippocampus, basal ganglia and other areas considered significant in mediating some symptoms of the disorder. Some clinically effective antidepressants are 5HT2C antagonists, while long-term administration of selective serotonin reuptake inhibitors results in downregulation of the 5HT2C receptor [12].

Regulation of the monoamine release can be modulated by serotonin [1]. In particular, 5HT2C receptors exert an inhibitory influence on frontal dopamine and noradrenaline pathways [26]. Agomelatine dose dependently increased levels

Table 18.3 Binding of agomelatine and melatonin to selected receptors

Binding site	Agomelatine	Melatonin	Activity	References
h-MT1	0.10	0.22	Agonist	Audinot et al. [4]
h-MT2	0.12	0.35	Agonist	Audinot et al. [4]
h-5HT2C	707	>10,000	Antagonist	Millan et al. [74]
h-5HT2B	257	5,750	Antagonist	Millan et al. [74]
h-5HT2A	447	>10,000	Antagonist	Millan et al. [74]

K_i values nM

of dopamine and noradrenaline in the prefrontal cortex of freely moving rats as shown by microdialysis experiments [74]. This effect was region specific as dopamine was not increased in nucleus accumbens or striatum. A similar effect was noted for noradrenaline concentrations, but not for serotonin. The effect on dopamine and noradrenaline in the cortex was not affected by a selective melatonin antagonist, S22153. Further, melatonin was without effect on dopamine or noradrenaline in cortex, suggesting that the effect of agomelatine was 5HT2C mediated. This antagonist action at 5HT2C receptors emerged in behavioural experiments as critical for determining antidepressant-like activity.

18.4.1.2 Behavioural Studies

Animal models have proven to be invaluable in the screening of new molecules for potential as antidepressant agents. While many suffer from the identification of false positives or false negatives, assessment of activity in several models has been useful in minimising these shortcomings. Agomelatine has been assessed for its activity in a number of animal models which possess high pharmacological predictive validity. Positive activity in the so-called forced swim test (FST) has become *de rigueur* for the assessment of the potential antidepressant activity of a new molecule [21]. Thus, agomelatine was shown to be active (prolonged swimming time) after acute and subchronic administration to rats and mice [10]. The effects were compared with that of melatonin (up to 64 mg/kg) as well as imipramine and fluoxetine. After repeated administration, agomelatine (as well as fluoxetine and imipramine) dose dependently reduced immobility time, an effect not noted with melatonin alone. In the learned helplessness model, agomelatine (10 and 50 mg/kg) was equivalent in its antidepressant-like effect to that of imipramine (64 mg/kg) [9]. In both of these studies, agomelatine and comparative agents were administered by the oral route. Chronic administration of agomelatine to transgenic animals with low glucocorticoid receptor function was successful in reversing the behavioural changes of transgenic mice in the FST [5].

In other models also dependent on chronic drug administration to detect activity, agomelatine was active. Thus, in the chronic mild stress model, chronic evening treatment with agomelatine or melatonin (10 and 50 mg/kg) for 5 weeks dose dependently reversed anhedonia (decreased sucrose consumption) induced by the effects of repeated stress [86]. Melatonin was less active than agomelatine. The effect was blocked by an acute injection of the MT1/MT2 antagonist, S22153. Administration of agomelatine during the morning was effective, but melatonin was not. Furthermore, the morning antidepressant-like effect was not blocked by the melatonin antagonist. As melatonin receptors show a diurnal rhythm, the data were interpreted to suggest that the antidepressant effects at night were dependent on agonist actions at the melatonin receptors but during the day were attributable to other properties of the drug, i.e. 5HT2C antagonism.

In the olfactory bulbectomised rat model, antidepressant-like activity of molecules is dependent on the ability, after chronic but not acute administration, to reverse hyper-locomotor activity of bulbectomised animals in an open field. Agomelatine (10 and 50 mg/kg) administered in the evenings was as active in this model

as the tricyclic antidepressant, imipramine (10 mg/kg). However, although melatonin (10 and 50 mg/kg) showed some activity, it was not as active as agomelatine [80]. In a dose-ranging study, the selective 5HT2C antagonist, S32006, was also less active than agomelatine alone [80]. The data again point the necessity for both melatonin agonist and 5HT2C antagonist properties of agomelatine to exert synergistic effects to accomplish an antidepressant-like effect in the model.

Neither melatonin nor S32006 alone, when chronically administered, was able to prevent the stress-related increase in glutamate release in prefrontal cortex of rats [109]. On the other hand, chronic administration of agomelatine was effective in completely preventing the stress-induced increase of glutamate. Acute agomelatine administration (40 mg/kg) was able to significantly increase the synthesis of mRNA for BDNF (brain-derived neurotrophic factor) in rat prefrontal cortex [75]. On the other hand, acute injections of melatonin (40 mg/kg) and the 5HT2C antagonist S32006 (10 mg/kg) were both without effect on BDNF expression, suggesting a synergistic effect of the two receptor activities in order to bring about the observed effects of agomelatine. Furthermore, the melatonin (MT1/MT2) antagonist, S22153, prevented agomelatine-induced increases in BDNF mRNA concentrations.

The consistency of the preclinical studies taken together suggests it is likely that a combination of melatonin agonist and 5HT2C antagonist activities is necessary for the antidepressant activity of agomelatine.

18.4.1.3 Clinical Studies Pertaining to Mechanism of Action

While binding, biochemical and animal behavioural experiments all point to the critical role played by the 5HT2C receptor in mediating the antidepressant-like effects of the drug, the relevance of this mechanism for human activity has been questioned. In a single-dose, double-blind, crossover study in healthy volunteers, subjects received agomelatine (25 mg) or placebo separated by a 7–14-day washout period [102]. Polysomnograms were recorded on each study night and the effect on slow wave sleep was measured. Acute administration of other 5HT2C antagonists has been shown to increase slow wave sleep [101]. Agomelatine failed to show any statistically significant effect on sleep parameters although subjects rated sleep quality as improved [102]. As there was no effect on the polysomnographic parameters, the authors concluded that 5HT2C antagonism is unlikely to be important in humans. This claim has been refuted [79] since the compounds which have been cited as putative selective 5HT2C antagonists in slow wave sleep studies in healthy volunteers are known to have actions at multiple receptor subtypes, in particular at the H1 receptor. Indeed, H1 antagonist effects are often more potent than the 5HT2C effects. An alternative hypothesis for the absence of an effect of agomelatine on slow wave sleep is that it lacks histamine H1 antagonist properties. The relevance of 5HT2C antagonism for the clinical effects of agomelatine needs further examination in clinical populations.

18.4.2 Clinical Efficacy of Agomelatine

It is not the intention here to discuss in detail clinical data addressing the issue of the antidepressant activity of agomelatine. This information is addressed elsewhere in this volume as well as in many previous publications, including a recent meta-analysis [110]. An extensive clinical trial programme has been conducted evaluating the antidepressant efficacy of agomelatine in patients with major depressive disorder (MDD). The general consensus of the majority of reviews of that data supports the notion that agomelatine possesses antidepressant activity [49, 54, 56, 103, 110]. An overview of selected acute clinical trials attesting to the efficacy of agomelatine is summarised in Table 18.4. Within the trial database presented to marketing authorities, not all trials are unanimous in supporting antidepressant efficacy [31]. This appears to be true for most recently approved antidepressant molecules and is certainly not unique to agomelatine. Thus, there are trials with the drug which have failed to separate from placebo, which might be

Table 18.4 Clinical trials of agomelatine in the treatment of major depression

Design, no subjects, duration of treatment	Medication	Objective	Endpoint	Results	Publication
Randomised, parallel groups, $n=28$, 4 weeks	5, 100 mg/day	Pilot study	MADRS	No difference between doses	Loo et al. [66]
DB, randomised, parallel groups, $n=711$, 8 weeks	1, 5, 25 mg/day; PBO; paroxetine 20 mg/day	Efficacy and safety	HAM-D	Ago > Pbo	Loo et al. [65]
DB, randomised, parallel groups; $n=238$; 6 weeks[a]	25, 50 mg/day; PBO	Efficacy and safety	HAM-D	Ago > Pbo	Ollie and Kasper [83]
DB, randomised, parallel groups; $n=212$; 6 weeks[a]	25, 50 mg/day; PBO	Efficacy and safety	HAM-D	Ago > Pbo	Kennedy and Emsley [55]
DB, randomised, parallel groups; $n=313$; 6 weeks	25 mg/day; sertraline 50 mg/day	Circadian rest activity cycle	HAM-D	Ago > Sert	Kasper et al. [52]
DB, randomised, parallel groups; $n=591$; 6–8 weeks; pooled data	25, 50 mg/day; PBO	Efficacy	HAM-D	Ago > Pbo severe depression HAM-D >22	Montgomery and Kasper [76]
DB, randomised, parallel groups; $n=339$; 34 weeks	25, 50 mg/day; PBO;	Efficacy and safety	HAM-D	Ago > Pbo	Goodwin et al. [39]
DB, randomised, parallel groups; $n=515$; 8 weeks	25 mg/day fluoxetine 20 mg/day	Efficacy	HAM-D	Ago > fluox	Hale et al. [41]
DB, randomised, parallel groups; $n=511$; 8 weeks	25, 50 mg/day; PBO	Efficacy	HAM-D	Ago > Pbo	Zajecka et al. [129]
DB, randomised, parallel groups; $n=503$; 8 weeks	25, 50 mg/day; PBO	Efficacy	HAM-D	Ago > Pbo	Stahl et al. [107]
DB, randomised, parallel groups; $n=419$; 6 weeks	25 mg/day; PBO; fluoxetine 20 mg/day	Efficacy and safety	HAM-D	No difference Ago v Pbo	Unpublished CL3-022[b] [31]
DB, randomised, parallel groups; $n=418$; 6 weeks	25 mg/day; PBO; paroxetine 20 mg/day	Efficacy and safety	HAM-D	No difference Ago v Pbo	Unpublished CL3-023[b] [31]
DB, randomised, parallel groups; $n=607$; 6 weeks	25, 50 mg/day; PBO; fluoxetine 20 mg/day	Efficacy and safety	HAM-D	No difference Ago v Pbo	Unpublished CL3-024[b] [31]

[a]Extension phase up to 46 weeks of additional treatment from the initial period
[b]Data sourced from EMEA submission

attributable to methodological issues such as trial design and patient selection.

The most recent meta-analysis of the agomelatine database examining double-blind, placebo-controlled studies concluded that agomelatine was an effective antidepressant with efficacy superior to placebo and similar to that of standard antidepressant drugs [110]. The authors calculated standardised mean differences to estimate treatment effect sizes using Hedges' g method and included both published and unpublished trials. Compared to placebo, response rates (defined

as a 50 % reduction in baseline depression rating scale, usually HAM-D) favoured agomelatine with an effect size of 0.24 (0.12–0.35 95 % CI). This is somewhat weaker than effect sizes calculated for other antidepressant agents of about 0.31 [113]. When effect sizes with respect to remission (defined as a HAM-D score <7 or MADRS score <12) were calculated, agomelatine was not different from placebo. In the analysis of data where comparisons of agomelatine to other antidepressants (sertraline, escitalopram, fluoxetine, paroxetine and venlafaxine) were performed, there was no difference between medications for response or remission. For the most part, these studies were of relatively short-term duration (6–12 weeks). Subgroup analyses of agomelatine efficacy have been conducted examining the efficacy versus placebo in patients with more severe forms of depression [56]. As the severity of depression increased from a baseline score of 25 on the HAM-D scale to 30 or more, the absolute drug-placebo differences also increased significantly. This attests to the efficacy of the drug in more severe forms of depression, a phenomenon noted for other antidepressants, namely: antidepressant medications work best in moderate to severe forms of the disorder [57].

Further convincing evidence for clinical antidepressant efficacy comes from relapse prevention trials, i.e. studies in short-term responders who are either maintained on medication or allocated to placebo in a double-blind study. The cumulative incidence of relapse in DSM-IV-TR-diagnosed patients who responded to 25 or 50 mg of agomelatine over 8–10 weeks was examined when patients were assigned to either placebo or continuation agomelatine [39]. Over the 24-week study period, 21.7 % of agomelatine patients and 46.6 % of placebo patients relapsed, a statistically significant difference in favour of agomelatine as assessed with a Kaplan-Meier survivor analysis. As in short-term studies, agomelatine was superior to placebo in maintaining response in patients with a baseline HAM-D depression score of 25 or more.

A convincing body of evidence has accumulated that agomelatine is an antidepressant agent in the clinic. From the preclinical pharmacology, both its melatonin agonist action and its 5HT2C antagonist properties would appear to make important contributions to this activity. While it has not been established from clinical studies that these receptor subtypes are needed for the antidepressant effects of the drug, indirect evidence suggests that this maybe the case clinically as well. While it can be argued that agomelatine has well defined circadian re-entrainment properties, data on the effects of melatonin given alone would suggest that this is not sufficient to bring about an antidepressant effect. Similarly, many known effective antidepressant agents have 5HT2C antagonist properties which must surely contribute to their antidepressant activity. Thus, both preclinical and inferential clinical data support the notion that agomelatine owes its antidepressant activity to a combination of receptor actions. Other melatonin agonists, lacking additional receptor actions, have not generally been shown to be antidepressant.

18.5 Other Melatonin Agonists as Antidepressants

Several melatonin agonists are available for clinical use, while a further group of agents are in development [16]. Ramelteon has been licenced for use in the treatment of insomnia [68]. Binding studies have established that the affinity of ramelteon for MT1 receptors is greater than for MT2 receptors (respective K_i values are 0.014 nM and 0.112 nM) [53]. It does not have appreciably affinity for other neurotransmitter and hormonal receptors [68]. As a consequence of the selectivity for MT1 over MT2, it is suggested that ramelteon targets sleep onset specifically [16]. There are no studies in which the drug has been used for the treatment of depression as a monotherapy. However, like melatonin itself, it could be anticipated that ramelteon might be useful for the treatment of insomnia associated with MDD. The addition of 8 mg at night to the sertraline regimen of an elderly patient with DSM-IV MDD resulted in sustained remission of symptoms for up to 6 months [36]. Euthymic patients with bipolar disorder and sleep disturbances were randomised

to receive adjunctive ramelteon or placebo in addition to their regular psychiatric medications in a double-blind study [81]. Patients were treated for up to 24 weeks or until relapse (defined as a depressed or manic event). A total of 83 subjects were randomised to either ramelteon ($n=42$) or placebo ($n=41$). During the study period, 40 (48.2 %) participants relapsed. Participants who received ramelteon were less likely to relapse than those who received placebo (OR = 0.48, $p<0.024$ Cox regression). Median survival times were longer in ramelteon-treated than placebo-treated patients (188 versus 84 days; $P<0.05$ chi-squared test). Thus, ramelteon as an add-on treatment was effective in maintaining stability for individuals with bipolar disorder through its action on sleep disturbance.

Tasimelteon is an investigative synthetic melatonin agonist with high affinity for both the MT1 and MT2 receptors in humans (respective K_i values 0.35 nM and 0.17 nM) [60]. These affinities are similar to that of melatonin for both receptors. It has been tested for its effects on insomnia [90]. Beneficial effects on sleep latency and maintenance were reported compared to placebo. The compound was effective in resetting the circadian rhythm, but there have been no studies of its use alone or as an adjunct in MDD.

Piromelatine (Neu-P11) is an investigational multimodal drug developed for the treatment of primary and co-morbid insomnia [61]. The drug interacts with MT1 and MT2 receptors (respective K_i values 22 nM and 34 nM) [46]. Additionally, the drug interacts with 5-HT1A ($K_i=1{,}110$ nM) and 5HT1D ($K_i=150$ nM) as well as enhances GABA activity, though not directly interacting with GABA receptors. A phase-II study in insomnia patients demonstrated that piromelatine significantly improved sleep maintenance based on polysomnographic assessments: wake after sleep onset, sleep efficiency and total sleep time [61]. In preclinical assessments, piromelatine demonstrated antidepressant-like and anxiolytic-like properties [112]. Thus, in the learned helplessness model, piromelatine and melatonin (25–100 mg/kg, ip) were administered to rats 2 h before the beginning of the dark phase once a day for 5 days. The number of escape failures and intertrial crossings during the test phase was recorded. Only piromelatine was effective in decreasing the escape deficit but had no effect on the intertrial crossings. In the forced swimming test, rats received a single or repeated doses of piromelatine (25–100 mg/kg, ip), and the period of immobility during was assessed. Single and repeated doses of the drug, either in the morning or in the evening, decreased immobility time. Taken together, these two tests suggest antidepressant-like effects for the drug. Anxiolytic-like effects were assessed in the elevated plus-maze test (EPM). Mice were injected with piromelatine (25–100 mg/kg) or melatonin in the morning or in the evening and tested 2 h later. Time spent in the open arms and the open arms entries were assessed. Piromelatine significantly increased the open-arm time and entries irrespective of the time of drug administration. Melatonin was effective only when administered in the afternoon. The data suggest an anxiolytic-like effect of the compound. Human trials are clearly required before conclusions can be drawn about the potential clinical use of the compound as an antidepressant or anxiolytic. Nevertheless, it is of note that additional pharmacological actions (effects on 5HT1A/1D) are present in this compound. This supports the notion raised previously that melatonin agonist effects alone are insufficient for antidepressant and/or anxiolytic activity.

Conclusions

There appears to be little value, from a diagnostic point of view, for the continued measurement of plasma melatonin or urinary melatonin metabolite concentrations alone as a potential biomarker of major depression. Demographic and medication factors have been shown to influence concentrations of the hormone confounding the interpretation of any pertinent findings. Initial findings of a low 'melatonin syndrome' as a subtype of major depression are not well supported by the literature. Furthermore, the initial enthusiasm for the concurrent assessment of multiple hormonal measures, such as cortisol and

thyroid hormone, in MDD does not appear to add any further diagnostic certainty, nor does it seem to aid especially in treatment choice decisions. The usefulness of evaluating serial nocturnal melatonin concentrations in MDD patients has the potential to identify a subset of patients who exhibit marked circadian phase abnormalities. Emerging evidence suggests that re-entrainment of the circadian system, if not an antidepressant of its own accord, aids in recovery from episodes of major depressive disorders. While melatonin and melatonin agonists are capable of such a re-entrainment, the weight of present evidence does not support a role for such agents as monotherapy. The reputation of melatonin as ineffective may be undeserved as it is based on a single study with what, in retrospect, seems to be a seriously flawed dosing strategy. Perhaps further clinical evaluations of melatonin are required which utilise lower doses and administration timed to take account of circadian factors. Nevertheless, data from other selective and non-selective MT1/MT2 agonists do not appear to offer the prospect of effective antidepressant treatments, although the database is sparse. Adjunctive melatonin trials seem also to suggest a lack of specific antidepressant effects of the hormone. Therapeutic gains that have been made in such trials have largely been attributed to the effects of melatonin on sleep parameters, but detailed analysis of individual symptom ratings or clusters of symptoms has not been performed. The recent development of agomelatine, a MT1/MT2 agonist, as an effective antidepressant suggests that additional pharmacological activities are required for antidepressant activity. In the case of agomelatine, the 5HT2C antagonist actions of the drug are reputedly important. This has been established preclinically, but in clinical studies, the relevance remains unclear. At present, there are no other exemplars of the genre of melatonin agonists/5HT2C antagonists to explore the notion that agonist effects at melatonin receptors alone are not antidepressant. Whether combining selective or non-selective agonist effects at melatonin receptors with other pharmacological actions (e.g. monoamine reuptake transporter inhibition, alpha-2 antagonism) might also provide an alternative class of antidepressant agents has not been explored. Synthetic manipulation of the basic melatonin chemical structure has resulted in a number of agents with pharmacological actions additional to agonist effects at melatonin. Few, if any evaluations, of these molecules have been conducted in animal models relevant to depression nor are there any relevant clinical investigations. Clearly, such molecules are of heuristic interest to establish the usefulness of melatonin agonist effects in the treatment of depression and whether additional pharmacological actions are essential.

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Neuropsychological Models of Depression

19

Stephan Moratti, Alberto Fernández, Rosa Jurado, and Gabriel Rubio

19.1 Introduction

Depression is the most commonly occurring disorder of emotion regulation and represents 4.3 % of global burden of disease [172]. Lifetime (LT) prevalence of major depressive episode in Spain was 10.6 %, while 12-month prevalence was 4.0 % [47]. Depression is an affective disorder that not only manifests affective symptoms like anhedonia, feelings of sadness, and low emotional arousal [68, 109] but also cognitive impairments in higher-order domains such as visual spatial attention, memory, and executive functions [68, 113].

During the past decade, there has been a growing interest in applying models of emotion and motivation emanating from basic research, like psychophysiology, to the study of psychopathology. Several of the most influential models posit higher-order dimensions that reflect the operation of biologically based systems that organize and activate responses to rewarding, appetitive, or otherwise positive hedonic stimuli and to aversive, threatening, or otherwise negative hedonic stimuli [159]. Such models are particularly relevant to unipolar depression in light of the proposal that this disorder is characterized by a combination of a hypoactivation in a biologically based approach system that mediates responses to appetitive stimuli and hyperactivation of a protective-defensive withdrawal system that mediates responses to aversive, threatening, or otherwise stressful stimuli [46].

Two of the most relevant models in this field have investigated the relationship between depression and positive and negative affects [142] and also the association between emotional functioning and cortical brain asymmetries in subjects with affective disorders [68].

S. Moratti
Department of Basic Psychology I, Faculty of Psychology, Complutense University, Madrid, Spain

A. Fernández
Department of Psychiatry, Faculty of Medicine, Complutense University, Madrid, Spain

R. Jurado
Department of Basic Psychology II, Faculty of Psychology, Complutense University, Madrid, Spain

Psychology Department, Faculty of Health Sciences, Universidad Camilo Jose Cela, Villanueva de la Cañada, Madrid, Spain

G. Rubio (✉)
Psychiatry Service, 12 de Octubre University Hospital, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Department of Psychiatry, Faculty of Medicine, Complutense University, Madrid, Spain
e-mail: gabriel.rubio@salud.madrid.org; grubiovalladolid@gmail.com

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19.1.1 Depression and Emotion Context Sensitivity

On self-report measures that assess mood states and traits, unipolar depressed, relative to nondepressed, individuals consistently report (a) lower positive affect, lower behavioral activation, and increased anhedonia and (b) heightened negative affect, behavioral inhibition, and generalized distress [85]. On measures of reactivity to emotional stimuli, however, data are not very clear. Depressed individuals consistently demonstrate deficits in the processing of and responses to positive hedonic stimuli on self-report [153], behavioral [75] and psychophysiological [32] measures. However, the evidence concerning reactions to negative affective stimuli is more equivocal. Whereas a few studies have found exaggerated responses to aversive or other negative stimuli [151], others have not and/or have found inconsistent results across dependent measures [143, 174]. Further, within-subject comparisons often indicate that depressed individuals are less responsive to variations in the affective valence of eliciting stimuli than are nondepressed individuals [142]. On the basis of these data, Rottenberg et al. have argued that depression is associated with a substantial *emotion-context insensitivity (ECI)* [141, 142]. They have argued that this view is consistent with evolutionary accounts of depression that conceptualize this syndrome as characterized by disengagement and a bias against action [127].

The ECI hypothesis can be contrasted with two other theories: the positive attenuation hypothesis and the negative potentiation hypothesis [142]. The positive attenuation hypothesis states that positive emotion is decreased in depression (i.e., anhedonia). The negative potentiation hypothesis states that depression is associated with high negative emotion. In their study of these three competing theories of depression, Rottenberg et al. found more support for the ECI hypothesis than for either the positive attenuation or negative potentiation hypotheses.

19.1.2 Cortical Brain Asymmetries and Depression

This model proposes that relative less left than right frontal brain activity is associated with reduced positive/increased negative mood in depression. Greater left frontal activity leads to positive valenced emotions, whereas greater right frontal activity is related to negative affect. Relative left frontal hypoactivation in depression not only has been related to low positive affect but also to cognitive deficits such as poor problem solving, memory problems, and tasks that involve effortful processing [68]. Further, right temporoparietal hypoactivation in depressive patients is associated with low emotional arousal and impairments of right posterior hemisphere functions such as emotional face processing [30] and sustained attention even during remission [170]. However, right temporoparietal activity is supposed to covary with co-morbid anxiety. High levels of co-morbid anxiety lead to a right temporoparietal hyperactivation as emotional arousal increases; cognitive performance is also differentiated and has been more severe for those patients showing co-morbidities [126]. Therefore, this model suggests that co-morbid anxiety has to be carefully controlled in studies investigating brain activity asymmetries in depression as different levels of anxiety can result in opposing effects. However, over the years the model proposed by the Heller group has been refined. For example, anxious apprehension is now differentiated from anxious arousal, and the two have been associated with different brain asymmetry patterns [152].

Interestingly, the Heller model adopts a dimensional approach of emotion that considers that all emotions can be described along a valence and an arousal dimension that are specifically related to frontal and posterior brain regions [72] (for a review).

This is in line with emotion research based on a motivated attention framework [98] that also considers emotions to be organized along valence and arousal dimensions. However, the motivated attention framework assumes that valence (pleasant vs. unpleasant) represents the activation of appetitive and defensive motivational circuits and

that arousal is associated with the intensity of the activation of one of the motivational systems. This accords with the notion that frontal asymmetries in brain activity reflect rather approach and withdrawal motivational tendencies than direct emotional valence [65]. Hereby, left frontal cortex activity is associated with approach behavior that usually provokes positive affect, whereas right frontal activation reflects withdrawal behavior that is accompanied by negative emotional experience [27].

In order to clarify the most relevant findings published in this field, the following sections will be presented in this chapter: (1) major depression studies based on startle reflex and (2) studies focused on brain asymmetry framework of depression, brain lesion and depression, electroencephalogram (EEG), and magnetoencephalogram (MEG) studies.

19.2 Startle Reflex and Major Depression

The magnitude of the startle reflex may be an endophenotypic marker for vulnerability to recurrent depression. The startle reflex, which is widely used in the investigation of the physiology of affect and attention, consists of a cascade of physical reactions to an intense stimulus with sudden onset, for example, a loud noise. One of the earliest and most stable components is the eyeblink response. This is a burst of electromyographic (EMG) activity in the orbicularis oculi muscle associated with the presentation of the startle stimulus [5]. One of its main features is that it is easily modulated, exhibiting different forms of plasticity, such as habituation, sensitization, or prepulse modulation. Variation in the affective and attentional modulation of the eyeblink startle reflex is associated with a number of psychopathologies [58]. The startle reflex is generally augmented while viewing unpleasant pictures and inhibited when viewing pleasant ones, compared with neutral stimuli, so the eyeblink response to startling noise has been used as an indicator of the emotional response as it is an easily quantifiable measure of behavioral reactivity to external stimuli.

19.2.1 Affective Startle Modulation: Comparisons Between Depressed and Nondepressed Individuals

The affective startle among not depressed individuals as response to an emotional context would occur as a reflection of the potentiation of a startle taking place in the presence of both aversive and appetitive stimuli when the startle probe and the affective picture are relatively long (i.e., 2,000 ms), suggesting associated anticipation processes. Nevertheless, when the amplitude of the startle is evaluated during the visualization of emotional stimuli, the response magnitude of the startle will be modulated by the stimulus valence; thus, unpleasant stimuli will potentiate the startle, and pleasant stimuli will potentiate inhibition. Besides, when the latency between the startle probe and the emotional stimulus is very short (i.e., 300 ms), the amplitude of the startle reflex is attenuated for both types of stimuli, placing the localization of the attentional resources on the emotional stimulus rather than on the startle [31].

Studies that have investigated affective modulation of the startle response in people with depression [3, 32, 44, 86] have found conflicting results. The most common finding has been a lack of affective startle modulation among depressed patients [3, 32, 86], a result that supports the ECI hypothesis. In contrast, although Forbes et al. found a significant linear trend in their depressed group's responses to different valences, like the controls, they found no potentiation of responses to unpleasant stimuli relative to neutral ones; this result only partially supports the ECI hypothesis. In addition, Goggin et al. [52] could not find a potentiation of the startle before words having a negative affective valence (e.g., alone, fright, lonely); hence, patients with depression showed a startle potentiation profile similar to controls, concluding that this fact could be the result of a low arousal level from stimuli or even following [14] statements that emotional stimuli could have lost relevance and consequently their capacity to activate the motivational defensive system [14, 52].

Finally, when the depressed group in the Allen et al. study was divided by severity of depression, only those with scores of 30 or higher on the Beck Depression Inventory (BDI) showed significantly greater startle responses to pleasant stimuli than to unpleasant stimuli, a result that supports the positive attenuation hypothesis. Following this approach, Vaidyanathan et al. [166] consider the greater number of recurrent depressive episodes as a determinant factor for the startle modulation. They then found that the recurrent group showed increased startle responses to both pleasant and unpleasant pictures while the nonrecurrent group evinced the expected linear pattern of responses, these groups did not differ from controls with regard to startle modulation [166].

All of these studies included at least some patients medicated with antidepressants. In the studies by Allen et al. [3] and Kaviani et al. [86], the majority of the participants were medicated (14 out of 14 in the Allen et al. study; 18 out of 22 in the Kaviani et al. study). Forbes et al. [44] had only 11 out 76 patients taking medication and reported that the results did not differ if those participants were removed. Dichter et al. [32] examined the effects of bupropion on affective ratings and affective modulation of startle and found that this drug had no effect on the participants' responses even though their scores on measures of depression improved markedly. But it is also known that among healthy volunteers, citalopram has been found to attenuate startle responses to negative stimuli [64], whereas both citalopram and reboxetine have been found to reduce the recognition of fearful and angry facial expressions [63]. Accordingly, different psychotropic medications may have different effects on startle modulation. In summary, in spite of these heterogeneous data, it seems that affective startle modulation paradigm may be a useful psychophysiological tool for studying depression.

19.2.2 Unmodulated Baseline Startle Reflex and Depression

Allen et al. [3] reported that those with more severe depression had significantly lower baseline startle magnitude and also that those with the lowest levels of positive affect (anhedonia) displayed an inhibited baseline startle amplitude. Additionally, Kaviani et al. [86] reported that while only startle reactivity to pleasant stimuli is reduced at low levels of depression and anhedonia, all reactivity is reduced at higher levels. Furthermore, and consistent with the findings of Allen and colleagues, it was also found that those with the highest levels of depression showed a lower startle amplitude. O'Brien-Simpson et al. conducted a 2-year follow-up study on depressed individuals and found that a relatively attenuated startle response at initial assessment was strongly predictive of both those who have depressive symptomatology and would experience relapse [129]. Taken together, these data show an association between depression and attenuated startle [118], but also baseline startle is, however, reported to be highly heritable [7, 9], suggesting that baseline startle may be a candidate as an endophenotypic marker of vulnerability to depression. Different mechanisms may account for the association between inhibited startle and depression. The serotonin transporter genotype has been involved in the dysfunction of both the startle reflex and depressive states [16, 62]. The lower startle reactivity displayed by depressive subjects may be considered a vulnerability marker to depression related to serotonergic and stress mechanism [82].

19.3 Studies Focused on Cortical Brain Asymmetry Framework of Depression

19.3.1 Frontal Brain Asymmetry and Depression

19.3.1.1 Classical Lesion Studies

Already in the late thirties, it was observed that left versus right anterior cortex damage could produce different mood changes [54]. Later studies confirmed that left hemisphere lesions are more likely to produce depressive symptoms [48, 139, 145]. Sackheim et al. observed by evaluating three restrospective studies that pathological laughing was associated with lesions of the right

hemisphere, whereas pathological crying was related to left hemispheric damage [145]. In the second study, right hemispherectomy produced euphoric mood changes. Finally, gelastic epilepsy patients, that present ictal outbursts of laughing, were characterized by left hemispheric epileptic foci. More specifically, Robinson and collaborators showed that severity of depression in poststroke patients was correlated with the proximity of the lesion to the left frontal pole [139]. However, within the right hemisphere, they found an inverse relationship with depression being more likely when the lesion location was more posterior (see below).

These findings were mirrored by observations during the WADA test. During the WADA test, sodium amobarbital is injected into the left or right carotid artery to anesthetize the left or the right hemisphere in order to determine the lateralization of brain function before neurosurgery. For example, Lee and collaborators reported more frequent laughter-related mood changes during injection of sodium amobarbital in the right carotid artery (right cerebral hemisphere) [100], while crying occurred more often after left-sided injections. In sum, suppression of left hemisphere functions by lesion or anesthesia produced depression-like symptoms. Right-sided lesions or anesthesia resulted in opposing euphoric behavioral reactions. These data were interpreted in the light of disinhibition. Thus, a lesion in the left hemisphere has been supposed to result in an overactive right hemisphere producing negative affect, whereas an overactive left hemisphere released from contralateral inhibition has been believed to generate positive affect.

19.3.1.2 EEG Alpha Asymmetry and Affective Style

In healthy humans the mutual interplay between left and right frontal cortex activity and its relationship with emotion has been mainly studied by using EEG measures of brain activation. Thereby, the power in the alpha band frequency has been considered as an inverse measure of tonal brain activation [130]. Higher alpha power at a specific electrode location has been interpreted as less tonal brain activity of the brain region beneath that electrode site [23]. Following the logic of the abovementioned lesion studies, alpha band power asymmetries between homologous left and right electrode sites have served as indicators of relative left and right hemisphere activity. Frontal (at frontal electrode sites) alpha band asymmetry measures were reported to be reliable, stable, and electrophysiological measures [165].

Usually, alpha band power is recorded during rest, while subjects have their eyes open and closed for the same amount of time. Davdison and collaborators [28] published the first paper linking positive and negative affect to frontal EEG alpha power asymmetry. Relative left frontal activity (less left frontal compared with right anterior alpha power) was associated with positive affect and relative right anterior activity with negative emotion. If frontal alpha band asymmetry indexes relative left vs. right frontal activity and this asymmetry is related to general mood, individual differences in resting tonal frontal brain asymmetries should explain the variance of affective styles between subjects. Tomarken and collaborators [161] assessed affective styles by measuring individual differences of general positive and negative affect using the Positive and Negative Affect Schedule (PANAS [169]). Participants that were characterized by increased relative left vs. right anterior activation reported increased positive and decreased negative affect in comparison with subjects showing the opposite EEG asymmetry. Baseline anterior alpha power asymmetries seemed to index general affective styles that can vary across subjects.

Affective styles should also be related to emotional reactivity to affective stimuli. As mentioned above, some authors have linked left frontal activity with approach motivational tendencies associated with positive affect, whereas right frontal activation reflects withdrawal tendencies accompanied by negative affective experience [27, 65]. A general disposition to approach behavior and positive affect should result in greater experience of positive emotion during positive affective stimulation, whereas negative biased motivational tendencies should provoke more negative emotional experience to unpleasant stimuli. Indeed, a greater relative right frontal baseline activity predicted greater fear response

to negative film clips [160]. Wheeler et al. [171] reported that subjects with greater left frontal baseline activity responded with more intense positive affect to positive film clips, whereas participants with greater right frontal activation reacted more negatively to unpleasant film material. A problem of these studies was that the samples consisted of female subjects only. However, these results were replicated in men later [81]. In the same line of research, Knyazev et al. [95] show how healthy individuals with high predisposition to depression exhibited an increased desynchronization of alpha and an evident difficulty to differentiate emotional information of stimuli [95].

These studies accord with the neuropsychological model of emotion proposed by Heller and colleagues [68, 70, 71] implicating the left frontal cortex with positive valence and the right frontal cortex with negative affect.

Frontal baseline alpha asymmetry seems to be a stable individual trait measure with good to excellent reliability [162, 165]. Up to 60 % of anterior alpha power asymmetry could be explained by individual differences on a latent trait variable [59]. As anterior alpha asymmetry measures trait-like emotional/motivational dispositions predicting emotive reactions to affective situations, it is of interest to know if this asymmetry is predominantly under genetic or environmental influence. Twin study data suggests that only a modest but significant amount of variance (11–28 % in children and 27 % in young adults) of frontal alpha power asymmetry is accounted for by genetic factors and that most of the variance is due to environmental influences [8, 49]. A recent review including studies of longitudinal high risk (LHR) summarizes how the high-risk (HR) youth demonstrate greater right lateral frontal EEG activation, whereas low-right (LR) youth demonstrate greater left lateral frontal EEG activation. Though it also informs about some inconsistencies regarding the influence of heritability, noticing in what way HR children between the ages of three and nine who completed resting-state EEG results reveal contrasting patterns including correlations between greater relative left frontal activity and higher levels of internalizing and externalizing problems [43]. Furthermore, Kovacs et al. [96] publish the first results from a longitudinal study about depression and dysthymia characteristics [96]; some of those HR youth diagnosed with infant depression were followed, but no significant differences were found among their children. However, relative right frontal EEG asymmetry both at baseline and in response to emotional (both happy and sad) film clips predicted elevated depression symptoms 1 year later [35, 82].

Collectively, these studies illustrate that differences in approach and withdrawal tendencies can be detected very early in development and may be predictive of a maladaptive trajectory in the development of emotion regulation that place a child at increased risk for MDD.

Given this trait-like behavior, the frontal alpha power asymmetry has been considered a good candidate to investigate affective disorders in the hope of finding a measure indexing risk of psychopathology. Hereby, the idea is that trait-like relative right frontal activity (and less left frontal activation) and its associated affective style predispose for affective disorders like anxiety and depression [26]. Nevertheless, the great environmental influence on alpha asymmetry offers the possibility to figure out environmental factors preventing the development of unfavorable frontal EEG asymmetries that could put an individual at risk for depression.

19.3.1.3 EEG Alpha Asymmetry and Depression

High levels of anhedonia, blunted affect [109], and low emotional arousal [68] characterize depressed patients. Therefore, depression is considered as an affective disorder implicating disturbed processing of emotional information. For example, depressed patients show abnormal startle reflex modulation during the presentation of affective pictures [44] and reduced facial expression [50]. From a more cognitive perspective, depressed people exhibit an attentional bias toward negative information and as a consequence fail to avoid negative valenced information [24, 56, 113]. Further, they better recall negative events [128] and make more negative

judgments about future and actual life events [11]. This resembles affective styles of subjects that react more intense with negative emotions to unpleasant stimuli and showed frontal EEG asymmetries reflecting relative greater right and less left frontal activation [160, 171]. It stands to reason that similar frontal EEG asymmetry patterns would be expected in depressed patients.

Numerous studies have shown that depressed patients exhibit relative less left and greater right anterior EEG activity [57, 73, 74, 147, 163]. Less left frontal activation has been interpreted as reflecting deficits in approach behavior associated with symptoms like sadness and depression. Greater right anterior activation has been supposed to index withdrawal behavior related to fear, disgust, and anxiety [65]. As co-morbid anxiety in depression is frequent, it is likely that anxiety levels influence anterior alpha power asymmetry patterns [66].

Remitted depressed patients showed the same frontal EEG asymmetry pattern as currently depressed subjects, suggesting that the observed anterior EEG asymmetry in depression represents a stable state-independent marker of depression [73]. Gotlib et al. [57] also reported that currently and previously depressed subjects showed the same left frontal hypoactivity compared to healthy controls. This accords with a more recent study [2] that showed that frontal EEG asymmetry stability over time in depressed patients is comparable to that observed in non-clinical samples [59]. EEG asymmetry scores remained stable over time, although patients improved clinically indicating that frontal alpha asymmetry represents a state-independent measure of depression [2]. If relative left frontal hypoactivity is a trait marker of depression, it should be able to identify people at risk for depression. In a longitudinal study, Pössel and collaborators [136] measured EEG alpha asymmetry and assessed depressiveness in 100 healthy adolescents at two time points that were 2 years apart. The research question was if left frontal EEG hypoactivity as indexed by alpha power asymmetry measures at time point, one can predict later increases in depressiveness. Indeed, left frontal and right parietal hypoactivity predicted depression supporting the notion that alpha EEG asymmetries can serve as a risk marker of depression [26].

However, as stated above, interindividual differences in frontal alpha power asymmetries were explained mainly by environmental factors during development [8, 49]. Thus, genetic factors probably predispose for the development of depression-like frontal asymmetries in unfavorable conditions. This is in line with the observation that left frontal hypoactivation was associated with lifetime depression, but not with parental depression status per se [20]. Stress could influence the development of frontal activity asymmetry. Frontal EEG asymmetry variance in adolescents was mainly explained by the lower socioeconomic status rather than by the history of depression of their mothers [163]. Lower socioeconomic status is associated with elevated stress levels as indexed by increased post-waking cortisol levels [106]. Cortisol is a key hormone during stress responses. Elevated cortisol levels are involved in the development of depression [55]. Kalin and colleagues [84] demonstrated that young rhesus monkeys with extreme cortisol levels showed also extreme frontal asymmetric electric activity with the right hemisphere being overactive compared to the left frontal cortex. In 6-month-old infants, extreme right vs. low left EEG activity was associated with elevated cortisol levels and fear reactions [22]. Right frontal and parietal hyperactivation has been related to anxiety [26, 68]. Anxiety often precedes depression. Thus, elevated environmental stress factors such as low socioeconomic status probably favor the development of right frontal hyperactivity patterns associated with withdrawal tendencies and anxiety. Later during chronic stress exposure, anxiety could turn into depression with a remaining EEG asymmetry of left frontal hypoactivation. Tops et al. [164] demonstrated a direct relationship between acute cortisol administration and changes in frontal alpha power asymmetry. In comparison with a placebo condition, cortisol provoked increased right and reduced left frontal EEG activity as measured by inverse alpha power. In sum, stable trait-like frontal alpha band asymmetry patterns that index risk for depression could

result from chronic stress exposure over lifetime, especially during childhood and adolescence.

Although studies of alpha asymmetry heredity point to environmental stress factors shaping the balance between left and right frontal cortex activity [8, 49], polymorphic gene variations affecting neurotransmitter systems that play an important role for the processing of stress-related information could determine the individual's vulnerability for psychopathology. Serotonenergic dysfunction is implicated in the onset, course, and recovery of depression (for a review, see [103]). If relative less left and increased right frontal brain activity put individuals at risk for developing depression, there should be a close relationship between interindividual genetic polymorphisms in serotonin genes and frontal alpha power asymmetries [12].

In sum, relative less left and greater right frontal EEG activity is considered a trait-like electrophysiological marker of motivational/emotional dispositions that represent a risk factor of depression. Less left frontal activity is associated with less approach-related behavior linked to less positive affect, whereas greater right frontal activity indexes greater withdrawal tendencies and negative affect. However, twin studies suggest that frontal EEG asymmetries linked to depression develop under environmental circumstances. Probably, an overexpression of HTR1a serotonin receptors in the right frontal cortex creates a vulnerability to develop frontal cortex activity asymmetries associated with depression in response to stressful life events.

19.3.2 Posterior Brain Asymmetry and Depression

The Heller's neuropsychological model of emotion [68] not only emphasizes left and right frontal brain activity associated with valence (positive and negative mood, respectively) but also assumes that right posterior cortex functioning is related to emotional arousal. From that perspective, depression has been associated with low emotional arousal due to right parietal cortex dysfunction [68, 70, 82, 120].

19.3.2.1 Classical Lesion Studies

Lesion studies investigating the association between lesion location and depression not only have found strong relationships between left frontal damage and the development of post-stroke depression. For example, Robinson et al. [139] observed the opposite pattern in the right hemisphere. The more posterior within the right hemisphere the damage was localized, the more likely patients developed depression. Interestingly, a follow-up of poststroke patients [150] revealed that the correlation between left frontal lesion site and depression only showed up during the hospitalization of the patients. At a 1–2 year follow-up of the same patients, depression was associated with right posterior damage, indicating that right posterior hemisphere lesion might be a more stable predictor of poststroke depression.

More specifically, right posterior lesions result in reduced skin conductance responses to emotional slides [114]. Skin conductance responses to emotional stimuli index autonomic arousal and vary as a function of arousal rather than valence. Therefore, poststroke depression after right posterior damage is probably related to low emotional arousal and reflects reduced affective arousal in depression per se [68].

19.3.2.2 Neuropsychological Findings of Right Hemisphere Dysfunction

To evaluate right temporoparietal cortex functioning in depressed patients, early studies utilized the chimeric face test (CFT), a free vision test to assess hemispatial biases in emotional face processing [105]. Thereby, split faces are used. One half of a face consists of a smiling, and the other half of a neutral expression. The smiling part can be either on the left or right side. Then two identical but mirrored chimeric faces are mounted on the top and the bottom of a page, and the participant has to judge which of the two faces looks happier. Normal subjects tend to choose the face with the smile on the left side [105]. As visual information from the left hemispace is projected to the contralateral hemisphere, this bias is believed to reflect an advan-

tage of right temporoparietal cortex in processing of emotional faces [19].

In the late 1980s, Jaeger, Borod, and Peselow [83] first observed that depressed subjects showed a reduced right hemisphere bias in the CFT test, suggesting a right temporoparietal dysfunction. Later, this was confirmed by comparing unipolar depressed, bipolar disorder, and left and right hemisphere-damaged patients. In this study, only unipolar depressed and right hemisphere lesion patients showed a reduced right hemisphere perceptual bias in comparison to the other patient groups and healthy controls [97].

As stated above, co-morbid anxiety has been hypothesized to result in opposite right vs. left posterior hemisphere activity [66]. Depressed patients with low anxiety should be mainly characterized by low emotional arousal. As the right temporoparietal cortex has been implicated directly in emotional arousal [70, 120], low-anxiety depressed people should show the above-mentioned reduced right hemisphere bias in the CFT test. However, depressed subjects with high anxiety associated with high-anxious arousal should show the opposite pattern. Therefore, it is important to carefully control for co-morbid anxiety as opposing effects could cancel out each other. Highly depressed students had smaller left hemispatial biases and highly anxious subjects increased right hemisphere advantages in the CFT task [67]. In a more recent study, Keller et al. [91] reported the same effects.

However, not only different levels of co-morbid anxiety seem to affect right hemispheric functioning in depression. Bruder and collaborators [19] applied the CFT test to a large sample of depressed patients. They divided the sample in those patients meeting the criteria for typical and atypical depression following the Columbia criteria for atypical depression. Thereby, typical depression is considered as melancholia subsuming symptoms like anhedonia, non-reactivity of mood, and vegetative symptoms like insomnia, anorexia, and psychomotor retardation. In contrast, atypical depression is defined as a non-melancholic subtype of depression that differs in reactivity of mood with a preserved pleasure capacity and opposite vegetative symptoms like hypersomnia and excessive eating. Comparing the hemispheric bias scores of typical and atypical patients obtained by applying the CFT test, typical depression was associated with the reduction of a right hemisphere advantage. Atypical patients even exhibited an increased right hemisphere bias compared to controls and the typical depression group. Interestingly, the same differences were observed after dividing typical and atypical patients into major depressive disorder (MDD) and dysthymia groups. Thus, with respect to right hemisphere function, typical and atypical depressed patients differ regardless if they meet DSM-IV criteria for MDD or dysthymia. In sum, right hemisphere dysfunction in depression seems to be associated with the melancholic subtype characterized by anhedonia and low emotional reactivity. This accords with the neuropsychological model of Heller et al. [68] that emphasizes that low emotional arousal in depression is related to right temporoparietal hypoactivation. However, this only seems to hold true for typical depression of the melancholic subtype (see also [18] using a dichotic listening task) and low-anxious patients.

19.3.2.3 Posterior EEG Asymmetries

Following the same line of reasoning as mentioned above, greater power in the alpha band at right vs. left parietal EEG electrode sites is considered to reflect relative less right than left parietal cortex activity. Mirroring the neuropsychological findings of right hemisphere dysfunction, many studies have found that depressed adults and also adolescents are characterized by a parietal EEG asymmetry indicating right parietal EEG hypoactivity [13, 17, 73, 82, 92]. Thereby, current and previously depressed patients showing reduced left frontal EEG hypoactivity exhibited also reduced right parietal EEG activity [73].

However, co-morbid anxiety in depression has proven to have a great influence on right parietal brain activity. High-anxious depressed patients can exhibit an opposite pattern of parietal EEG asymmetry than low-anxious depressed subjects [17] or at least attenuate the asymmetry pattern associated with less right posterior activity [92].

It has been proposed that especially anxious arousal is indicated by increased right posterior brain activity, whereas anxious apprehension is indexed by greater left posterior activation [69]. Augmented right parietal cortex activity in patients with high levels of anxious arousal is in line with the notion that the right parietal cortex is part of a cortical attentional vigilance system [36]. Thus, high-anxious patients probably have an overactive attentional vigilance system scanning the environment for potential danger. Posttraumatic stress disorder patients were characterized by increased right posterior EEG activity probably representing such a hyperactive vigilance system [116]. In contrast, depressed patients with low-anxious arousal are characterized by low emotional arousal and show right parietal EEG hypoactivity [17]. Children with low emotionality at risk for depression also show reduced right parietal hypoactivation [149]. The processing of high-arousing emotional stimuli (pleasant and unpleasant) depends on activity in cortical attention systems that probably exert top-down influences onto brain areas of basic visual processing [168]. The right parietal cortex is part of such a network active during processing of high-arousing emotional stimuli [119]. It seems that cortical attention networks are not properly engaged by motivationally significant stimuli in depressed patients.

As mentioned above, frontal EEG asymmetry seems to develop more likely under environmental influences [8, 49], whereby stress could result in the development of depression-like frontal alpha power asymmetries. In a study by Bruder et al. [20], left frontal EEG hypoactivation was associated with lifetime MDD and not with parental MDD status, whereas reduced right parietal activity depended on MDD status of the participants' parents. This indicates that right parietal dysfunction in depression probably is under a heavier genetic influence than frontal EEG asymmetries. This is in line with the observation that grandchildren with family history of MDD but no own lifetime history of depression already show posterior EEG asymmetries indicative of relative less right parietal activity [21]. However, no genetic hereditability studies of posterior EEG asymmetry have been conducted so far [121].

19.3.2.4 Event-Related Potentials and Fields

However, neuropsychological findings and EEG alpha power asymmetries are rather indirect measures of brain activity. More critically, they do not measure directly activation during emotional arousal. Thus, a better test of right temporoparietal hypofunction and its association with low emotional arousal would consist of measuring brain activity in that brain region during high vs. low emotional arousal in depressed and healthy subjects. From the motivated attention perspective of emotional picture processing, numerous studies using evoked potentials and hemodynamic brain responses investigated emotional arousal-related brain responses (a complete review of this literature is not the objective of this chapter so please refer for a review to [98]). The Lang Laboratory at the Center for Emotion and Attention Study developed the International Affective Picture Set (IAPS [99]) with normative ratings for valence and arousal. The advantage of the vast literature about brain responses and affective arousal is that they all used comparable stimulus material [98].

Electrophysiological studies recording evoked potentials generated by affective pictures from the IAPS have reported arousal modulations of early and late visual ERP components such as the N100 (between 100 and 150 ms after stimulus onset), the early negative potential (ENP, about 200 ms after stimulus onset), and the late positive potential (LPP, about 300 ms after stimulus onset). Whereas early effects were reported as less consistent in the literature, arousal modulations of the LPP were reliably observed (for a review, see [132]). The LPP is a P3-like-positive slow wave that develops between 300 and 700 ms (or longer) after stimulus onset and shows its maximum at central/parietal electrode sites [148]. The LPP has been shown to be greater for high-arousing pleasant and unpleasant stimuli when compared with low-arousing neutral pictures [25, 29, 33, 60, 61, 77, 88, 134, 144, 148]. The LPP represents an extremely replicable and stable

electrophysiological measure that varies with emotional arousal. Thus, the LPP would be ideally suited for clinical research.

Indeed, ERP studies of emotional perception in depression found impaired P3 modulations at right hemisphere electrode locations in depressed patients [10, 30, 87]. The consistent ERP findings in healthy controls obtained by using the IAPS could help to elucidate differences in the processing of high-arousing emotional stimuli in depression. Given the above-revised literature, reduced arousal modulation of the LPP across right parietal electrodes would be expected for depressed patients.

In clinical research, not only stable and reproducible experimental protocols are needed, but they also should be of short duration. In EEG and MEG (magnetoencephalography is a technique measuring the magnetic fields generated by the electric activity of synchronized neurons) research, the steady-state response represents a good candidate to fulfill those criteria. The steady-state response is an oscillatory evoked potential/field that develops by presenting the affective pictures in a luminance-modulated mode. One could imagine the same picture presented for several seconds but flickering. The flickering occurs at a stable frequency of, e.g., 10 Hz (others frequency can be used as well). That means that the same picture is presented in an on/off cycle of 50 ms, showing the picture for 50 ms followed by 50 ms of background color. This cycle is then repeated for several seconds. Presenting visual stimuli in that way generates the so-called steady-state visual evoked potential (ssVEP in EEG) or field (ssVEF in MEG). It is an oscillatory brain response with the same frequency as the driving stimulus [138]. Thus, a 10 Hz flickering picture evokes an oscillatory brain wave at a frequency of 10 Hz.

The advantage of this technique is that within a short recording time, one can obtain very good EEG and MEG signals [112, 167]. The flickering mode allows a densely repeated presentation of the stimuli during a short recording time. Imagine that a specific picture is depicted at a flickering mode of 10 Hz. Thus, the same picture is shown ten times within 1 s (one cycle is 100 ms). If the picture is presented for 6 s, the same picture is shown 60 times. This results in a very good signal to noise ratio with respect to EEG/MEG evoked potentials/fields. To obtain an evoked EEG/MEG response, the stimuli have to be repeated many times and the stimulus locked signals to be averaged. More repetitions lead to higher signal to noise ratios. Steady-state paradigms implicate many stimulus repetitions.

Another advantage of the steady-state paradigm is that attentional processes across several seconds of picture viewing can be observed. Numerous studies have found that attended visual stimuli that are presented in a steady-state fashion produce steady-state oscillations of greater amplitudes than non-attended stimuli [122–124]. The motivated attention approach to emotion emphasizes that high-arousing emotional stimuli attract automatically attention [90]. This leads to top-down facilitation of the visual processing of high-arousing stimuli, involving the visual cortex and higher-order cortical attention networks, such as the frontal and parietal cortex of the right hemisphere [168]. Keil et al. [89] demonstrated that high-arousing pleasant and unpleasant pictures generated greater ssVEP responses than low-arousing neutral pictures. Using MEG, Moratti et al. could show that arousal-modulated ssVEF responses were generated in the right occipital and frontoparietal brain regions [120]. This group tested the hypothesis of temporoparietal dysfunction and low emotional arousal in depression more directly by using the abovementioned steady-state paradigm. The rationale behind this study was that ssVEF responses of healthy subjects recorded by MEG were modulated by emotional arousal [119]. This arousal modulation was observed in cortical attention networks of the right hemisphere. As the right temporoparietal cortex is part of that network, depressed patients should show a reduced modulation of their ssVEF responses in the right temporoparietal cortex. Indeed, low-anxious clinically depressed patients exhibited reduced emotional arousal modulation of their ssVEF response in right temporoparietal cortex; in contrast, healthy subjects showed the strongest emotional arousal modulation of ssVEF activity in that brain region [120].

19.4 Overview of Perspectives of EEG and MEG Analysis

The acknowledgement that "mental illnesses" are, at least in part, caused by some kind of brain dysfunction yielded a radical change in the methods of basic research and clinical practice within the fields of psychiatry and psychopathology [4]. Major depression (MD) is a good example of this radical change. During the last three decades, considerable investigation efforts focused on brain imaging and neurophysiological/psychophysiological techniques, such as positron emission tomography, structural and functional magnetic resonance imaging, quantitative EEG (QEEG), event-related potentials (ERPs), magnetoencephalography (MEG), etc. These brain imaging methods proved that MD has a definite neurobiological correlate. In fact, pharmacological interventions are usually the first choice for MD treatment, and such interventions are based on the assumption of an underlying neurobiological dysregulation. Nonetheless, the attempts to use brain imaging techniques to assist the diagnosis, to select treatment alternatives, or to objectively evaluate clinical outcomes are scarce. EEG, ERPs, and MEG may be of particular importance since they offer a set of noninvasive tools to assess brain activity. The number of EEG-/MEG-based studies on MD is difficult to estimate, but they undoubtedly represent a fundamental body of replicated evidence showing significant differences between patients with depression and healthy controls. In addition, EEG is an easily available and relatively unexpensive technique with widely utilized and replicated analysis algorithms. MEG is a more expensive and less broadly employed technique, but it presents some technical advantages when compared to EEG, specially its capability to calculate the sources of brain electromagnetic activity, and consequently should be considered a suitable research and clinical tool in MD. Importantly, the suitability of EEG-/MEG-based techniques in MD investigation may have some physiological determinants. Hughes and John [79] reviewed in a key article the underlying physiology of EEG (and indirectly MEG) dynamics and its potential relationship with the pathophysiology of mental illnesses. According to their point of view, EEG rhythms derive from the synchronous activity of neural groups localized in some key brain structures such as the thalamus or the mesencephalic reticular formation which project to the cortex. Those key structures are mediated by neurotransmitters and neuromodulators such as the gamma-aminobutyric acid (GABA), serotonin, acetylcholine, norepinephrine, or dopamine, all of them involved in neurobiological models of psychiatric diseases, including MD. As a consequence, any dysregulation in the neurochemical homeostasis underlying EEG generators should produce, in turn, a modification of the EEG spectrum. Since neurotransmitter perturbations are widely believed to contribute to the psychiatric pathophysiology, EEG-/MEG-based techniques should be considered as first-election tools in psychiatry.

Despite this particular sensitivity, the acceptance of EEG (or QEEG) and MEG within the psychiatric community has been slow and scarce. Hughes and John proposed two major reasons to explain the situation:

1. The abnormalities reported in early EEG studies of visual inspection have been considered too unspecific. Recent quantitative investigations showing more stable and robust differences have not been published in psychiatry journals but rather in neurology or neurophysiology publications. Consequently, result presented in those investigations might have not reached the right target audience.
2. Position papers published by professional organizations over the last decades concluded that EEG is of limited and adjunctive clinical use in psychiatric practice.

Here we will present an overview of EEG and MEG studies on MD. We will focus not only on basic research but also on potential clinical applications. In this vein, we consider that some specific fields of research, such as those devoted to the prediction of the therapeutic response to antidepressant drugs, have attained sufficient importance to receive close attention from clinical practitioners.

19.4.1 Frequency Bands Other Than Alpha

As it was described in the previous section, the majority of EEG studies on MD focused on alpha activity and particularly on the so-called alpha asymmetry. However, other frequency bands, especially in the low-frequency range, deserved the attention of investigators. The presence of sporadic or generalized low-frequency activity, particularly in the delta range, is considered a major sign of brain disturbance (see, e.g. [51]), and increased low-frequency activity is systematically observed in dementias, brain trauma, hypoxia, etc. Interestingly, low-frequency investigations on MD tended to show reduced power or current density values. Studies, such as [15], showed that depressed patients differed from demented patients at the lower end of the spectrum, having significantly less delta and theta activity. The comparison of demented and aged depressed patients is important since both groups share some cognitive and emotional symptoms. Pozzi et al. [137] performed QEEG evaluations in depressed Alzheimer's disease patients, nondepressed Alzheimer's patients, nondemented depressed patients, and healthy aged controls. Similarly to Brenner et al.'s results, they found reduced delta power in nondemented depressed patients as compared to both demented groups. Moreover, in Pozzi et al.'s study, delta power values were also lower within the nondemented depressed group compared to healthy aged controls.

More investigations are employing analysis techniques such as the low-resolution electromagnetic tomography (LORETA) [133]. Mientus et al. found significantly lower source-current densities in unmedicated depressed patients in all bands, but delta and theta shared the characteristic that the center of that reduced current density was the anterior cingulate areas [117]. We will see that theta current density in anterior cingulate cortex has a critical role in the prediction of response to antidepressants. Lubar et al. [110] obtained current density values by means of LORETA analysis in a group of depressed females and healthy controls. Overall, they found reduced current density values in the delta band within the depressed group. This effect was more evident in the right temporal lobe, and authors interpreted the finding as produced by a lack of basic modulation properties in this region probably due to a lack of "small-scale local organization." LORETA solutions were also utilized by Flor-Henry et al. [42]. In their interesting study, they hypothesized that depression can be characterized by a prefrontal hypoactivation, and, as usual, the hypoactivation is reflected by an alpha asymmetry. However, results did not support their hypothesis. The expected left anterior hypoactivation (increased resting alpha current density) was not seen in their results, but a reduced current density in the delta band was observed in the left hemisphere.

Decreased low-frequency activity has been also observed in MEG investigations [38, 173]. Wienbruch et al. calculated dipole density values within the delta and theta range in a group of schizophrenic patients, depressed patients, and healthy controls. While schizophrenics showed accentuated delta and theta activity in temporal and parietal regions, depressives exhibited reduced frontal and prefrontal delta and theta dipole densities. As in previous investigations, such reduced low-frequency activity was associated with a dysfunctional pattern of local activation. Wienbruch et al. pointed out that previous EEG and MEG studies demonstrated increased delta power and dipole densities after effective electroconvulsive therapy [146, 154]. Interestingly, the increase of delta dipole density was also observed after effective pharmacological treatment but to a lesser and nonsignificant extent (see also [94]). This line of evidence suggests that the activity in delta and theta bands seems to be reduced in MD, and an effective treatment modifies this abnormal pattern making low-frequency values closer to those of controls.

High-frequency activity was also investigated in MD. For example, Knott et al. [93] performed a comprehensive study trying to investigate not only the alpha asymmetry but also other parameters such as absolute and relative power in all bands, mean frequency values, hemispheric coherence, etc. Their results failed to show the

expected alpha asymmetry. MD patients exhibited higher absolute and relative beta values, a higher mean frequency (tendency to values in the higher range of the EEG spectrum), and reduced interhemispheric coherence (coherence results will be discussed elsewhere). Pizzagalli et al. utilized LORETA solutions to investigate the influence of concomitant factors such as anxiety, melancholic features, severity, etc. [135]. They found no effects in alpha band. Instead, depressed patients showed more beta3 (21.5–30 Hz) than controls on Brodmann areas 11 and 9/10 of the right frontal lobe and less beta3 in the posterior cingulate gyrus and precuneus. Of note, Beck Depression Inventory values were positively correlated with frontal asymmetry in beta3. According to Pizzagalli et al.'s interpretation of their results, depressed patients were characterized by relative hyperactivity in the right frontal regions and hypoactivity in the posterior cingulate and precuneus. Right frontal hyperactivity was most conspicuous in melancholic patients who were severely depressed. These results demonstrate that clinical characteristics (i.e., severity, melancholic features) are somehow modulating the brain activity of MD patients. Following up the investigation of high frequencies in MD [156], they reported that the power of gamma rhythm in the frontal and temporal areas of the cortex was significantly greater in patients with depression than in normal subjects. These results confirm the tendency to elevated power and current density values in the high-frequency bands.

19.4.2 Connectivity in MD: Coherence and Synchronization Studies

EEG and MEG connectivity studies are noninvasive tools for studying functional relationships between brain regions. Therefore, they are assumed to reflect functional interactions between neural networks represented on the cortex. Similar to other imaging techniques, connectivity investigations on MD are relatively recent. Coherence algorithms have been widely utilized in psychophysiology, and they usually compute the squared cross-correlation in the frequency domain between two EEG/MEG time series measured in two different scalp locations [76]. Reduced coherence values in MD are a common finding. Roemer et al. [140] obtained interhemispheric coherence values in elderly depressives and found that patients had lower than normal anterior interhemispheric coherence in all frequency bands (i.e., delta, theta, alpha, beta). Knott et al. [93] also found reduced interhemispheric coherence values in MD patients for all frequency bands. Furthermore, the combination of delta and beta interhemispheric coherence and alpha interhemispheric asymmetry yielded a 91.3 % of overall classification capability in a depressives vs. control discriminant analysis. As authors pointed out, the main problem with coherence estimates is their interpretation in terms of functional and/or anatomical connectivity. According to Roemer et al.'s and Knott et al.'s results (see also [45, 108], it might posed that interhemispheric synaptic connection is reduced in MD and other affective disorders [45] or that an imbalance exists between the functional processes of both hemispheres. However, coherence results should be interpreted with caution.

Fingelkurts et al. performed a fine synchronization studies on EEG in unmedicated depressive patients [40]. First, they criticized the current "state of the art" in the field of MD psychophysiology, mostly dominated by the alpha asymmetry paradigm. Authors claimed that modern models of mind disorders consider the disease as produced by a change in the autonomy and connectedness of different brain systems that sustain health. According to this, connectivity estimates seem to be more appropriate to untangle the pathophysiology of MD. Fingelkurts and coworkers utilized a synchronization measure called "structural synchrony index" (see [39]) which estimates general nonlinear interdependences between dynamic systems. Contrary to coherence findings, authors hypothesized that MD is characterized by an increase of functional connectivity. Their results confirmed the hypothesis that depressive patients showed a state of increased and "strengthened" functional connectivity of brain processes, especially for what they

called "short-range distances" (i.e., closer electrode pairs). Other authors find similar results; Leuchter et al. [104] recently reported an increased topographic EEG coherence between frontal brain areas in MDD in the EEG alpha, beta, and theta bands [104]. Further Lee et al. [101] found that responders to antidepressant treatment, compared with nonresponders, showed increased coupling of EEG power at the delta and theta frequencies between right frontoparietal electrodes [101]. Olbrich [131] et al. show an increased functional connectivity by means of EEG lagged phase synchronization during rest between the subgenual prefrontal cortex and the left dorsolateral prefrontal cortex and the left medial prefrontal cortex within the EEG alpha frequency band in MDD in comparison to findings in healthy controls [131].

Leistedt et al. [102] utilized synchronization likelihood, a measure of generalized synchronization (see [155]), to assess connectivity patterns in depressed patients and healthy controls. Results indicated that (1) the acute state of depressive disease was characterized by a decrease in the global mean levels of the EEG sleep synchronization and (2) a major depressive episode displays a significant reorganization of the neural brain networks. These findings may be of particular relevance, since slow-wave activity synchronization may play a role in the hypothesized importance of slow-wave activity during sleep for cognitive performance [78]. Cognitive problems, including executive dysfunctions, attention alterations, memory impairments, etc., are strongly associated with a depressive episode.

19.4.3 Brain Complexity in MD

Complexity analysis is an emerging field of investigation within the theoretical background of nonlinear analysis methods. The fundamental assumption of nonlinear analysis is that EEG or MEG signals are generated by nonlinear deterministic processes with nonlinear coupling interactions between neuronal populations. Complexity analysis is a particular form of brain activity nonlinear analysis. Brain complexity, as estimated by EEG-MEG signals, has received different interpretations which usually highlight the degree of randomness-predictability and/or the number of independent oscillators underlying the observed signals [53, 111] Particularly, Lempel-Ziv complexity (LZC), one of the most frequently utilized complexity measures, represents an estimate of the number of different frequency components that actually compose the brain signals [1]. Overall, higher variability and number of independent oscillators yield higher complexity scores.

Early studies of complexity in MD [125] indicated a higher predictability of EEG signals in depressive patients and consequently a decrease of complexity scores. This lower complexity was interpreted as associated with a lower level of environmental interaction, but ulterior investigations contradicted Nadrino et al.'s results. For example, Thomasson et al. observed that averaged global entropy slightly decreased during treatment in patients with depression, suggesting an important significant correlation with mood modulation in all depressive patients [157]. The main objective of Thomasson and coworkers was the demonstration of a longitudinal covariance between brain dynamics complexity (represented by global entropy levels) and mood using different treatment approaches in patients with depression. This study demonstrated a clear association between clinical outcome and brain dynamics reorganization. An ulterior paper by Thomasson and coworkers [158] confirmed such association. In their intriguing article, Thomasson's group presented a case study of a 48-h cyclic manic-depressive patient where they investigated the correlation between mood variations and EEG nonlinear characteristics, such as entropy values. The correlation between entropy values and clinical self-assessment scale's values was impressive ($\rho = 0.92$); but even more interestingly that so high correlation was explained because higher entropy levels were clearly associated with depressive phases, while lower entropy levels were clearly associated with hypomanic phases. According to these results, it may be hypothesized that depression is associated with higher

brain's complexity. Two recent investigations using LZC methodology supported this hypothesis. First, Li and coworkers [107] measured LZC derived from EEG signals in patients with schizophrenia, patients with psychotic depression, and healthy controls. Both patient groups showed higher complexity values when compared to healthy controls. Such tendency to higher LZC in MD was further confirmed by Méndez and coworkers [115] in their very recent study. Authors performed baseline and follow-up evaluation of spontaneous MEG scans in MD patients. At baseline, depressives showed higher LZC values, as compared to controls, and failed to exhibit the "normal" tendency to increased complexity values as a function of age observed in controls and described in previous investigations [6, 37]. After 6 months of treatment with mirtazapine, LZC values were reduced in all patients, making them more similar to controls' LZC scores. Moreover, MD patients recovered that "normal" tendency to increased complexity values as a function of age. Thomasson's and Méndez's articles seem to prove that depressive phases are associated with a higher complexity of EEG/MEG signals and also that symptoms' remission produces a reduction of the initially elevated complexity levels.

How can we explain this abnormal increase of brain's activity complexity in MD? As it was above mentioned, complexity estimates assess the variability and number of generators of EEG/MEG signals. Some early electrophysiological studies in schizophrenia emphasized a so-called dysrhythmia [80], defined as abnormally elevated frequency variability and reduced amplitude of EEG traces. Such abnormally elevated frequency variability in schizophrenia correlates with higher complexity scores (see, e.g., [34]). Is higher-frequency variability a common finding in depression studies? Certainly it is not, but the analysis of frequency variability was not a matter of interest in the field of depression, dominated by traditional power spectrum analyses. Recently, Fingelkurts and coworkers [41] published a key investigation on this issue. They employed a novel method for EEG analysis, called "probability-classification analysis of short-term EEG spectral patterns," which is supposed to "extract" the exact composition of brain oscillations. Two major findings were reported: (1) MD patients' EEG traces were characterized by a reorganization of their oscillatory activity which affected not only the frontal lobes but more importantly the posterior cortex of the brain and (2) the reorganization of the oscillatory activity in MD was characterized by more segments of polyrhythmic/disorganized activity, as compared to control subjects. This study supports the notion of increased frequency variability in MD which might explain the elevated complexity levels observed in EEG and MEG studies. Here it is important to note that complexity estimates demonstrated a "particular" sensitivity to detect the dynamical changes observed in MD (see, e.g., the association between symptoms' remission and complexity variations), and such sensitivity might play an important role in the clinical application of EEG/MEG within this field.

19.5 Summary

Studies based on startle reflex paradigms agreed with the Rottenberg's *emotion-context insensitivity model: d*epressed individuals consistently displayed deficits in the processing of and responses to positive hedonic stimuli on self-report behavioral and psychophysiological measures. These deficits may be considered a vulnerability marker to depression related to serotonergic and stress mechanisms.

The neuropsychological model of depression as put forward by Heller et al. [68] emphasizes left frontal and right posterior hypoactivation as a key feature of cortical activity pattern in depression. Lesion, EEG, and MEG studies have supported this model. However, co-morbid anxiety and depression type exert an important influence on frontal and parietal activity asymmetry. Low-anxious melancholic depression seems to result in the previously described cortical asymmetry pattern of left frontal and right parietal hypoactivation indexing negative affect and low emotional arousal, respectively. High-anxious depressed patients and atypical depression-type

patients may be characterized by increased right temporoparietal activity indexing augmented emotional arousal. Whereas depression-like frontal EEG asymmetry may develop under chronic environmental stress exposure, posterior activity asymmetry could depend more strongly on genetic factors.

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Chronobiology of Mood Disorders

20

Felice Iasevoli, Livia Avvisati, Valentina Gilardi, Gianmarco Latte, Emiliano Prinzivalli, Domenico de Berardis, Alessandro Valchera, Michele Fornaro, Carmine Tomasetti, and Andrea de Bartolomeis

20.1 Anatomy and Physiology of the Suprachiasmatic Nucleus as a Circadian Rhythm Central Regulator

20.1.1 The Suprachiasmatic Nucleus and Its Interaction Pathways

Several biological and behavioral processes have cyclic rhythms, and many of these are characterized by variable duration. Some examples are circadian rhythms (from the Latin term *circa diem*), which last 24 h and present a light-dark alternation; ultradian rhythms (which last less than 24 h); and infradian rhythms (which last more than 24 h) [1]. Beyond being the result of fluctuations in the natural environment, biological rhythms are due to the influence of other elements. The main ones are circadian clocks, synchronizing inputs, and various central and peripheral oscillators [2, 3]. The organization of

F. Iasevoli, MD, PhD (✉) • C. Tomasetti
Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Building 18, 3rd floor, Naples 80131, Italy

Polyedra Clinical Group, Teramo, Italy
e-mail: adebarto@unina.it

L. Avvisati • V. Gilardi • G. Latte • E. Prinzivalli
A. de Bartolomeis
Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Building 18, 3rd floor, Naples 80131, Italy

D. de Berardis
NHS, Department of Mental Health, Psychiatric Service of Diagnosis and Treatment Hospital "G. Mazzini", Teramo, Italy

Department of Neurosciences and Imaging, and Clinical Sciences, University "G. D'Annunzio", Chieti, Italy

A. Valchera
Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Building 18, 3rd floor, Naples 80131, Italy

Polyedra Clinical Group, Teramo, Italy

Villa S. Giuseppe Hospital, Hermanas Hospitalarias, Ascoli Piceno, Italy

M. Fornaro
Polyedra Clinical Group, Teramo, Italy

Department of Education Science, University of Catania, Catania, Italy

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the periodicity of biorhythms is based on the action of structures having an intrinsic rhythmicity, which are named *pacemakers*.

Pacemakers are mutually interacting structures located in the central nervous system (CNS). In turn, CNS is the central oscillator and controls rhythms of endocrine nature, blood pressure, body temperature, liver metabolism, and, finally, the sleep-wake cycle [4]. Among CNS structures, the hypothalamic suprachiasmatic nucleus (SNC) is an essential element in regulating biological rhythms. The SNC acts as a master pacemaker (which means it generates biological cycles in all mammals with an intrinsic rhythmicity that normally lasts over 24 h), and the autonomic nervous system plays an important role as a hand of this pacemaker.

The SNC consists of approximately 20,000 neurons, each of them displaying independent rhythms of firing rate and gene expression [5]. The SNC derives its name from the fact it is located in the hypothalamus above the optic chiasma [4–6]. More precisely, it is located between the neural structures that receive signals from the outside environment, mainly through the retina and the optic pathway, and the effectors that regulate rhythm functions. Interestingly, animals undergoing an experimental alteration of the SNC display a loss of circadian cycles, including rest activity or drinking habits [4]. Alterations are reverted to the functioning before the lesion once the animals receive a transplant of fetal SNC [7].

An essential element in the rhythmicity of the central pacemaker is represented, through the process of "entrainment," by *zeitgebers* (or external time givers). One of the main zeitgebers is light, which has an essential role since it influences the functioning of several oscillator systems by entraining the master clock [1, 3].

Three main input pathways influence the functioning of the SNC, the first of which is the retinohypothalamic tract (RHT). The SNC receives a direct photic input from photoreceptor cells called "intrinsically photoreceptive retinal ganglion cells" (ipRGCs). These cells generate melanopsin, a photopigment that causes them to be intrinsically photosensitive to short wavelength irradiation and to release glutamate [5, 8].

The second and the third input pathways to the SNC are, respectively, the GHT (geniculohypothalamic tract) and the 5HT (serotonergic) inputs. The GHT and the raphe nuclei (both the dorsal raphe and the median raphe nuclei) send nonphotic timing stimuli, and in this process a central role is played by the serotonergic system, suggesting a key role of serotonin in the circadian activity of the hypothalamus and the epiphysis. In their action on the SNC, each of these essential inputs seems to inhibit the phase changes induced by the other [1, 2, 9–11].

20.1.2 Functional Significance of Anatomical Connections

The SCN has a fundamental role in activating circadian rhythms, such as body temperature, motor activity, sleep, nutritional and sexual behavior, and the secretion of ACTH, prolactin, gonadotropin, and melatonin. The biological actions mediated by SCN are in turn modulated by multiple input and output projections.

The autonomic nervous system plays an important role as a hand of the SCN master pacemaker [2]. The paraventricular nucleus of the hypothalamus (PVN) is the main output projection of the SCN. PVN regulates visceral functions, and it turns the signals sent by the SCN into hormonal and autonomic signals for peripheral organs through the autonomic neurons and the release of corticotropin-releasing factor from secreting neurons that compose the hypothalamus-pituitary-adrenal axis [1, 3].

SCN contributes to control the secretion of several hormones and neuropeptides. The SCN uses neural and hormonal signals from output projecting fibers to activate the circadian secretion of glucocorticoids [12], as shown by the control of daily corticosterone rhythm. In addition to the neuroendocrine control of the adrenal cortex by the PVN-CRH-ACTH cascade, the autonomic projections by which the SCN reaches the intermediolateral cell columns through the PVN promote the infradian modifications of adrenal gland's sensitivity to ACTH [2]. Another set of output projections from the SCN reaches the MPOA (medial preoptic

area), which is enriched of gonadotropin-releasing hormone (GnRH)-containing neurons and causes the rhythmic release and reproduction of GnRH and prolactin [3].

SCN is also pivotally involved in the regulation of melatonin secretion. The melatonin synthesis pathway is known to be stimulated by the noradrenaline released from the sympathetic nerve terminals of the pineal gland. However, the synthesis of melatonin is modulated by the SCN through a multisynaptic neural pathway, which includes the pre-autonomic neurons of the PVN, the sympathetic preganglionic neurons of the intermediolateral cell column of the spinal cord, and the noradrenergic sympathetic neurons of the superior cervical ganglion [3, 13].

The SCN-mediated modulation of melatonin secretion occurs mostly by GABAergic projections from the SCN to the PVN. Exposure to daylight activates SCN neurons, thereby promoting GABA release from SCN output fibers as a reaction to the light-induced activation of SCN neurons. SCN-mediated GABA release on PVN neurons results in an inhibition of the production of melatonin [3, 14]. Despite being only indirectly controlled by the SCN, melatonin has remarkable importance in mediating the chronobiological rhythms that are hand-mastered by the SCN. Melatonin interacts with two subtype receptors (Mel_{1a} and Mel_{1b}, respectively, known as MT1 and MT2), which are mainly localized in the CNS. Noticeably, melatonin receptors have also been detected outside the CNS, e.g., in blood platelets, in granulosa cells from preovulatory follicles, in lymphocytes, in the mucosa and submucosa of the colon, in prostate epithelial cells, and in spermatozoa [13]. As a consequence of the widespread distribution of melatonin receptors, melatonin may be intended as a peripheral effector of chronobiological rhythms tuned by the SCN. Therefore, melatonin acts in colligating the photic-mediated circadian rhythms controlled by the SCN to a wide range of biological functions, including immune system, cardiovascular, reproductive, or metabolic functions, emotional behaviors, and sleep regulation [15–18].

SCN also has an "oscillatory" role, since it coordinates and synchronizes the activity of other oscillators (defined as "slave") located in other areas of the brain (i.e., the cortex) and in peripheral organs (i.e., the liver or the kidney), each of which is a multi-oscillator unit [19]. Altogether, there anatomical structures appear to be organized in a SCN-headed multi-oscillator hierarchical system, which gives stability and a specific phase control to the physiological systems that it regulates.

Several researches in the past years have attempted to clarify the molecular steps involved in biological rhythm generation by the SCN. The circadian oscillator system consists of several interconnected loops having a positive and negative feedback that interacts at the levels of gene transcription, translational and posttranslational regulation [4].

The main proteins and their coding genes implicated in this system are the CLOCK (Circadian Locomotor Output Cycles Kaput); the BMAL1 (Brain and Muscle ARNT-like protein 1); the Period, Timeless, and Cryptochrome; the NPAS-2 (Neuronal PAS domain protein-2); and the Fer2 and nocturnin genes [1]. Among these, the *CLOCK* and the *BMAL1* genes have an important role in generating biological rhythms. The products of these genes form heterodimeric protein complexes that are translated into the nuclei and bind to the promoters of various genes, by means of specific E-box motifs. The CLOCK/BMAL1 heterodimeric complex stimulates the transcription of the period genes *per1*, *per2*, *per3*, as well as of the cryptochrome genes *cry1* and *cry2* [20]. In turn, PER and CRY proteins form the PER/CRY cytoplasmic complexes that, once reached sufficient levels, move to the nucleus inhibiting the action of the CLOCK/BMAL1 complexes and, consequently, the transcription of PER and CRY.

The length of this cycle of transcription and translation is determined by the stability of the heterodimeric complexes. The inhibitory PER/CRY complexes are subsequently degraded by proteasome enzymes following their phosphorylation by CKIε (casein kinase I epsilon) and CKIδ (casein Kinase I delta), which in turn remove the inhibition on CLOCK and BMAL1 genes' expression. This mechanism allows the

feedback loop to restart in a 24-h cycle. However, several other genes and proteins (e.g., RORA, Rev-erbα) are critically implicated in generating rhythmicity [20, 21].

20.2 The Sleep-Wake Cycle

20.2.1 Neurobiology of the Sleep-Wake Cycle

Among the multiple circadian rhythms that take place, the sleep-wake cycle is most presumably the more apparent one and that whose effects are most clearly noticeable on mood changes. Also, sleep disturbances are among the core clinical manifestations in mood disorders, represent common residual symptoms in partial remitting mood disorders, and are often early signs of a re-exacerbation of an affective episode. Therefore, the neurobiology of sleep-wake cycle is integral to the chronobiology of mood disorders.

Sleep is an alternating pattern of neural activity that arises from multiple brain regions, neurotransmitter systems, and modulatory hormones. Collectively, sleep is organized in rapid eye movement (REM) and non-rapid eye movement (NREM) stages. Upon the initiation of sleep, an individual goes slowly across stages 1–4 NREM sleep and then rapidly ascend these stages into REM sleep, which is characterized by a considerable motor atonia. Every night, four to five NREM/REM sleep cycles take place, with period lengths between 80 and 110 min [22]. This complex behavior is supposed to arise from the functional interplay between the ascending arousal circuitry – which promote vigilance and wakefulness – and the hypothalamic sleep circuitry, according to both circadian and homeostatic mechanisms [23].

20.2.2 The Ascending Arousal Circuitry

The ascending arousal system is located in the upper pons and consists of two pathways, one projecting to the thalamus and the other projecting to hypothalamus and basal forebrain, both of which activate the cerebral cortex. The first pathway derives from cholinergic neurons in the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei and projects to the relay nuclei (ventroposterior or mediodorsal nuclei) and to the reticular nucleus of the thalamus, which primarily contains GABAergic neurons. Since these GABAergic neurons project in turn to thalamus by a feedback inhibitory loop, the activation of the reticular nucleus facilitates the thalamocortical transmission of sensory inputs, promoting the cortical activation and the wakefulness state [24–26]. The second main arousal pathway consists of monoaminergic projections from noradrenergic locus coeruleus (LC), glutamatergic parabrachial nucleus (PB) and precoeruleus area (PC), serotonergic dorsal raphe (DR), dopaminergic ventral periaqueductal gray matter (vPAG), and histaminergic tuberomammillary nucleus (TMN) [25, 26]. Their ascending axons converge to the lateral hypothalamic orexin neurons and to the cholinergic and GABAergic neurons of basal forebrain, which in turn directly innervate the cerebral cortex and contribute to behavioral arousal [27]. The orexin neurons, which are located in the posterior lateral hypothalamus, contact neurons at multiple levels of the sleep-wake regulatory system [27]. Because the two types of orexin receptors actually recognized are excitatory, orexin is supposed to activate its targets, including the ascending monoaminergic systems, the basal forebrain cholinergic system, and the REM-off neurons located in the vlPAG and lateral pontine tegmentum (LPT). Therefore, the net effect of orexin pathway is to stabilize the normal waking state and prevent rapid transition in REM sleep [28, 29].

20.2.3 The Hypothalamic Sleep Circuitry

Normal transitions from wakefulness to sleep involve sleep-related inhibition or disfacilitation of the multiple arousal systems. The neuron clusters that switch off the arousal network, at sleep onset, reside in the ventral lateral preoptic area (VLPO) and in the median preoptic nucleus (MnPO) of the hypothalamus [30]. These two

nuclei, whose projections are inhibitory, strongly fire during sleep and innervate most of the components of the arousal network. VLPO neurons contain both GABA and galanin, an inhibitory neuropeptide, while MnPO neurons contain only GABA [30, 31]. Evidence indicates that these two nuclei have complementary functions and may synergistically cooperate to inhibit the arousal systems; indeed, during sleep deprivation in animals, only the firing of MnPO neurons is elicited, but the VLPO neurons are not activated until the animal actually falls asleep [32]. Thus, the MnPO neurons seem the first to receive homeostatic signals indicating accumulated need of sleep, i.e., increased levels of extracellular adenosine in the basal forebrain, and could in turn activate the VLPO neurons [26, 32].

Other hypothalamic neurons, which contain the peptide melanin-concentrating hormone (MCH) as well as GABA, also are most active during sleep. These neurons specifically inhibit the monoamine systems and the REM-off cell groups, thus contributing to promote the transition from waking to sleepy state [26, 33].

20.2.4 The "Flip-Flop Switch" Model

Preoptic hypothalamic neurons are activated during sleep and exhibit sleep-wake state-dependent discharge patterns that are reciprocal of that observed in several arousal systems [30].

The GABAergic neurons in the VLPO nucleus are also innervated by inhibitory projections deriving from the arousal systems. Indeed, during wakefulness, the activity of the monoaminergic cell groups (i.e., LC, TMN, DR, PB/PC, and vPAG) inhibits both the preoptic sleep-promoting neurons (VLPO and MnPO) and pontine REM-promoting neurons (located in PPT and LDT nuclei) [24–26].

Therefore, the arousal network seems to act in both anatomic and functional antagonism to the sleep hypothalamic circuitry, producing a condition called *flip-flop switch*. In this model of switching system, when one side gains control, it turns off the other side, thus stabilizing its own firing. Such switches are conceived in electronic engineering to obtain two stable states with rapid transitions between them. Most people spend 98 % or more of their day in one state or the other and less than 2 % in transition [26]. This seems to occur because of the mutual antagonism between sleep-promoting and wake-promoting systems in the brain [25, 26, 34].

20.2.5 The Generation and Regulation of Sleep-Wake Cycle

Normal cycling between sleep and wakefulness is mediated by the combined influence of (i) a physiological homeostatic sleep pressure that increases with continued wakefulness and dissipates with sleep and (ii) an intrinsic 24 h circadian oscillation [26, 30]. According to recent evidence, the former represents a homeostatic mechanism possibly triggered by an extracellular increase of sleep-promoting metabolic products (i.e., adenosine) in the basal forebrain [35]. The latter represents a baseline neurobiological rhythm driven by the central master pacemaker in the SCN in response to environmental stimuli, mainly represented by the light-dark cycle [30]. Under normal circumstances, the two processes work in concert to induce wakefulness during the day and sleep during the night [36]. In addition to light levels, also social cues, stress hormones, orexin, melatonin, and sleep-promoting metabolic products, such as adenosine, interleukin 1 (IL-1), and tumor necrosis factor-alpha (TNF-alpha), all play key modulatory roles in sleep-wake cycle [35, 36].

During daytime, both SCN and the arousal network are active and reduce, via direct and indirect mechanisms, the VLPO and MnPO firing, with a net push toward wakefulness. For instance, the SCN directly reinforces monoaminergic activation with efferent tracts to the LC and DR nuclei; moreover, the SCN-mediated secretion of corticotropin-releasing factor (CRF) by PVN inhibits the secretion of melatonin from pineal gland. In addition, orexin neurons strongly fire during wakefulness, further stabilizing the monoaminergic activation [26, 36].

Under conditions of dim light, the SCN induces, via efferent projections to the PVN, the

production of melatonin in the pineal gland. As night approaches, the combination of (i) circadian increase in melatonin levels, (ii) circadian decrease in CRF levels, and (iii) homeostatic build-up of sleep-promoting molecules in basal forebrain (such as adenosine, IL-1, and TNF-alpha) cooperates to activate the VLPO and MnPO firing, with the net effect of turning-off the arousal circuitry and shifting the global balance toward sleepiness [36].

20.2.6 Neuroimaging of Sleep

In order to describe the global and regional brain activity patterns during NREM and REM sleep, imaging studies of sleep stages have been initially conducted with positron emission tomography (PET) by using H2(15)O or 18FDG, and more recently with functional magnetic resonance imaging (fMRI), in some cases combined with simultaneous EEG recording. A brief summary of these findings is provided below.

In the past two decades, PET studies have shown that NREM sleep is associated with global or regional decreases of cerebral metabolic rate of glucose (CMRglu) or regional cerebral blood flow (rCBF), when compared to wakefulness, in specific subcortical (i.e., brainstem, thalamus, basal forebrain, basal ganglia, cerebellum) and associative cortical (i.e., prefrontal, anterior cingulate, precuneus) regions [37–41].

Notably, decreases were observed in brain areas representing the most active ones during wakefulness [42]. REM sleep, on the other hand, has been associated with regional increases and decreases of brain activity as compared to wakefulness [39, 43–46]. Regional increases in rCBF or CMRglu were observed in thalamus, pons, basal forebrain, amygdala, hippocampus, anterior cingulate cortex, and temporo-occipital cortices. Decreases were found in associative cortices including dorsolateral prefrontal cortex, posterior cingulate gyrus, precuneus, and inferior parietal cortex. These results have been hypothesized to reflect REM sleep generation mechanisms in animals, based on cholinergic processes starting from brainstem structures and activating the cortex via the thalamus and basal forebrain [47].

Early fMRI studies recorded during sleep also compared brain activity patterns between stages of sleep and wakefulness, confirming the localized cortical and subcortical decreases observed during NREM sleep with PET studies [48, 49]. More recently, fMRI studies have assessed the brain activations occurring within sleep stages by using simultaneous EEG recordings, suggesting new functional implications.

It is well known that NREM sleep consists of spontaneous and coalescent brain oscillations, called spindles and slow waves [50]. The former waves represent a hallmark of NREM sleep stage N2 and are recorded at EEG as waxing-and-waning waves oscillating at a frequency of 11–15 Hz, arising from reticular nucleus of the thalamus [50]. Human EEG studies have revealed two spindle subtypes: slow spindles, prominent on frontal areas, and fast spindles, prominent in centro-parietal regions [50]. The latter ones, i.e., slow waves, predominate during NREM sleep stage N3 and show, at EEG, low frequency (0.5–4 Hz) and high amplitude. Slow waves arise from almost any cortical regions, including primary sensory, associative, and motor cortices [50].

On the other hand, in animals, phasic activity during REM sleep has been characterized by bioelectrical potentials most frequently recorded in the pons, lateral geniculate bodies of the thalamus, and occipital cortex. PGO complexes, therefore, represent a hallmark of REM sleep in animals, and similar neural correlates have been observed in humans, since EEG recordings demonstrated transient occipital and parietal potentials time-locked to rapid eye movements [51].

In a recent combined fMRI and simultaneous EEG recording study of spindles, 14 healthy and non-sleep-deprived participants were scanned while trying to sleep [52]. During NREM sleep stages N2–N3, significant increases in BOLD signal, time-locked to spindles, were found in the thalamus (lateral and posterior parts) and in specific cortical areas including paralimbic (anterior cingulate cortex, insula) and neocortical (superior temporal gyrus) structures. In contrast to

PET data, showing NREM sleep as a state of brain deactivation compared to wakefulness, fMRI studies revealed that NREM sleep is also characterized by a transient increase of brain activity in phase with sleep oscillations such as spindles. The activation of the thalamus is perfectly in agreement with the key role of this region in spindle generation [50]. Moreover, fast spindles were associated with larger activation in precentral and postcentral gyri, medial prefrontal cortex, and hippocampus, suggesting a putative specific role of fast spindles in sensorimotor processing and sleep-related memory consolidation [42]. Such hypothesis could be reinforced by a recent fMRI study showing an increased functional connectivity between the hippocampal formation and neocortical areas during sleep stage N2, specifically in phase with fast spindles [53]. Moreover, another recent study evaluated the effects of sleep spindles on the neural activations induced by acoustic stimulation during NREM sleep. Similar to wakefulness, the global sensorimotor processing of acoustic stimuli was preserved during NREM sleep, except in phase with the occurrence of sleep spindles, when no significant thalamocortical activation was observed [54]. It has been hypothesized that spindle activity, during NREM sleep, could isolate the cortex from environmental stimuli and promote the processing of endogenous information, thereby favoring memory consolidation and brain plasticity [54]. Regarding neuroimaging of REM sleep stage, a H2(15)O PET study in 12 healthy volunteers has reported an association between the density of rapid eye movements during REM sleep and rCBF in the lateral geniculate bodies of the thalamus and occipital cortex [55]. Subsequent studies have also observed BOLD signal increases associated with rapid eye movements in the pons, thalamus, and primary visual cortex, in line with the neurobiological and bioelectrical findings observed for PGO generation in cats [56, 57].

The functional significance of such processes in humans, for instance, in regard to brain plasticity and memory consolidation, remains to be further explored. Little is known about the networks recruited by sensory stimulation during REM sleep. One fMRI study conducted by Wehrle and coll. investigated the brain responses to acoustic stimulation during REM sleep [58]. In this study, periods of phasic REM sleep, with high density of rapid eye movements, were distinguished from periods of tonic REM sleep, with low level of rapid eye movements. The study showed that the activation of the auditory cortex in response to acoustic stimulation was observed during tonic REM sleep, but no activation was recorded during phasic REM sleep. The lack of reactivity during phasic REM sleep was considered as a transient state of functional isolation during which the brain acts as a "closed intrinsic loop" not open to the modulation by environmental stimuli [58].

20.3 Neurobiology of Stress

Stress is an adaptive response which comprises internal mechanisms developed throughout evolution in order to allow the individuals to cope with events threatening their homeostasis (i.e., the "stressors"). Whenever a stressful event is triggered, it generates a well-defined pattern of neuroendocrine responses, which are highly conserved across species. These responses involve complex interactions among hormones and neurotransmitters, which modulate the activation and plasticity of specific neural networks targeted with precise spatial and temporal patterns [59, 60]. The coordination in space and time of stress-related brain responses allows the selective activation of neural networks with precise time shifts, in order to provide a complete and accurate reallocation of processing resources to cope with homeo-destabilizing environmental stressors.

20.3.1 The Timing of Stress: Coordination of Neuroendocrine Responses

Until the first identification of the corticotropin-releasing hormone (CRH) in 1981 [61], and thanks to successive studies on hormonal reactions to stress events, the hallmark of stress

responses has always been the activation of the autonomic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis. Thus, the "fight-or-flight" response is the classical way to conceive the physiological and behavioral adaptations resulting from a threatening event. However, the most part of studies on stress reactions agrees that physiological stress responses have precise time domains, which can be subdivided in two major classes: quick responses and delayed responses. Each of the processes activated in the two major time domains implies specific modulation of neural networks involved in stress management [62].

The first stage of quick stress response involves the activation of the autonomic nervous system (ANS), which activated the release of adrenaline (epinephrine, E) and noradrenaline (norepinephrine, NE), by the medullar region of adrenal gland. As a consequence of E and NE adrenal secretion, all basal metabolic functions are boosted; thus, blood pressure results elevated, heart rate and breathing are accelerated, and the final outcome is the increase in blood flow to crucial vital organs (i.e., eyes, heart, skeletal muscles), which essentially subserves the "fight-or-flight" response.

A later phase of stress response involves the activation of the HPA that starts a complex interaction among cortical, limbic, and hypothalamic brain structures, which finally determines the multifaceted behavioral and neurocognitive response to stress. It is worth to notice, although it will be extensively explained below, that a major role in these final responses to stressors is played by corticoid hormones (mineralcorticoids and glucocorticoids), which mediate fast (non-genomic) and retarded (genomic, i.e., by transcriptional regulators) actions essential to brain plasticity remodeling.

Finally, other systems are involved in late responses to stress, such as gonadal axis, adipose tissue, and the immune system, which all concur to the complete homeostatic restoration after threat.

The whole process of actively protecting and maintaining homeostasis by means of neuroendocrine modulation is called *allostasis*, and the *allostatic load* or *overload* is the direct consequence of a dysregulated allostasis, in which neuromodulation and neuroplasticity are not synchronous or adequately performed.

20.3.2 Synchronized Effects of Stress-Related Neuromodulators

As above mentioned, the early first response to stress threatening is the activation of catecholamine secretion. Indeed, acute stress is able to activate NE secretion from the locus coeruleus (LC) [63]. The central secretion of NE concurrently stimulates peripheral activation of the ANS, with the subsequent secretion of E and NE from the adrenal medulla. Further, adrenal E and NE in turn increase NE central secretion though a vagal feedback response [64]. The effects of NE and E on body and brain are obviously determined by local distribution of different types of adrenergic receptors. For instance, whereas high affinity alpha2A receptors in the prefrontal cortex (PFC) may be early activated, the low affinity beta1 amygdala receptors need stronger catecholamine secretion, thus determining possible differential PFC and amygdala responses to stress [65–67].

Recent reports have described that also the dopaminergic system is essentially involved in stress response, because threatening events have been found to early increase dopamine release in PFC, nucleus accumbens, and dorsal striatum [68]. Although classic studies assert the uncontested and well-known role of dopamine in reward stimuli modulation, recent works have demonstrated that both rodents and primates hold specifically distinct dopaminergic neuron subpopulations, localized in the most dorsal part of midbrain, which are peculiarly activated by aversive stimuli [69].

As in the case of NE, dopamine effects on target tissues tightly depend on regionally expressed receptor profile. Indeed, PFC dopamine release by stress events tends to reduce neuronal activity through a D1 receptor-mediated mechanism, whereas D1 receptors induce the expression of conditioned fear in the amygdala [70].

As previously reported, corticosteroid hormones play a role in both quick and retarded stress responses. Recent studies have reported that both mineralcorticoids (MCs) and glucocorticoids (GCs) may exert rapid actions on a large set of target tissues by membrane-associated receptors [71]. Indeed, receptors for both MCs and GCs have been found in the paraventricular nucleus of hypothalamus, as well as in the hippocampus, amygdala, and PFC. In all these regions, MCs and GCs have been reported to modulate neuron excitability by rapid action [72, 73], with differential regional effect persistence depending on receptor type and distribution [74]. Moreover, GCs have been demonstrated also to rapidly modulate catecholamine actions in different brain regions, such as NE in the amygdala [75] or dopamine in the PFC [76].

However, the most explored effects of GCs and MCs are genomic and are initiated at least after 1 h and may last for several hours [62]. These effects, which are mediated by intranuclear GCs receptors, induce profound neuroplastic changes in target brain regions, mediated by GC-induced transcriptional modulation. For instance, GCs may induce facilitation in working memory PFC functions [77], as well as they may induce modifications in hippocampal neurons excitability [78].

20.3.3 Brain Regional Effects of Stress: The Salience Network and the Executive Control Network

Although the first studies on neurocognitive effects of stress focused on individual brain region involvement, recent studies have shifted attention on the fact that neurocognition stems from a complex interaction among interconnected brain network, which coactivate or co-deactivate during cognitive tasks [79]. Along this view, the most recent evidence asserts that the adaptive changes recognized in neurocognitive domains due to stressful events may not be localized in distinct brain regions, but they originate from the interaction among different network deputed to the responses to external stimuli: the salience network, which comprises amygdala, and the executive control network, comprising PFC [80].

Properly, the salience network, which is originated from the interaction among anterior cingulate cortex, thalamus, insula, amygdala, and infratemporal/temporoparietal cortex, has been regarded to integrate complex functions of rapid reprogramming of threat responses, such as reorient attention and take quick unreasoned decisions [80].

A crucial role in the modulation of salience network functions is played by LC. Indeed, as previously mentioned, the tonic NE secretion induced by stress from LC has been demonstrated to provide a rapid increase in the ability of environment examining, as well as a facilitation in quick attention reorienting to threatening stimuli [81]. The rapid shift from a regular phasic NE secretion from LC to a tonic secretion has been suggested to underlie the increase in attention vigilance by acute stress [82, 83].

Furthermore, some studies implicated also the striatal system in stress responses. Indeed, the rapid shift during life-threatening events from a flexible behavior to a stimulus-response behavior leading to unpremeditated actions may be possibly provided by striatum activation [84].

Therefore, the salience network appears to be hyperactivated during the early phases of stress responses, above all in the amygdala circuits [85]. Moreover, the hyperstimulation of salience neurocircuitry is concurrent to the progression of the classical physiological markers of stress, such as heart rate and blood pressure increase [86, 87].

However, the main characteristic of the adaptation to stress response is its self-limiting ability. Indeed, several studies have demonstrated that, after the immediate response, any physiological stress reaction tends to be slowly suppressed, above all regarding brain neurocognitive processes. A fundamental role in these effects is played by GC-mediated late responses. Corticosteroids have been demonstrated to reduce late stress-induced dysregulated responses, such as anxious behaviors [88], as well as to exert protective effects on stress

responses [89]. These effects have been reported to be possibly due to a corticosteroid-mediated reduction in stress responsiveness of salience network principal brain regions, such as amygdala [90], which has been found "decoupled" from LC overstimulation during GCs-modulated late response [91].

Differently from the salience network, which elaborates rapid reorienting functions, the higher-order attention and cognition functions stem from the interconnections among prefrontal areas (e.g., dorsomedial PFC, dorsolateral PFC, frontal cortex, posterior parietal cortex), which controls executive processes [92]. These executive control networks appear definitely compromised during stress responses. Indeed, the increase in catecholamine activity has been demonstrated to impair the persistent patterns of neural PFC activity that form the substrate for working memory [93]. Moreover, the whole "normal" flexible goal-directed behavior seems to be impaired during stress response, shifting to a more rigid stimulus-directed response [94].

Oppositely to the salience network, the executive control network appears to be quickly downscaled during early phases of stress responses, but a slow recovery of higher-order cognitive functioning may be observed after 4 h from a threatening event, with improved working memory and neurocognition [95], thus reversing the complete functions of the executive network.

A particular role in stress responses is played by hippocampus, which expresses receptors for both GCs and MCs and which has been demonstrated to be involved in both early and late responses to stress [96].

GCs, indeed, have been demonstrated to impair memory in rodents exposed to training-associated emotional arousal [97]. Thus, in light of abovementioned observations, it is possible that stress may impair memory formation by shifting neurocognitive performances on a focused attentional vigilance.

Moreover, some studies demonstrated that stressful events may definitely alter hippocampal neuroplasticity, with a progressive remodeling in dendrite shape and synapse morphology [98]. A GC-mediated reduction in brain-derived neurotrophic factor (BDNF) has been correlated to this hippocampal remodeling by stress response [99].

20.3.4 Stress and Circadian Networks' Interactions

As previously seen, stress system has the essential role of restoring homeostasis in response to threatening stressful events, thus remodeling functions of selected brain networks to counteract the destabilizing effects of stressors. A similar role is played by the circadian clock physiological system, which modulates the internal responses to external light-dark cues, thus synchronizing tissue-specific responses to daily cycles. The main molecular mechanisms by which light-dark internal homeostasis is maintained are based on the activity of biological clock genes, which are basically transcriptional activators and repressors that modulate nuclear translocations creating continuous interlocking loops [100], as previously described.

Recent studies have found that stress and circadian systems may interact at multiple molecular crossroad sites. Specifically, late evidence reported that CLOCK transcription factor may directly interact with the intracellular receptor for GCs in humans (human glucocorticoid receptor, hGR) [101]. Through this interaction, CLOCK impairs the cytoplasm-to-nucleus translocation of hGR and represses the hGR-induced transcription of GC-responsive genes, thus providing evidence for transcriptional interconnection between HPA axis and clock systems. Moreover, hGR acetylation by CLOCK (which is responsible for impaired translocation of the receptor to the nucleus) seems to follow circadian rhythms, similarly to the same CLOCK activity, thereby being higher in the morning and lower in the evening and mirroring the diurnal fluctuations of blood cortisol [102].

The neurocircuit bases for HPA/clock interaction have been demonstrated in the direct synapses between the SCN neurons, which represent the "sensors" for circadian rhythms, and PVN neurons, which are deputed to CRH secretion [103]. Moreover, central clock genes may directly influ-

ence corticosteroids adrenal secretion by modifying the sensitivity of adrenal cortex (zona fasciculata) to the adrenocorticotropic hormone (ACTH) [104]. These control mechanisms seem to add to the intrinsic peripheral circadian control of adrenal cortisol secretion. Indeed, adrenal glands hold internal clock genes controlling the rhythmic expression of molecules belonging to the ACTH-responsive steroidogenic pathway [105].

On the other hand, HPA axis has been repeatedly demonstrated to influence circadian systems by acting on clock genes. Indeed, *Per1* and *Per2* have been reported to hold GC receptor-responsive binding elements (GREs) in their regulatory sequences [106]. Therefore, this modulation of clock genes by HPA axis allows a direct override of circadian rhythmic regulation in response to stressful events, and it has been demonstrated to play an essential role in time-restricted feeding. Indeed, GCs are highly secreted during time-restricted feeding in order to exert protective effects on the concurrent uncoupling of the central SCN clock and the peripheral oscillators, thus representing a fundamental stress-protecting mechanism [107].

The crucial role of circadian/HPA systems' interactions may be easily deduced by the large amount of studies reporting different kinds of metabolic dysregulations (e.g., dyslipidemia, insulin resistance, or hypertension) in individuals with disturbances of clock gene functions, such as in rotating shift workers [108]. Indeed, the circadian dysregulation in these individuals represents the molecular basis for their propensity to myocardial infarctions and metabolic syndrome. Finally, HPA axis interactions with circadian system may also influence immune responses. Indeed, several cytokines are secreted in a circadian fashion, and this clock-dependent induction has been regarded as the underlying mechanism of symptom as morning flares in autoimmune patients [109]. On the other hand, GC treatment may reduce the morning joint stiffness and pain in rheumatoid arthritis [110].

Circadian rhythm impairment has been also suggested to have a role in the psychiatric symptoms (such as anxiety and depression) of rotating shift workers [111]. Learning and attention impairment, as well as mood alterations, have been demonstrated in animal models of circadian impairments (e.g., aberrant light exposition) and have been correlated to high levels of circulating GCs, which may be restored by antidepressant treatments [112].

20.4 Circadian Rhythms, Affective States, and Susceptibility to Mood Disorders

The term "chronotype" refers to the time of the day in which individual patterns of physical functions (e.g., eating, sleeping, hormone levels) are predominantly active. However, the term is most commonly used to define the sleeping habits [36].

Two main chronotypes have been described: morning and evening ones. People with morning chronotype are also defined as *larks*. They tend to wake up early in the morning, show the most behavioral activation in the late morning, and then have a lack of energy in the late afternoon. Approximately 50–60 % of the population falls in this category.

People with evening chronotype are defined *owls*. They usually go to sleep very late and have trouble waking up early in the morning. Also, they feel great difficulty in being immediately active and alert, and their highest productivity occurs in the afternoon and evening. Only 2–6 % of the population shows this chronotype, while the most part of general population falls in between the two extremes [113].

Chronotypes are considered tightly connected with circadian rhythms, also including affective states, and are thought to confer different susceptibility to different types of mood disorders. Individual chronotype can be assessed using specific questionnaires. The first one was developed by Horne and Östberg in 1976 and still remains the most widely used one for research in chronobiology. The MEQ (morningness-eveningness questionnaire) is a self-report questionnaire consisting of 19 multiple-choice questions. Each question has four response options and a scaled score that is summed to calculate the individual's

morningness-eveningness rating. Scores range from 16 to 86 and can be categorized as *definitely evening* (i.e., 16–30), *moderately evening* (i.e., 31–41), *neutral* (i.e., 42–58), *moderately morning* (i.e., 59–69), and *definitely morning* (i.e., 70–86) chronotypes [114]. Over the years, many other questionnaires have been developed, such as the Circadian Type Inventory [115]; the Composite Morningness Questionnaire [116]; the Lark-Owl Chronotype Indicator (LOCI) [117]; and the Munich Chronotype Questionnaire (MCTQ) [118]. These tools have been instrumental in studying several putatively chronotype-related biological and behavioral outcomes, such as adaptations at night shifts [119], cortisol secretion patterns in different chronotypes [120], or cognitive and high school performances [117].

20.4.1 Chronotype and Affective Behavior

The close correlation between sleep and mood is easily experienced by healthy population. Sleep, such as feeding and reproduction, is a fundamental biological function, and sleep disturbances cause irritability, fatigue, moodiness, and negative affect. In a study investigating the relationship between sleep loss and emotional reactivity in a sample of medical residents, sleep deprivation was found to intensify fatigue and negative emotions following daytime disruptive events and to reduce positive emotion following goal-enhancing events [121]. Sleep loss also has a negative effect on the individual's ability to process emotional information, including emotional empathy [122], and events disturbing the circadian rhythm, such as shift work or jet lag, negatively impact on mood and also increase the risk of depressive illness [123]. Cumulative nocturnal sleep debt has detrimental effects on psychomotor vigilance performance and leads to mood disturbance [124], while extended sleep significantly improves daytime alertness, reaction time, and mood [125].

Current data suggest that morning and evening chronotypes have different and peculiar associations with personality styles and temperamental traits and may confer distinct susceptibility to psychiatric illness [126, 127]. In healthy population, morning chronotype is associated to greater subjective well-being compared to evening type [128]. With respect to personality traits, subjects with morning chronotype show a stable personality [129] and tend to be higher in self-esteem, more agreeable and conscientious than subjects with evening type. These latter, contrarily, show higher tendency to be more neurotic and opened to experiences [130]. Evening-type subjects exhibit less affinity in their relationships with other people and less respect for behavioral and social norms compared to morning and intermediate types [131]. Morning types also tend to be less pessimistic and more responsible than evening types [132], while evening types are more creative and emotionally unstable and find more difficulties in social adaptation [133].

Adan et al. [134] explored the relationships between circadian typology groups and the seven personality dimensions of Cloninger's model, using Temperament and Character Inventory (TCI-56). Morning-type subjects showed more industriousness, ambition, perseveration despite frustration (PS), and more ability to control, modulate, and adapt their behavior in relation to chosen objectives and values (SD). Conversely, evening-type subjects tended toward exploratory activity in response to novelty. They also showed impulsive decision-making and presented lower behavioral inhibition in relation to potentially dangerous stimuli and anticipation of negative effects (HA).

Konrad S. Jankowski [135] in 2014 explored the role of temperament as defined by the Regulative Theory of Temperament (RTT) in the relationship between morningness-eveningness and mood. Compared to morningness, he found that eveningness is strictly linked to lower endurance (EN), higher emotional reactivity (ER), lower hedonic tone (HT), energetic arousal (EA), and higher tense arousal (TA). Among RTT traits, EN was the most strongly related to eveningness and completely mediated the relationship between eveningness and mood. This finding embraces the hypothesis that social jetlag mediates eveningness' impact on mood [136]. According to this concept, evening types,

especially those ones with lower levels of EN, experience a discrepancy between their internal and social clocks and are particularly susceptible to stress and depressive mood. Therefore, high eveningness associated to low EN could be considered as a risk factor of depressed mood. Nonetheless, none of these temperamental variables in themselves constitute a clinical disorder, but presumably the association among some of them contributes to increase risk and susceptibility to behavioral problems and psychiatric illness [137–139].

The relationship between chronotypes and affect was widely investigated over the years. Morning chronotype has been linked to higher positive affect and greater behavioral activation compared to individuals with later time of day preferences [127]. This association was found not only in young adults but also in older adults [140]. In contrast, evening chronotype has been linked to depressive mood, lower behavioral activation, and lower positive affect [127, 136, 141–143]. Eveningness is also found to intensify susceptibility to depression [144] and increase alcohol and stimulant drugs use [136].

20.4.2 Circadian Rhythms, Sleep-Wake Cycle, and Mood Disorders

Mood disorders have been related to circadian system and sleep-wake function disorders. These, in turn, have been related to malfunctioning of three relevant biological systems, namely, the circadian system, the sleep homeostat, and the stress system. The most clinically relevant disorders in circadian system and sleep-wake function include circadian phase shifts, blunting of circadian rhythms, impaired emotional regulation due to sleep deprivation, and overriding of circadian function by abnormal stress system activity [36].

The phase shift hypothesis states that a misalignment between circadian rhythms and sleep time (assessed as the time between dim light melatonin onset and mid-sleep) may represent a core malfunction in mood disorders [145]. Clinical and experimental observations initially led to the inference that this shift may occur in advance (i.e., with circadian rhythms in advance compared to sleep time) leading to the initial name of phase advance hypothesis [146]. Phase shifts may occur in both phase delay or advance in mood disorders: major depressive disorder (MDD) has been associated with increased likelihood of evening chronotype and with phase shift (usually delay), whereas bipolar disorder (BD) has been associated with both phase delay (in depression) and phase advance (in mania) [36, 127, 147].

Indeed, mood disorders show similar neurobiological features with circadian dysfunctions, suggesting both increased limbic activity (such as elevated activity and volume loss of the hippocampus, orbital and ventral prefrontal cortex) and decreased prefrontal activity (prefrontal cortex hypometabolism). These biological correlates of mood disorders have been conceptualized as a loss of top-down control over limbic structures or alternatively as a disinhibited limbic drive which overrides cortical regulation [148]. Furthermore, an abnormal clock gene function has been implicated in mood disorders, including seasonal affective disorder [149], unipolar depression [150], bipolar disorder [150–152], and depression vulnerability [153]. Circadian clock gene variants associated with seasonal affective disorder include NPAS2 and CRY2 gene variants [154, 155]; variants associated with depressive disorder include RORA and CRY1 gene variants [150, 153]. Gene variants associated with bipolar disorder include RORB and NR1D1gene variants [156, 157], with CRY2 being associated with rapid cycling [158]. Finally, PER2 variants have been associated with depression vulnerability [153].

Mood disorders have also been associated to dysfunctions in the sleep-wake cycle. Between 60 % and 84 % of MDD patients report symptoms of insomnia [159], whereas hypersomnia in MDD varies widely, ranging from 8.9 % in childhood to 75.8 % in young adulthood [159]. In 1,170 Japanese individuals, extreme evening chronotype has been associated with increased incidence of depressive states, whereas an extreme morning chronotype has been associated

with the decreased incidence of depressive states, suggesting that evening preference might increase susceptibility to depressive states [144]. In another study, it has been observed that depressed patient may show increased homeostatic sleep pressure and sensitivity to sleep deprivation. Indeed, young women suffering from MDD undergoing a multiple nap paradigm showed higher frontal EEG delta activity, a marker of homeostatic sleep pressure, compared to healthy young and older women [160].

Despite this body of evidence, however, studies so far carried out have not consistently found that the endogenous circadian pacemaker is either abnormally phase advanced or phase delayed in individuals with depression [161, 162]. Nevertheless, phase delay has been reported to be the most common phase shift in nonseasonal unipolar depression [36, 163], with a more delayed circadian misalignment linked with more severe depressive symptoms [162]. Moreover, a blunting of endogenous circadian rhythms has been described in depression. Depression has been associated with a disruption in circadian regulation of thermoregulatory function, with a blunting of the normal fall in core body temperature and higher average temperatures, thus suggesting a blunting of melatonin secretion. Indeed depressed subjects show elevated nocturnal core body temperature and higher mean temperatures [164]. Notably, remission in depressive symptoms has been associated with temperature normalization [165, 166]. Furthermore, in 126 MDD outpatients, a flattened diurnal cortisol pattern has been demonstrated, which was positively associated with shorter total sleep, more severe depression, and higher suffering levels [167].

BD has been associated with circadian system phase delay or advance (in depression or mania, respectively) and stress system dysregulation (both in depression and mania) [36]. During mania, patients experience reduced need for sleep [168]; rates of insomnia in BD vary across studies, with up to 100 % of depressed bipolar patients reporting insomnia and between 23 and 78 % reporting hypersomnia during the depressive phase [168, 169]. In bipolar patients during interepisodic intervals, a rate of hypersomnia of approximately 25 % has been assessed [170].

Both bipolar disorder and bipolar depression may be associated with sleep phase delay. Bipolar patients have been demonstrated to be more likely evening chronotypes, thus suggesting a relationship between circadian phase delay and BD [171]. Furthermore, a delayed sleep phase has been shown in young unipolar and bipolar patients during the depressive phase, with sleep offset times delayed in subjects with mood disorders compared to the control group and in bipolar patients compared to unipolar patients [172].

In mania, there may be a phase advance [36]. Evidence exists that circadian system features may vary in bipolar patients depending on whether they are experiencing a maniac or depressive phase. During a manic phase, the daily profiles of melatonin in saliva differ compared to healthy controls and patients in a depressive phase, with elevated melatonin levels during the daytime [173]. Daily profiles of Per1 and Nr1d1 clock gene expression from buccal mucosa may be advanced in manic compared to depressive phases [173]. Bipolar patients may persist experiencing a range of sleep abnormalities during remission, when compared with nonpsychiatric controls, with significant differences in sleep latency, sleep duration, wake after sleep onset, and sleep efficiency [174].

Another model that has been hypothesized to explain the correlation between circadian rhythms and mood disorders is the so-called *social zeitgeber* theory. Zeitgebers (time givers) are stimuli that may set the circadian clock, the most powerful zeitgeber being light. Several stimuli are also crucial to set the circadian clock (such as exercise, food intake, arousal, and social activity) [175]. Social zeitgeber theory posits that life stressors may destabilize social rhythms, thus disrupting circadian rhythms and leading to affective episodes. If this is the case, the disturbances of biological rhythms in mood disorders may actually be secondary to previous disruptions in social interactions or other life-stressor events, which would constitute the primary alteration. At present, data in support of this hypothesis remain limited and

suggest more complex causes in affective episodes and relapses [176].

Depression has been associated with stress system dysregulation, and evidence suggests that different diagnostic subtypes may show opposite dysregulation patterns. Hyperactivity of the HPA axis is one of the most replicated biological findings in major depression. Nonetheless, different lines of evidence suggest stress system and HPA axis overactivity in the melancholic subtype and stress system and HPA axis underactivity in the atypical subtype, consistent with their different clinical profile [177].

Different lines of evidence exist for HPA dysfunction in bipolar disorder. Hypercortisolism may be crucial for the pathogenesis of depressive symptoms; furthermore, abnormalities in urinary and cerebrospinal fluid cortisol levels, and decreased dexamethasone test suppression, have been shown in bipolar patients [178, 179].

20.4.3 HPA Axis and Psychosocial Stress

The hypothalamic-pituitary-adrenal axis (HPA or HTPA axis) is a complex circuit of interactions between three glands: the hypothalamus, anterior pituitary gland, and suprarenal glands. As the most important part of neuroendocrine system, HPA axis plays a key role in many biological functions, such as regulation of body temperature, immune system, mood, and sexuality, and also represents the main effector of the individual response to stress, in concert with the sympathetic and parasympathetic branches of the autonomic nervous system. HPA axis has found to be involved in many psychiatric disorders and especially in major depression [180, 181]. Particularly, the hyperactivity of HPA is one of the most consistent biological findings in depressive illness. The specific mechanisms underlying the association between HPA axis, affect, and mood disturbances remain still contested and unclear. Nevertheless, it is well known that psychosocial stress activates the stress system and thus HPA axis, leading to increased negative affects [182], and it is also demonstrated a strong association between psychosocial stress and increased risk of depression [183].

Intriguingly, mounting data support the hypothesis that HPA axis hyperactivity is not only a consequence or an epiphenomenon of depressive illness but also constitutes by itself a risk factor for depression [181]. Early life traumatic events [184–186], together with inflammatory processes [187], and genetic predisposition [188] can lead to persistent neurobiological abnormalities that may increase the risk of depression.

20.4.4 SCN and Reward

The relation between mood and reward behaviors is extremely complex and beyond the purpose of this chapter. There is a general agreement on the fact that the activation of the reward systems is generally experienced as a positive affect, while the activation of the threat system is experienced as a negative affect. As the broad range of mood disorders (from depressive personality traits to major depressive disorders) is tightly bond with chronobiology, it is critical to explore the bidirectional relation existing between the circadian system, with its regulators, and the reward system.

Rewarding behaviors, like sexual activity or food assumption, show some rhythmicity. Also risky behaviors have their own chronobiology, as it has been observed that a progressive disruption in circadian rhythms during adolescence may be related to addictive behaviors, including alcohol and substance misuse [189].

The idea that rewarding behaviors could be cyclically regulated has been addressed in rodents, showing that the time of the day affects amphetamine-induced conditioned place preference. The observation that behavioral activation follows a rhythmic daily pattern is consistent with metabolic activity in several dopaminergic areas within the limbic system, including the ventrotegmental area (VTA) and the nucleus accumbens (NAc). A large body of evidence has described rhythmic activity of dopamine metabolism in those areas, including rhythmic expression of tyrosine hydroxylase and peaking levels

of dopamine and its metabolites [190]. Whether this cyclic metabolic activity is extrinsically (i.e., from the hypothalamus) or intrinsically regulated is a matter of debate.

On the one hand, projections from the SCN to different limbic and mesolimbic structures have been confirmed [191]. On the other hand, a relevant expression of clock genes in the limbic system has been reported [19]. As clock genes are expressed also in other cortical and subcortical areas, a unified model has been proposed, positing that SCN exerts an *in-phasing* activity on extra-hypothalamic clock genes rhythmicity.

Although mounting data in literature corroborate the strong and bidirectional relationship between circadian rhythm and mood, the mechanisms underlying the association between chronotypes and mood are still not completely understood. It has been recently proposed that emotional chronobiology could be organized along two systems, i.e., threat and reward models of motivation. Accordingly, circadian rhythm has been associated with reward and therefore with behavioral activation and positive affect and the stress system with threat and thus with behavioral inhibition and negative affect [127, 192]. Appetitive motivation and positive affects, but not negative affect [193], show systematic daily variations according to circadian rhythm. In an evolutionary perspective, this mechanism may have adaptive functions in terms of motivating organisms toward goal-seeking activities at optimal times to reward, that coincide with daytime, and could explain the demonstrated association between morningness, positive affect, and lower risk of depression.

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Bipolar Disorders and Biological Rhythms

21

Robert Gonzalez

21.1 Introduction

All organisms exhibit rhythmic oscillations in a variety of physiological processes. Central to this process is the biological time-keeping system which functions to relay environmental signals necessary for organisms to synchronize physiological and behavioral processes and thus adapt to their environment. Disruptions in this timing system result in disturbances of biological rhythms [1–8] and may have clinical and pathophysiological relevance to bipolar disorder.

Rhythm disruption is a hallmark of bipolar disorder. These disturbances in rhythms are fundamental components of the diagnosis [9]. Bipolar disorder is marked by fluctuations and disturbances in activity levels, energy, subjective speeds of thought, and sleep-wake cycles. Even some specifiers used to describe the illness such as rapid cycling and seasonality suggest that rhythm disturbances are core features of the disorder. There has long been the notion of a relationship between disturbances in biological rhythms and the illness. For example, Goodwin and Jamison [10] postulated, "the genetic defect in manic depressive illness involves the circadian pacemaker or systems that modulate it." In this chapter, we will exam the relationship between bipolar disorder and rhythm disturbances. We will review some of the prevailing theories regarding rhythm disturbances in bipolar disorder, clinical signatures of rhythm disruption, physiological markers of rhythm disturbances, possible links between circadian genes and the illness, and possible treatment implications of biological rhythms.

21.2 Theories of Biological Rhythm Disturbances in Bipolar Disorder

The significant rhythm disturbances noted in bipolar disorder have lead many to hypothesize that disturbances of biological rhythms play a fundamental role in the etiology of the disorder. Multiple models have been proposed to explain the disruptions in rhythms associated with bipolar disorder including intrinsic variations in the biological timing system, phase variations of biological rhythms, and general instability of rhythms.

R. Gonzalez
Department of Psychiatry, Texas Tech University Health Sciences Center El Paso, El Paso, TX, USA
e-mail: robert99.gonzalez@ttuhsc.edu

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21.2.1 Intrinsic Variations of Biological Rhythms

Some have hypothesized that there are intrinsic signatures in the biological rhythms of bipolar patients [11, 12]. For example, although not universally demonstrated [13], early temporal isolation studies noted that some bipolar disorder patients demonstrated an intrinsic period of rhythms that was shorter than the close to 24-h period normally observed [11, 12]. A shortened circadian period could result in a state of desynchronization. For example, hamsters with a genetic mutation in the gene *CKIε* have demonstrated a circadian periodicity running approximately 20 h as compared to the normal 24 [14–18]. These hamsters demonstrated a significantly lowered ability to entrain rhythms, particularly in reference to the light-dark cycle. Studies involving the reciprocal grafting of SCN between *CKIε* mutants and wild-type mice demonstrated that the genotype of the grafted tissue, and not of the host, dictated the periodicity of the organism as a whole [15]. A similar process is could possibly be occurring in patients with bipolar disorder.

21.2.2 Phase Variations of Biological Rhythms

It has been suggested that shifts in the phase may result in misalignments between circadian phase of certain biological rhythms and both physiological and environmental rhythms. The opposing models of phase delays [19, 20] and phase advances [21–25] of circadian rhythms have been proposed as the primary circadian rhythm disturbances in the disorder. Phase delays have been reported in melatonin secretion [20] and are suggested by the preference for evening activities [19] in patients with bipolar disorder. Several lines of evidence point to phase advancement as playing a significant role in the pathophysiology of bipolar disorder [26]. Phase advancements in catecholamine secretion [21], temperature [21], the nadir of ACTH and cortisol secretion [23–25], and of activity rhythm [22] have been reported in subjects with bipolar disorder.

21.2.3 Instability of Biological Rhythms

While studies suggest that there are phase shifts or inherent differences in the period of biological rhythms, other research findings point to an inherent instability in the biological rhythms of those suffering from the illness. Wide variability in the phases of circadian rhythms have been reported in bipolar disorder [27–31]. Patients with bipolar disorder have demonstrated significant variation and fluctuation in body temperature, in mania [27, 28], and in depression [27–30] and less stable and more variable circadian activity patterns as compared to controls [31, 32]. A less robust circadian oscillator may result in the greater likelihood of rhythm disruption. In a similar phenomenon to that observed in individuals intolerant to shift work [33], patients with bipolar disorder may be more susceptible to the disruption of biological rhythms secondary to the blunting or weakening of biological rhythms [34]. People who demonstrate an inability to adapt to shift work share some of the core symptomatology associated with bipolar disorder including alterations in sleep such as insomnia, short sleep duration, poor sleep quality, and mood alterations including irritability and mood lability [33, 35–40]. Patients with bipolar disorder may, therefore, be more susceptible to the disruption of biological rhythms by disruptions in environmental factors (i.e., sleep, daily routines) [33, 41–61] as a result of a less robust biological timing system [34, 62–64].

21.2.3.1 Summary

While they differ in the proposed pathophysiological mechanisms, all of the above proposed mechanisms may result in a predisposition for patients with bipolar disorder to develop a state of desynchronization with internal or external time cues or of various physiological processes. Uncertain is whether the rhythm disturbances are primary pathophysiological processes or secondary to other pathophysiological mechanisms of the illness. Presented in the remainder of the chapter are clinical, biological, and physiological findings supporting the relationship between bipolar disorder and biological rhythm disturbances.

Theories of biological rhythm disturbances in bipolar disorder	
Inherent less than 24-h rhythm	Bipolar disorder subjects free-run with circadian rhythms shorter than near 24-h rhythm usually noted [11, 12]
Phase delay	Later time to melatonin secretion in bipolar disorder compared to controls [20]
	Preference for evening activities in bipolar disorder subjects as compared to controls [19]
Phase advance	Phase advance in urinary 3-methoxy-4-hydroxyphenylglycol in bipolar disorder subjects as compared to controls [21]
	Phase advance in temperature in bipolar disorder subjects as compared to controls [21]
	Early timing of the nadir of ACTH and cortisol secretion in bipolar disorder subjects as compared to controls [23–25]
	Phase advancement of activity rhythms in manic and mixed bipolar disorder subjects compared to controls and euthymic patients [22]
	Phase advancement of activity rhythm in euthymic bipolar disorder subjects compared to controls [22]
Instability/variability	Significant variation and fluctuation in body temperature in mania [27, 28]
	Significant variation and fluctuation in body temperature in depression [27–30]
	Less stable and more variable circadian activity patterns in bipolar disorder subjects as compared to controls [31]
	Greater variability of activity patterns in bipolar disorder subjects as compared to controls [32]

21.3 Clinically Related Rhythm Disturbances in Bipolar Disorder

As noted above, rhythm changes are core diagnostic phenomena of bipolar disorder. Research in this area has led to the discovery of clinically related rhythms disturbances and characteristics of the illness. These include relationships between chronotype, social patterns, and seasonality.

21.3.1 Chronotype

Recently, studies have begun to assess the relationship between chronotype, or the preference for diurnal activities, and bipolar disorder. Preliminary studies suggest that patients with bipolar disorder may have an evening preference for daily activities [19, 65]. It has been reported that chronotype in bipolar patients is stable over time suggesting that this is a trait characteristic. An evening chronotype may suggest a phase delay of circadian rhythms [19]. Chronotype as a trait may have significant clinical relevance in bipolar disorder. A greater degree of eveningness has been associated with rapid mood swings [66], greater recurrence rates [66], and an earlier age of illness onset [66].

There is mounting evidence to suggest that chronotype is associated with variations in physiological parameters [67, 68] that may be important to the underlying pathophysiology of bipolar disorder. These include variations in body temperature [67, 69, 70], catecholamine secretion [67, 71], sleep patterns [67, 72–74], subjective activation and arousal [67, 71, 75], and circadian rhythms of hormone secretion [70]. It remains to be seen if the clinical observations concerning chronotype are associated with underlying physiological characteristics in patients suffering from bipolar disorder. Unclear are the potential influences that imposed social schedules and medications may have on the assessment of chronotype and the expression of associated physiological parameters.

21.3.2 Social Rhythms

Disruptions in the social rhythms of bipolar patients have been described. Bipolar patients demonstrate a lower degree of regularity in social

rhythms when compared to controls [54]. Consistent with the social zeitgeber theory [41–43], the literature also suggests that in patients with bipolar disorder, a disruption of social factors (i.e., personal relationships, work, hobbies, daily routines, and life events) could destabilize overt biological rhythms and eventually lead to the exacerbation of mood episodes [53]. Several studies have noted a relationship between adverse events and lifestyle disruption and affective episodes in bipolar disorder [44–53]. Of particular interest are findings that suggest associations between life events that disrupt social rhythms and the onset for mania, but not depressive episodes [52, 53].

21.3.3 Seasonality

Light is a prominent zeitgeber in humans as well as other organisms. Disruption of this most basic of entrainment signal can cause serious disruption in circadian rhythms [76]. While there are conflicting reports [77], a subset of bipolar patients do demonstrate a seasonal pattern to their mood episodes [78–82]. A greater degree of seasonality has been reported in bipolar subjects as compared to healthy controls and unipolar depression subjects [80]. The types of mood episodes may also follow seasonal patterns. It has been reported that depression peaks in autumn [79], mixed mania peaked in late summer [78], and mania in early spring [78]. Seasonality may also represent a heritable phenomenon associated with the illness. A greater overall degree of seasonality as well as greater seasonal changes in the total sleep time and mood disturbances has been reported in the twins with bipolar disorder as compared to age- and gender-matched twins [82]. Taken together, these studies suggest that patients with bipolar disorder demonstrate an altered sensitivity to photic stimuli which could act to destabilize biological rhythms.

Clinically related rhythm disturbances in bipolar disorder	
Chronotype	Evening chronotype in bipolar disorder subjects as compared with controls [19, 65]
	Rapid mood swings in patients associated with an evening chronotype [66]
	Evening chronotype associated with greater 1-year mood episode rates [66]
	Greater degree of eveningness associated with earlier age of illness onset [66]
Social rhythms	Life events associated with social rhythm disturbances were associated with the onset of mania [52, 53]
	Less regularity of social rhythms in rapid cycling bipolar disorder subjects compared to controls [54]
	Phase delays noted in depression as compared to hypomanic and euthymic states [54]
Seasonality	Mania with peak in early spring and nadir in late fall [78]
	Mixed mania peaked in the late summer and nadir in the late fall [78]
	Preponderance of depression in autumn [79]
	Greater degree of seasonality in bipolar disorder subjects as compared to controls and unipolar depression subjects [80]
	Bipolar disorder subjects exhibit intermediate seasonality between controls and seasonal affective disorder subjects [81]
	Bipolar disorder twins demonstrate greater seasonality and seasonal changes in sleep and mood as compared to control twins [82]

21.4 Biological and Physiological Markers of Rhythm Disruption

The significant rhythm disturbances comprising the diagnostic criteria and clinical presentation of the illness have led to a search for the underlying pathophysiological markers of rhythm disturbances associated with the illness. Research has explored the potential links between bipolar disorder and physical activity levels, melatonin secretion, sleep-wake cycles, and hormone secretion.

21.4.1 Physical Activity

Variability and disturbances of psychomotor activity have been noted in patients suffering from bipolar disorder. Patients suffering from bipolar disorder demonstrate a greater variability [31, 32], less stable circadian patterns [31, 32], and a greater fragmentation of activity patterns [31], increased movement during sleep [83], and a lower relative activity amplitude [83], when compared to controls.

Disturbances in the levels of locomotor activity are common in both manic and depressive phases of bipolar disorder [84]. Bipolar subjects show less locomotor activity when depressed and greater locomotor activity when manic [21, 84]. Phase advancement of activity rhythms has been reported in patients suffering from both manic and mixed episodes as compared to patients in a euthymic state [22], and, when compared to control subjects, euthymic bipolar patients demonstrated a phase advancement of activity rhythms [22] suggesting that phase advancement of activity rhythms is a trait phenomenon that are amplified in affective states. Research findings also suggest that differences in locomotor activity may distinguish bipolar and unipolar depressions [85, 86].

Recent research suggests a relationship between mood state symptom severity and rhythm disturbances of locomotor activity in subjects suffering from bipolar disorder. A greater severity of manic symptoms has been correlated with a lower degree of rhythmicity and less robust rhythms of locomotor activity [87]. The relationships between rhythm disruptions and clinical characteristics of mania such as decreased need for sleep, disturbances in content of thought and thought disorder, increase in the rate and amount of speech, and increased motor activity and energy [87] have been reported.

21.4.2 Melatonin Secretion

The hormone melatonin is a fundamental component of the circadian timing system. Melatonin is produced and secreted by the pineal gland in a diurnal fashion [88]. The production of melatonin is only significantly influenced by endogenous circadian rhythms [89] and ocular light exposure [88] that acts to inhibit melatonin production in a dose-dependent fashion [88, 90].

It has been suggested that a dysfunction in the rhythmic secretion of melatonin may underlie some of the pathophysiology of bipolar disorder [91]. Even though melatonin has proven to be a reliable marker of circadian phase [92], few studies have focused on this measure in bipolar disorder and have yielded contradictory findings. While some studies report phase disturbances [20, 93] in the melatonin secretion in bipolar patients, others report no phase variations [94]. Bipolar patients have demonstrated a phase delay in melatonin secretion when compared to unipolar depression patients and controls [20]. When compared to healthy controls, bipolar patients have demonstrated significantly lower peak nocturnal melatonin levels [20, 94]. Since no appreciable differences in nocturnal melatonin secretion have been found between mood states [94], a trait phenomenon is suggested. In addition, both euthymic and acutely ill bipolar patients have also demonstrated a hypersensitive pineal response to ocular light exposure when compared to controls [95] and unipolar depression patients [20] as noted by a significantly lower plasma melatonin after nocturnal light exposure.

21.4.3 Sleep-Wake Cycles

Sleep disturbance is a hallmark of bipolar disorder. Somnographic findings in both manic and depressed bipolar subjects include a disruption in sleep continuity [96], increased time spent in stage 1 sleep [96], shortened REM latency [96], and an increase in the density of REM sleep [96, 97].

Sleep disturbances in bipolar disorder have demonstrated an association with clinical and course of illness characteristics. Sleep disruptions have been associated with a worse course of illness [97, 98], an increased symptom severity [97–99], and impairments in functioning and quality of life [97–99]. The types of sleep disturbances may also be associated with specific mood states. The variability in

sleep latency has been associated with depressive symptoms [98] and lower and more variable sleep efficiency associated with more lifetime depressive episodes [98]. Manic symptoms have been associated with decreased sleep efficiency [98] and the duration of REM and slow-wave sleep [97]. Decreased sleep duration has been reported to be a predictor of manic symptoms on the subsequent day [100]. While these studies suggest a causal relationship between sleep deprivation and the switch to mania, some studies have reported that the decreased sleep time and insomnia present prior to the switch [100–102] suggesting that decreased sleep may be a naturally occurring characteristic of the switch process.

Sleep characteristics may also prove to be important phenotypic signatures of the illness. Short sleep duration is associated with more severe symptoms [99] while both short and long sleep durations associated with poor functioning and quality of life [99].

Sleep disturbances may also be a heritable phenomenon as evidenced by the reports of higher rates of sleep disturbance in high-risk offspring of parents with BD [103]. Sleep disturbances may be initial prodromes [103–105] and trait markers [99] for the illness. For example, reported prodromal sleep disturbances have been reported in bipolar disorder that include general sleep disturbances [104, 106], lack of sleep [107], decreased sleep [108], insomnia [109, 110], and poor sleep efficiency [109, 110].

21.4.3.1 Functioning of the HPA Axis

While the theoretical evidence for disruption in the normal timing of hormone secretion would be expected, studies to date do not provide significant data in support of this. The strongest support comes from the examination of the cortisol secretion. It has long been suggested that dysregulation of hypothalamic-pituitary-adrenal (HPA) axis function may, in part, be responsible for mood episodes in bipolar patients [111]. Hyperfunctioning of the HPA axis, hypercortisolemia, and dysfunctions in glucocorticoid receptor feedback mechanisms have all been suggested as possible mechanisms underlying etiology in bipolar disorder [112]. Some evidence that suggests that there is an early nadir of cortisol secretion in bipolar patients in both the depressive [23] and manic [25] states of the illness. Some research findings also suggest that there may be abnormalities in the ultradian rhythms of cortisol secretion [25, 113]. Increased afternoon cortisol levels have been reported in the children of bipolar parents as compared to children whose parents had no history of psychiatric disorder [114]. There is also evidence that suggests an increased sensitivity of the HPA system resulting from disturbances in sleep in patients with bipolar disorder. An increase in cortisol secretion has been reported in patients with bipolar disorder in association with nocturnal awakenings [115].

In spite of the theoretical implications, studies have failed to find consistent and characteristic rhythm disturbances of the HPA axis in bipolar patients In addition, it has not been clearly demonstrated that the rhythm disturbances noted in HPA axis functioning in bipolar disorder are resulting from primary dysfunction of the circadian timing system.

Biological and physiological markers of rhythm disruption in bipolar disorder	
Psychomotor activity	Greater variability of activity in when compared to controls [31, 32]
	Less stable circadian patterns [31, 32]
	Greater fragmentation of activity patterns [31]
	Lower relative activity amplitude [83]
	Increased movement during sleep [83]
	Phase advancement of activity rhythms in manic and mixed patient compared to controls and euthymic patients [22]
	Phase advancement of activity rhythm in euthymic bipolar disorder subjects compared to controls [22]
	Less locomotor activity when depressed and greater locomotor activity when manic [21, 84]
	Greater activity levels in unipolar as compared to bipolar subjects [85, 86]

Biological and physiological markers of rhythm disruption in bipolar disorder	
Melatonin	Bipolar disorder subjects had significantly lower melatonin levels compared to unipolar and control groups with nocturnal light exposure [20]
	Later peak time for melatonin on dark night [20]
	Lower levels of melatonin in bipolar disorder subjects compared to controls [94]
	No differences in melatonin secretion between mood state [94]
	Twofold greater decrease in euthymic unmedicated bipolar disorder subjects compared to controls after nocturnal light exposure [95]
Sleep	Manic and depressed bipolar disorder subjects exhibit disturbed sleep continuity, increased stage 1 sleep, shortened REM latency, and increased REM density when compared to controls [96]
	Lower and more variable sleep efficiency associated with more lifetime depressive episodes [98]
	Variability in falling asleep time associated with concurrent depressive symptoms [98]
	Decreased sleep efficiency associated with manic symptoms [98]
	Greater REM density in bipolar disorder subjects compared to controls [97]
	Duration of REM and SWS was positively correlated with manic symptoms [97]
	Short sleep duration associated with more severe symptoms [99]
	Short and long sleep duration associated with poor functioning and quality of life [99]
	Higher rates of sleep disturbance in high-risk offspring of parents with bipolar disorder [103]
	Sleep disturbances noted as prodromes of the illness [104, 105]
	Decreased sleep prior to manic switch [100]
	Decreased sleep time and insomnia present prior to the switch [100–102]
	Prodromal sleep disturbances [103–105] have been reported in bipolar disorder including:
	General sleep disturbances [104, 106]
	Lack of sleep [107]
	Decreased sleep [108]
	Insomnia [109, 110]
	Poor sleep efficiency [109, 110]
	Trait markers [99]
	Manic switch secondary to sleep deprivation [102, 116]
Cortisol	Early nadir of cortisol secretion in bipolar patients in both the depressive [23] and manic [25] states of the illness. Some research findings also suggest that there may be
	Abnormalities in the ultradian rhythms of cortisol secretion [25, 113]
	Increased afternoon cortisol levels have been reported in the children of parents diagnosed with bipolar disorder as compared to children whose parents had no history of psychiatric disorder [114]
	Increase in cortisol secretion has been reported in subjects with bipolar disorder in association with nocturnal awakenings [115]

21.5 The Relationship Between Circadian Genes and Bipolar Disorder

The precision of the circadian timing system is in large part dictated by the expression of circadian genes and the interactions of their protein products [117, 118]. Alterations in these core circadian genes can change the expressed circadian period and phase [15] and disrupt normal circadian rhythmicity [119]. Evidence has begun to emerge indicating that alterations in these genes can have just such an impact in humans [120]. Variations in circadian genes have been associated with diurnal preference for daily activities [121–124], delayed [125] and advanced [126] sleep phase syndromes, and schizophrenia [127–130].

Given the social, behavioral, and physiological rhythm disturbances which characterize bipolar disorder, genes that encode the components of endogenous clocks or systems that modulate them would suggest them to be good candidate genes. Recent studies suggest an association between variations in circadian genes and bipolar disorder [131–147]. In addition, genetic variations in circadian genes have been associated with clinical characteristics of the disorder including insomnia [134, 140, 141], decreased need for sleep [140], rapid cycling [134], diurnal preference [139, 142], diurnal mood variation [134], greater recurrence rates of mood episodes [148], and age at onset [132, 146]. Preliminary studies categorizing functioning of molecular clocks in bipolar patients also seem to indicate that less robust molecular clocks may be associated with the illness [149].

Perhaps the most compelling evidence implicating circadian genes in the pathophysiology of bipolar disorder comes from a unique animal model. Roybal, McClung, and colleagues have mice with a point mutation in the gene CLOCK that yields an inactive protein [150]. The behavioral profile of the CLOCK mutant mice is strikingly similar to manic symptomatology. These mice demonstrate a decreased time spent in all sleep stages, a decreased anxiety-like behavior, and an increased sensitivity to the rewarding effects of cocaine. From a physiological perspective, the ventral tegmental area (VTA) dopaminergic neurons in the CLOCK mutants show increased firing rates, an increase in the expression of tyrosine hydroxylase activity, and a decreased expression of other clock genes such as Per 1, Per 2, Cry, and CKIε. Interestingly, both the delivery of a functional CLOCK gene to the VTA dopaminergic neurons via viral gene transfer and lithium treatment returned many behaviors of clock mutants to near wild-type levels. Taken together, these results suggest an important role for circadian genes in regulating complex behavior and may represent an animal model for mania.

Associations between circadian genes and bipolar disorder

Circadian gene (OMIM nomenclature) (Chromosomal location)	Genetic association
BHLHB2 (DEC1) Basic helix-loop-helix domain containing, class B, 2 (3p26.1)	SNP associated with bipolar disorder [134]
BHLHB3 (DEC2) Basic helix-loop-helix domain containing, class B, 3 (12p12.1)	SNP associated with bipolar disorder [137]
ARNTL1 (BMAL1) Aryl hydrocarbon receptor nuclear translocator-like 1 (11p15)	SNPs associated with bipolar disorder [135–137]
	Haplotype associated with bipolar disorder [138]
	SNPs and haplotypes associated with rapid cycling [134]
ARNTL2 (BMAL2) Aryl hydrocarbon receptor nuclear translocator-like 2 (12p12.2-p11.2)	SNP associated with bipolar disorder [137]
	SNP associated with diurnal mood worse in the evening [134]
CK1ε Casein kinase 1 epsilon (22q13.1)	Haplotype associated with rapid cycling [134]
	SNP associated with bipolar disorder [136, 137]
	SNPs associated with morningness [139]
CSK1δ Casein kinase 1 delta (17q25)	SNP associated with bipolar disorder [139]
CLOCK Circadian locomotor output cycles kaput (4q12)	SNP associated with increased recurrence rates [148]
	SNPs associated with insomnia [134, 140]
	Haplotypes associated with insomnia [134]
	SNP associated with greater insomnia with SSRI treatment for depression [141]
	SNPs associated with bipolar disorder [139]
	SNPs and haplotypes associated with rapid cycling [134]
	SNP associated with bipolar disorder [137]
	SNP associated with diurnal preference of daily activities [142]
CRY1 Cryptochrome 1 (12q23-q24.1)	SNP nominal association with bipolar disorder [137]

Associations between circadian genes and bipolar disorder	
Circadian gene (OMIM nomenclature) (Chromosomal location)	Genetic association
CRY2 Cryptochrome 2 (11p11.1)	SNP associated with bipolar disorder [136]
DBP D site of albumin promoter-binding protein (19q13.3)	SNP associated with diurnal mood worse in the evening [134]
GSK3-β Glycogen synthase kinase 3 beta (3q13.3)	SNP associated with later age at onset [132]
	SNP associated with greater response to total sleep deprivation [132]
	SNP associated with response to lithium [133]
	SNP associated with female gender in bipolar type II disorder [143]
NPAS2 Neuronal PAS domain protein 2 (2q11.2)	SNPs associated with bipolar disorder [136, 137, 139]
NPAS3 Neuronal PAS domain protein 3 (14q12-13)	SNPs and haplotypes associated with bipolar disorder [144]
	Haplotypes associated with increased risk and protective attributes [144]
NR1D1 Nuclear receptor Subfamily 1, group D, member 1 (17q11.2)	SNP associated with female bipolar disorder patients [145]
	SNPs associated with bipolar disorder [139]
	Haplotype associated with bipolar disorder [146]
	Haplotype associated with age at illness onset [146]
PER1 Period homologue 1 (*Drosophila*) (17p13.1)	SNP associated with bipolar disorder [139]
PER2 Period homologue 2 (*Drosophila*) (2q37.3)	SNPs associated with bipolar disorder [139]
PER3 Period homologue 3 (*Drosophila*) (1p36.23)	SNPs associated with bipolar disorder [135, 137]
	Haplotype associated with bipolar disorder [138]
	SNP associated with eveningness [139]
RORα RAR-related orphan nuclear receptor alpha (15q22.2)	SNP nominal association with bipolar disorder [137]
RORβ RAR-related orphan nuclear receptor beta (9q22)	SNPs associated with bipolar disorder [136]
	SNPs and haplotypes associated with bipolar disorder in a pediatric population [147]
TIM Timeless homologue (*Drosophila*) (12q13.3)	SNPs associated with bipolar disorder [135]
	SNP associated with insomnia in mania [134]
	Haplotype associated with rapid cycling [134]

SNP Single Nucleotide Polymorphism

21.6 Chronobiologically Based Treatment Perspectives

Given the significant rhythm disturbances noted in bipolar disorder, interest has been shown in delineating the role that biological rhythms may have in monitoring the illness and in developing directed treatments for the illness.

21.6.1 Physical Activity and Treatment Response

Pharmacological treatments of the illness impact these alterations in activity with these changes in activity levels possibly serving as markers of treatment response. For example, lithium has been shown to increase activity levels in depressed bipolar subjects [86, 151] and decrease activity in the manic phase of the illness [151, 152]. In addition, greater relapse rates have been noted in remitted lithium-treated bipolar subjects who presented with greater baseline activity levels [153].

21.6.2 Social Rhythms Treatment

Given the impact that disturbances of social rhythms affect the course of illness in bipolar disorder, the stabilization of social rhythms

could be a positive adjunct for the treatment of bipolar disorder [55]. Frank and colleagues have developed a novel psychotherapeutic treatment, the Interpersonal and Social Rhythms Therapy (IPSRT) [56]. IPSRT emphasizes the maintenance of daily rhythms and monitoring the impact that life events have on daily routines. Randomized control trials have compared IPSRT with intensive clinical management consisting of medication management and psychoeducation [57–59]. While no difference in survival time was reported, patients receiving IPSRT in the acute phase of illness have demonstrated a longer time to the appearance of a new episode, an increase in regularity of social rhythms, and a more rapid improvement in occupational functioning [154]. Interestingly, subjects who switched treatment modalities had significantly higher recurrence rates than subjects who continued with the same treatment throughout the course of the study [57]. Collectively, the findings of these studies suggest that the course of illness could be influenced by the stabilization or destabilization of social rhythms or daily routines and suggest possibilities for non-pharmacological interventions for the illness.

21.6.3 Phototherapy

Variations in response to photic stimuli could have clinical as well as diagnostic implications. Light therapy has long been used for the treatment of affective disorder. It is hypothesized that the therapeutic effects of light therapy may work by shifting phase of circadian rhythms [155].

Phototherapy has demonstrated efficacy in the treatment of both seasonal and nonseasonal depression [156, 157]. An increasing body of literature suggests that bright light therapy may be efficacious in the treatment of bipolar depression [158–167]. One study has reported that bipolar patients suffering from depression had a significantly decreased hospital stay when exposed to greater degrees of morning sunlight [162]. Phototherapy has demonstrated efficacy in the treatment of nonseasonal bipolar depression [165] and as adjunctive treatment in rapid-cycling bipolar patients [166]. Phototherapy has also been reported to enhance the efficacy of sleep deprivation in treating bipolar depression [160].

While some have reported light therapy to be well tolerated, of concern are several reports of serious adverse events being associated with bright light therapy. Suicidality [168], mania [169–171], mixed states [167], and mood instability [166, 172] have been reported in relation to phototherapy.

In regard to the development of side effects versus beneficial effects secondary to phototherapy, the timing of light administration, the intensity of light, and biological predisposition may be important. The administration of bright light at midday may be of most benefit to patients with bipolar disorder [166, 167], whereas light administered in the morning is associated with a greater risk of producing manic symptoms [166, 167, 169]. Other studies suggest that a history of seasonality, rather than an underlying psychiatric diagnosis, is associated with mania after bright light treatment [173].

The studies of phototherapy in bipolar disorder varied considerably in the intensity, wavelength, and duration of administered light. While more rigorous randomized control trials are required to evaluate the efficacy of phototherapy [157] and the phenotypic and pathophysiological implications of the response of light in bipolar disorder, both the reported seasonality and the side effects associated with bright light therapy suggest that patients with bipolar disorder may demonstrate a hypersensitivity to photic stimuli.

21.6.4 The Role of the Melatonergic System in Treatment

Given its key role that melatonin plays in the maintenance of circadian rhythms and diurnal behaviors, it has been postulated that treatments that target the melatonergic system may be of significant clinical benefit. Melatonin and related compounds may have clinical benefits. In addition, some medications commonly used to treat bipolar disorder may impact this system as well.

21.6.4.1 Melatonin Treatment

Melatonin has been hypothesized to be of potential therapeutic benefit. While there is significant theoretical basis for the study of melatonin administration, only two small open-label adjunctive treatment studies have examined the therapeutic effects of melatonin in bipolar disorder. One study reported that melatonin administration was associated with increased sleep duration as well as a decrease in manic symptoms [174]. The second study reported that melatonin did not have a beneficial effect and that melatonin withdrawal was associated with both delayed sleep onset and mild mood elevation [175]. Given the fact that melatonin administration was not what is currently recommended in terms of entraining the circadian clock, it is not clear whether the effects noted with melatonin were secondary to somnolence, effects on the circadian clock, or whether other mechanisms were involved.

21.6.4.2 Agomelatine

Some of the most compelling evidence for manipulation of the melatonergic system in the treatment of bipolar disorder has emerged from studies of the novel antidepressant, agomelatine, a potent melatonin receptor agonist [176]. Agomelatine has shown efficacy in the treatment of seasonal and nonseasonal depression [176]. One open-label study of adjunctive agomelatine treatment in bipolar depression [177] reported significant efficacy. Agomelatine administration has been reported to increase rapid eye movement and result in a phase advance of circadian rhythms [176] in healthy individuals. Agomelatine administration has also been shown to result in improvements in sleep quality and sleep efficiency as well as a possible normalization of NREM sleep [176] in depressed patients. As with melatonin, the exact mechanisms of efficacy in bipolar disorder are not known.

21.6.4.3 The Effects of Mood Stabilizers on the Melatonergic System

Commonly used mood stabilizers for the treatment of bipolar disorder may act via influence on the melatonergic system. Literature suggests that both valproic acid and lithium may exert some of their therapeutic effects by decreasing the sensitivity of melatonin secretion to nocturnal bright light administration [178, 179]. While these results are promising leads for potential mood-stabilizing properties of mood stabilizers, it should be noted that these initial studies were conducted in healthy controls and have yet to be conducted in patients with bipolar disorder. Further research is required to assess if mood stabilizers act, at least in part, by decreasing hypersensitivity of bipolar patients to nocturnal light exposure.

21.6.5 Effects of Psychiatric Medications on Sleep

Many psychiatric medications significantly alter some of the rhythmic properties of sleep. For instance, lithium, valproic acid, and carbamazepine have been noted to increase slow-wave sleep [180, 181]. Valproic acid has been shown to decrease REM sleep [182], and lamotrigine and gabapentin have been reported to increase REM sleep [183, 184]. A decreased REM latency has been reported after administration of electroconvulsive therapy [185], and a less robust treatment response to ECT has been reported in patients with continued delayed REM latency after treatment [186]. It has been reported that most antidepressants decrease slow-wave sleep [26]. Antidepressants have also been reported to have variable effects on sleep continuity. Interestingly, antidepressants that improve sleep continuity have been associated with phase delays [26].

21.6.6 Effects of Lithium on Circadian Rhythms

Lithium has been demonstrated to significantly impact biological rhythms. Lithium has been found to lengthen the circadian periods of multiple organisms [187, 188] including humans [189–192]. In patients suffering from bipolar depression, lithium has been shown to phase delay REM sleep and temperature in depressed bipolar patients

[193]. Characteristics of circadian phase may also be important markers associated with treatment response. It has been suggested that bipolar patients with a shorter circadian period respond to lithium while those with longer circadian periods do not [12].

21.6.7 Sleep Deprivation

While the detrimental effects of sleep disturbances in bipolar disorder have been well documented, the manipulation of the sleep-wake cycle may yield potential clinical benefits. While the antidepressant effects of sleep deprivation are transient, significant data has accumulated which indicates that sleep deprivation is an effective treatment for bipolar depression [116, 194]. Some reports suggest that patients with bipolar depression may demonstrate a more robust treatment response to sleep deprivation when compared to unipolar depressives [116, 194]. The antidepressant effects of sleep deprivation are robust and occur rapidly usually within the course of 24 h [195, 196]. Both lithium [197, 198] and sleep phase advancements [197] have been shown to sustain the antidepressant effects of sleep deprivation.

Even though reports of therapeutic benefits of sleep deprivation are encouraging, it should be noted that sleep deprivation has been reported to precipitate a manic or hypomanic episodes [116]. While these studies suggest a causal relationship between sleep deprivation and the switch to mania, some studies have reported that the decreased sleep time and insomnia present prior to the switch [100, 102] suggesting that decreased sleep may be a naturally occurring characteristic of the switch process.

Some have hypothesized that sleep deprivation owes its antidepressant effects to the resetting of abnormalities in endogenous molecular clocks [196]. Although not yet fully explored in bipolar disorder, sleep deprivation may impact the functioning of various physiological systems implicated in the pathophysiology of the illness. Sleep deprivation has been associated with activation of the HPA axis [199] with subsequent increase in EEG frequency and wakefulness [199]. The effects of sleep deprivation occur by affecting neurotransmitter systems thought to be associated with bipolar disorder such as the serotonergic system [200, 201] and the limbic dopaminergic pathways [202–204]. Sleep deprivation may also elicit its effects via the activation of certain brain regions such as the locus coeruleus and reticular activating system [199], the frontal cortex and anterior cingulate [205], and the hippocampus [202]. The physiological sequelae on these systems may explain both the therapeutic effects and detrimental effects of sleep deprivation in patients with bipolar disorder.

21.6.8 Circadian Genes and Treatment

Emerging literature suggests that certain pharmacological treatments for the illness may exert some of their therapeutic action via their influence on endogenous molecular clocks. For example, both lithium [206] and valproic acid [207] have been shown to influence the rhythmic expression of circadian genes and the rhythmic properties of molecular clocks. Lithium [208] and valproic acid [209] may exert some of these actions via inhibition of glycogen synthase kinase 3-beta (GSK3-β). Inhibition of GSK3-β results in modification of phosphorylation patterns in circadian proteins with subsequent lengthening of circadian period. Interestingly, a single-nucleotide polymorphism located in the GSK3-β promoter region has been associated with response to the total sleep deprivation during depressive episodes [132] and long-term response to lithium treatment [133] in bipolar patients. In addition, variations in CLOCK gene SNP (C/C) was found to be associated with insomnia associated with SSRI treatment for depression [141].

Perhaps the most compelling evidence implicating circadian genes in the pathophysiology of bipolar disorder comes from a unique animal model. Roybal, McClung, and colleagues have mice with a point mutation in the gene CLOCK that yields an inactive protein [150]. The behavioral profile of the CLOCK mutant mice is strikingly similar to manic symptomatology. These mice demonstrate a decreased time spent in all sleep stages, a decreased anxiety-like behavior,

and an increased sensitivity to the rewarding effects of cocaine. From a physiological perspective, the ventral tegmental area (VTA) dopaminergic neurons in the CLOCK mutants show increased firing rates, an increase in the expression of tyrosine hydroxylase activity, and a decreased expression of other clock genes such as Per 1, Per 2, Cry, and CKIε. Interestingly, both the delivery of a functional CLOCK gene to the VTA dopaminergic neurons via viral gene transfer and lithium treatment returned many behaviors of clock mutants to near wild-type levels. Taken together, these results suggest an important role for circadian genes in regulating complex behavior and may represent a novel understanding about mechanisms of treatment of the illness.

Chronobiologically based treatment perspectives germane to bipolar disorder	
Psychomotor activity	Lithium increases activity levels in depressed bipolar subjects [86, 151]
	Lithium decreases activity in the manic phase of the illness [151, 152]
	Greater relapse rates have been noted in remitted lithium-treated bipolar subjects who presented with greater baseline activity levels [153]
	Lithium slowed and lengthened this rhythm [12]
Social rhythms	Randomized control trials have compared IPSRT with intensive clinical management consisting of medication management and psychoeducation [57–59]
	No difference between IPSRT and intensive clinical management in adjunctive treatment after 2 years [154]
	More rapid initial improvement [154]
	Greater improvement in occupational functioning [154]
	Altering treatment was related to increased recurrence risk [57]
Phototherapy	*Treatment*
	Efficacy in the treatment of nonseasonal bipolar depression [165]
	Phototherapy enhanced the efficacy of sleep deprivation in treating bipolar depression [160]
	Light therapy showed some benefits and side effects in adjunctive treatment in rapid-cycling bipolar patients [166]
	Midday exposure provides some improvement [166, 167]
	Morning sunlight decreased hospital stay in bipolar depression [162]
	Adverse effects
	Manic symptoms associated with phototherapy [169–171]
	Mixed states associated with morning administration of phototherapy [167]
	Mood instability associated with morning administration of phototherapy [166, 172]
	Mood swings associated with uncontrolled light exposure [172]
	Phototherapy associated with suicidality [168]
	Morning light exposure is associated with a greater risk of producing manic symptoms [166, 167, 169]
Melatonin	*Treatment*
	Adjunctive melatonin treatment for insomnia in bipolar disorder increased sleep time [174]
	Adjunctive melatonin treatment for insomnia in bipolar disorder decreased mania [174]
	Adjunctive treatment with melatonin in rapid-cycling bipolar disorder showed no improvement in mood or sleep [175]
	Agomelatine adjunctive therapy for bipolar depression demonstrated efficacy [177]
	Decreased sensitivity of melatonin secretion to nocturnal light exposure in healthy controls after treatment with lithium [178]
	Decreased sensitivity of melatonin secretion to nocturnal light exposure in healthy controls after treatment with valproic acid [179]
	Adverse effects
	Melatonin withdrawal was associated with delayed sleep onset [175]
	Melatonin withdrawal was associated with possible mood elevation [175]

Chronobiologically based treatment perspectives germane to bipolar disorder	
Sleep deprivation	*Treatment*
	Efficacy of sleep deprivation in bipolar disorder subjects [194]
	Efficacy of sleep deprivation greater in depressed bipolar subjects when compare to unipolar depressives [116, 194]
	Lithium sustains the antidepressant effects of sleep deprivation [197, 198]
	Sleep phase advance sustains the antidepressant effects of sleep deprivation [197]
	Adverse effects
	Manic switch secondary to sleep deprivation [102, 116]
	Decreased sleep time and insomnia present prior to the switch [100, 102]
Effects of psychiatric treatments on sleep	Lithium, valproic acid, and carbamazepine have been noted to increase slow-wave sleep [180, 181]
	Valproic acid has been shown to decrease REM sleep [182]
	Lamotrigine and gabapentin have been reported to increase REM sleep [183, 184]
	A decrease REM latency has been reported after administration of electroconvulsive therapy [185]
	Less robust treatment response to ECT has been reported in patients with continued delayed REM latency after treatment [186]
	Antidepressants decrease slow-wave sleep [26]
	Antidepressants that improve sleep continuity have been associated with phase delays [26]
Effects of lithium on circadian rhythms	Phase delay REM sleep and temperature in depressed bipolar patients [193]
	Bipolar subjects with a shorter circadian period respond to lithium while those with longer circadian periods do not [12]
	Lithium increases activity levels in depressed bipolar subjects [86, 151]
	Lithium decreases activity in the manic phase of the illness [151, 152]
	Greater relapse rates have been noted in remitted lithium-treated bipolar subjects who presented with greater baseline activity levels [153]
	Lithium slowed and lengthened this rhythm [12]
Circadian genes and treatment	*CLOCK*
	SNP associated with greater insomnia with SSRI treatment for depression [141]
	GSK 3-beta
	SNP associated with greater response to total sleep deprivation [132]
	SNP associated with response to lithium [133]

21.7 Summary and Future Directions

Rhythm disturbances are hallmarks of bipolar disorder. Abundant clinical experience as well as mounting scientific research suggests that this may indeed be an etiological component of the illness. These observations seem to implicate a dysfunction or altered sensitivity of the circadian time-keeping mechanism.

Even though there is compelling evidence to suggest that there is a disruption of biological rhythms in bipolar disorder, as of yet no consensus has been reached as to the exact nature of these disturbances. There are several factors that may contribute to the apparently contradictory findings and the interpretation of studies conducted to this point. Many of these initial studies were conducted in small sample sizes and mixed populations (i.e., bipolar and unipolar patients). In addition, it should be noted that many of these studies used different methodologies and focused on testing different physiological parameters. In addition, many current analytical methods (i.e., cosinor analysis) require that data fit a specific mathematical model or set time frame (i.e., 24 h). The use of more refined methods of analysis that do not adhere to predetermined mathematical models or time constraints may better describe rhythm disturbances especially in a population such as bipolar disorder where there is a great degree of rhythm disturbance. For example, the

use of functional principal component analysis may allow for a more refined quantification of the biological rhythm data and the flexibility to search for functions representing activity patterns that may distinguish between subgroups and between-subject variability in bipolar patients [210].

Chronobiological studies in bipolar disorder present many formidable challenges. There are multiple methodological difficulties associated with chronobiological research. First is the issue of masking or the influence that an environmental stimulus has on the expression of a biological variable. Indeed, environmental stimuli can have an impact on biological rhythms. For instance, cortisol secretion can be increased by stress and nocturnal awakenings. Melatonin secretion can be suppressed by light particularly in the evening. Temperature rhythms are quite sensitive to food intake, motor activity, and postural changes. Specific protocols have been developed in order to control for the environmental factors possibly masking biological rhythms but require controls of multiple variables (i.e., constant dim lighting, subjects being required to remain supine, isocaloric meals, etc.). The implementation of these protocols in bipolar disorder presents significant challenges, especially when conducting them in patients with significant manic symptoms. In addition, some of these protocols involve sleep deprivation or disturbances of the normal 24-h sleep-wake cycle. Even though masking is reduced in these protocols, it presents some ethical concerns when considering conducting these experiments in bipolar subjects.

Many of the studies conducted to this point in bipolar disorder have focused on circadian or diurnal rhythms. Future research should focus on ultradian (<24 h) and infradian (>24 h) rhythms as well. This will require study designs that may vary greatly based on the specific type of rhythm one is interested in studying. This will require differences in the sampling density required as well as the period of time considered.

While both clinical and translational research point to a disruption of biological timing system in bipolar disorder, the possibility of rhythm disturbances being an epiphenomenon exists. As with all biological systems, the biological timing system is heavily intertwined with other physiological systems. The biological timing system not only works on its own complex feedback-feed forward system to affect the functioning of other biological systems but its own functioning is modified by various physiological systems and environmental factors as well. It is not beyond the realm of possibility that the rhythm disturbances seen in bipolar disorder may have their etiology in other internal or external environmental signals. In fact, an intact and functioning biological timing system may be a necessary conduit required to relay the rhythm disturbances noted in bipolar disorder.

Even though no definitive studies have been conducted, the biological timing system could potentially play a fundamental role in our understanding of the development of the illness as well as potential points for intervention. Disruption of the circadian time keeping system could potentially explain the pathophysiology of bipolar disorder on multiple levels from genetic to behavioral. Dysfunction at any level in the system may have a profound effect on biorhythms and potentially lead to the rhythm disturbances noted in bipolar disorder.

Greater knowledge of biological rhythms and circadian genes may enhance our understanding of bipolar disorder on multiple pathophysiological levels and help improve diagnostic criteria and in the development of novel and directed treatments for the disorder. The use of specific clinical phenotypes and intermediate phenotypes based on chronobiology may provide a directed approach to examining the possible pathophysiological mechanisms of bipolar disorder.

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Circadian Clock Genes and Mood Disorders

22

Timo Partonen

Abbreviations

ARNTL	Aryl hydrocarbon receptor nuclear translocator-like
ARNTL2	Aryl hydrocarbon receptor nuclear translocator-like 2
BHLHE40	Basic helix-loop-helix family, member E40
BHLHE41	Basic helix-loop-helix family, member E41
CLOCK	Clock circadian regulator
CRY1	Cryptochrome circadian clock 1
CRY2	Cryptochrome circadian clock 2
CSNK1E	Casein kinase 1, epsilon
GSK3B	Glycogen synthase kinase 3 beta
NPAS2	Neuronal pas [period–aryl hydrocarbon receptor nuclear translocator–single-minded] domain protein 2
NR1D1	Nuclear receptor subfamily 1, group D, member 1
PER1	Period circadian clock 1
PER2	Period circadian clock 2
PER3	Period circadian clock 3
RORA	RAR [retinoic acid receptor]-related orphan receptor A
RORB	RAR-related orphan receptor B
RORC	RAR-related orphan receptor C
TIMELESS	Timeless circadian clock
VIP	Vasoactive intestinal peptide

22.1 Introduction

Circadian clocks are universal and evolve their properties when subjected to selection [1]. Behavioral traits that tell about the preference to time the daily activities emerge as the result of interactions between the circadian clocks and the natural environmental cycles [2]. Species have developed these mechanisms to match their cellular oscillations with environmental variations. Such synchrony helps in anticipation of routine fluctuations in environmental conditions and in adaptation to these changes. When life emerged 3.5–3.9 billion years ago, the length of day was about 14 h and ultraviolet radiation was not filtered by the atmosphere. So, in the beginning of life, circadian clocks met light–dark transitions

T. Partonen
Department of Health, National Institute for Health and Welfare, P.O. Box 30, Helsinki FI-00271, Finland
e-mail: timo.partonen@thl.fi

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with the approximate period of 14 h and were readily involved in protection from ultraviolet radiation. Thereafter, the period has lengthened, and circadian clocks have adopted periods longer than 14 h up to the current one of approximately 24 h as the reference.

Thus, circadian clocks are built on properties generating metabolic oscillations within the ultradian, i.e., shorter than 24 h range, having the primary task for protection from ultraviolet radiation. Of the circadian clock components, the cryptochromes evolved from the family of DNA photolyase proteins, implying that control of the light-induced effects and protection from ultraviolet radiation share a common evolutionary origin [3]. Cryptochromes react not only to ultraviolet radiation [4] but also to light exposure [4, 5] and to geomagnetic activity [4, 6] and take part in regulation of the thyroid-regulated energy balance involving brown adipose tissue in mammals [7]. Therefore, a pacemaker generating the ultradian rhythms might be the primary one for the body, as it is the oscillator with a shorter period which sets the pace for oscillators with longer periods and not vice versa. The master ultradian clock, which normally cycles in harmony with the master circadian clock but may become misaligned when dopamine tone elevates over a critical level, is probably located in the dopaminergic neurons of the midbrain [8]. Currently, in mammals, there are a set of genes whose transcription cycle has an approximate period length of 8–24 h [9].

The oldest components of these timekeeping mechanisms are non-transcriptional in nature and well conserved throughout evolution across kingdoms [10]. The repetitive cycles of day and night provide the basis for assessment of the time of day, and the length of night relative to that of day yields information on season. Circadian clocks do not only passively measure the length of day but also actively generate and maintain the routine variation in a range of functions of the organism. New properties were needed when the routine change of the seasons, with the routine variations in photoperiod and geomagnetic activity, started to challenge the functions of circadian clocks among those species that were living in locations further away from the equator. These conditions may have evolved the properties of temperature compensation for circadian clocks in animals and non-shivering thermogenesis for mammals in particular. Not only internal or social stimuli but also physical time givers that act through the ultradian or circadian, or both, clocks can to a great extent modify complex behaviors such as eating, drinking, and sleeping in humans among other animals [11].

22.1.1 Circadian Rhythms in Mood Disorders

Disrupted or misaligned circadian rhythms and circadian gene expression are often found in major depressive disorder and bipolar disorder, with or without a seasonal pattern [12]. As the sleep–wake rhythm is dictated [13, 14] by the master circadian clock in the neurons that are located in the suprachiasmatic nucleus of the anterior hypothalamus in the brain, these disruptions are often seen as sleeping problems. Documentation of circadian rhythm disruptions in patients with mood disorders relies on valid markers that are generated by the master circadian clock and display a reliable circadian rhythm, such as continuous recording of core body temperature and repeated assessments of melatonin concentration [15].

Because nearly all the individuals suffering from mood disorders have disruptions in circadian rhythms, dysfunction of the proteins encoded from the circadian clock genes is hypothesized to play a role in the etiology of mood disorders. Depending on the definition for the core circadian clock genes, their number varies from study to study, but usually it is around 20. Genetic variants in the core circadian clock genes, or circadian clock-related genes, have been analyzed in a range of phenotypes that display, or are suggested to display, abnormal circadian rhythms [16–19]. Many of these genetic variations tend to associate also with sleep and metabolic disorders [11] that are frequently comorbid conditions with mood disorders.

Since the original finding [20], there has been a growing evidence that genetic variations in the core circadian clock genes associate with mood

disorders [21]. Circadian data have pointed at the circadian clocks as a key, first, to bipolar disorder; thereafter, to seasonal affective disorder; and, finally, to depressive disorder. Here, I consider those proteins that are repressors of transcription to be most important, since they are essential to the normal function of circadian clocks [22]. Among them, NR1D1 has a key position as a connecting node in the transcriptional and translational loops that constitute the circadian clock in a cell [23–25]. Further, CRY2 and CRY1 are the key repressors in the core of the circadian clock [26–32].

During the past 15 years, genetic variants in circadian clock genes have been analyzed in depressive disorders and in bipolar disorders as well as in the aforementioned disorders with a seasonal pattern that are called seasonal affective disorder [33–36]. Since the winter type of seasonal affective disorder, i.e., recurrent depressive episodes that occur during winter year after year, is by far the most frequent one, seasonal affective disorder is often called also as winter depression. Materials for these studies have been derived from clinic-, family-, or population-based samples, and their main findings are presented in the table (Table 22.1). It lists those findings that are based on studies in which the distributions of genotypes were assessed for both cases and controls and in which the statistical significance of results survived the multiple testing correction. The names of the genes are according to the Human Genome Organisation (HUGO) Gene Nomenclature Committee (HGNC) database (see list of abbreviations).

There are two concerns. Concerning the methods for genetic investigation, a clear strength of genome-wide association studies is their unbiased approach, whereas disadvantages of genome-wide association studies are the lack of hypothesis-driven analysis and the fact that their gene coverage is not complete. However, the results from genome-wide association studies are currently conflicting, and subsequent replication studies often fail to confirm the earlier findings and tend to produce a list of novel areas of interest and new candidates therein. Concerning the study designs, the case-control assessment of population-based samples provides the advantage of having a reference for comparison, whereas patient-only studies suffer from the lack of such reference and can provide information only on the clinical picture, course of illness, and treatment response that may not be specific to the disorder. Therefore, in the following, I do not summarize the results from patient-only studies.

22.1.2 Depressive Disorder

Since the beginning, it has been known that patients with depressive disorder may have opposite signs of sleep (insomnia or hypersomnia), appetite (decreased or increased), and weight (loss or gain). It is likely that this kind of clinical heterogeneity has slowed down the progress in identification of a gene or a network of genes along a pathway that contributes to the disease.

There are six studies of depressive disorder in which association with more than one candidate of the circadian clock genes has been analyzed. Kripke et al. [41] analyzed 198 single-nucleotide polymorphisms (SNPs) of 26 genes in cases and controls from family-based samples and a clinic-based sample, but none was associated with depressive disorder. Utge et al. [45] analyzed 113 tagging SNPs of 18 genes in 384 cases with depressive disorder and 1,270 controls from a population-based sample and found none to be associated with depressive disorder. However, their best findings of indicative significance included the associations of TIMELESS rs7486220 A-allele with depressive disorder with excessive daytime fatigue among women and of TIMELESS rs1082214 T-allele with depressive disorder with early-morning awakening among men and the two-way interaction of TIMELESS rs2291739 and ARNTL rs1868049 in association with depressive disorder with early-morning awakening among men [45]. Lavebratt et al. [37] analyzed 115 tagging SNPs of 18 genes in 459 cases with depressive disorder and 926 controls from a population-based sample and demonstrated that RORA rs2028122 G-allele was associated with depressive disorder and with the development of depression within 3 years independent of financial strain.

Table 22.1 The associations of mood disorders with circadian clock gene variants

Disease	Gene	SNP	Risk allele	Reference
Depressive disorder	RORA	rs2028122	G-allele	[37]
	CRY1	rs2287161	C-allele	[38]
		rs2287161	C-allele	[39]
Dysthymia	CRY2	rs7121611	A-allele	[40]
		rs10838524	G-allele	[40]
		rs7945565	G-allele	[40]
		rs1401419	G-allele	[40]
Bipolar disorder	NR1D1	rs2314339	C-allele	[41]
	RORA	rs782931	G-allele	[42]
	RORB	rs7022435	A-allele	[43]
		rs3750420	T-allele	[43]
		rs1157358	T-allele	[43]
		rs3903529	A-allele	[43]
	TIMELESS	rs774045	A-allele	[42]
Seasonal affective disorder	NPAS2	rs11541353	A-allele	[20]
	CRY2	rs10838524	A-allele	[44]

In addition, PER2 rs10462023 C-allele and NPAS2 rs1374324 G-allele associated with depressive disorder, in comparison to resilient individuals, and with the development of depression within 3 years independent of financial strain or negative life events [37]. Soria et al. [38] analyzed 209 SNPs of 19 genes in 335 cases with depressive disorder from a two-hospital-based sample and 440 controls from a population-based sample and found that CRY1 rs2287161 C-allele was associated with depressive disorder. In addition, the results suggested that NPAS2 rs11123857 G-allele was associated with depressive disorder [38]. From a population-based sample being representative of the adult general population, Kovanen et al. [40] analyzed 43 tagging SNPs of three genes in 5,737 individuals among whom there were 354 cases with depressive disorder (major depressive disorder or dysthymia) and 3,871 psychologically healthy controls. CRY1 or CRY2 was not associated with major depressive disorder, but four of the ten SNPs of CRY2 did associate robustly with dysthymia [40]. This association of CRY2 with dysthymia was further supported by haplotype analysis, yielding that the risk haplotype of CRY2 spans almost the whole gene [40]. Hua et al. [39] replicated the association of CRY1 rs2287161 with major depressive disorder, the C-allele being the risk allele, with the use of a sample of 105 cases and 485 controls. In this study, CRY2 rs10838524 was not associated with major depressive disorder [39].

In six case-control studies, only one candidate gene was analyzed. With regard to depressive disorder and CLOCK rs1801260 (T3111C), the original study by Desan et al. [46] tested the hypothesis that given the close involvement of circadian rhythms in depression, a polymorphism which affects the phase relationship of the sleep–wake cycle, as had been demonstrated by Katzenberg et al. [47], also affects susceptibility to depression. There was no difference in the genotype distribution between 143 patients with major depressive disorder and 137 controls [46]. This negative finding has thereafter been repeated in four studies [48–51]. In addition to the CLOCK gene, the NR1D1 gene has been studied by analyzing three tagging SNPs in 322 patients with major depressive disorder and 360 controls, but there was no association with major depressive disorder [52].

Li et al. [53] demonstrated that, compared with controls, of the seven circadian clock genes analyzed mRNA expression remained blunted for two genes (CRY1 and PER1) and opposite in phase for one gene (NPAS2) in patients with major depressive disorder, after 8 weeks albeit response to treatment with an antidepressant. Another study of

gene expression, with the use of postmortem brains and the analysis of six cortical and limbic regions of 34 patients with major depressive disorder and 55 controls, found that the 24-h patterns were markedly weaker in the cases [12]. Furthermore, some genes that normally coincide with each other by their expression timetable were discovered to be out of phase, such as the pair of BHLHE40 with PER2, whereas some that normally are out of phase were in phase with each other, e.g., the pair of BHLHE41 with insulin-induced gene 1, among the depressed [12].

Using the summary statistics for the genome-wide association studies of major depressive disorder from the Psychiatric Genomics Consortium as of February 2013, Byrne et al. [54] reported that the 34 SNPs of NR1D1 had a nominal association with major depressive disorder, the best association being with rs7502912. Their analysis included altogether 21 core clock genes as well as 322 clock-controlled genes in 9,240 cases and 9,519 controls [54].

22.1.3 Bipolar Disorder

Despite the fact that bipolar type 1 disorder is usually identified with ease, it has been challenging to identify a gene which contributes to the disease. Having the estimates of very high heritability for bipolar type 1 disorder [55] and the data on that bipolar disorder is sensitive to environmental stimuli and the seasonal effect [56], this is unexpected. However, this disease has attracted growing research efforts lately.

There are eight case-control studies of bipolar disorder in which association with more than one candidate of the circadian clock genes has been analyzed. In the analysis of 198 SNPs of 26 genes, Kripke et al. [41] found the association of NR1D1 rs2314339 C-allele with bipolar disorder. In addition, PER3 rs228697 G-allele was associated with the increased preference to the evening hours in daily activities among the patients with bipolar disorder [41]. In the study by Soria et al. [38], the analysis of 209 SNPs of 19 genes in 199 cases with bipolar disorder from a hospital-based sample and 440 controls from a population-based sample yielded that none was associated with bipolar disorder. However, their report indicated that VIP rs17083008 A-allele had a suggestive association with bipolar disorder [38]. Mansour et al. [57] analyzed 44 SNPs of eight genes and found that none associated with bipolar type 1 disorder, but of note their best findings of indicative significance included the associations of TIMELESS rs2291738 and TIMELESS rs2279665 as well as ARNTL rs7107287 with bipolar type 1 disorder. In their further analysis of 252 tagging SNPs of 18 genes in 523 cases with bipolar type 1 disorder and 477 controls, Mansour et al. [58] found no association with bipolar disorder, but their best findings of indicative significance suggested the associations of NPAS2 rs17025005, RORB rs10491929, and CRY2 rs1554338 with bipolar type 1 disorder. Nievergelt et al. [59] analyzed 52 SNPs of ten genes, of which none was associated with bipolar disorder. Shi et al. [60] in their analysis of 81 SNPs of 15 genes demonstrated the three-way interaction of BHLHE40 rs6442925 and TMEM165 (transmembrane protein 165) rs534654 and CSNK1E rs1534891 with bipolar disorder. McGrath et al. [43] in their unique analysis of 355 tagging SNPs of the RORA and RORB genes found that RORB rs7022435, RORB rs3750420, RORB rs1157358, and RORB rs3903529 were associated with bipolar type 1 disorder. In this study of a pediatric cohort [43], special attention was paid to comorbid conditions such as attention-deficit hyperactivity disorder, but in general there is still a need for in-depth evaluation of genetic variants in case of comorbid phenotypes. In their analysis of 353 SNPs of 21 genes in 239 patients with bipolar disorder and 873 controls, Etain et al. [42] found a nominal association for 14 SNPs. These SNPs were thereafter analyzed using a replication sample of 240 patients with bipolar disorder and 886 controls [42]. Finally, three SNPs had a nominal association similar to the first analysis, and the combined meta-analysis pointed out the significant associations of TIMELESS rs774045 and of RORA rs782931 with bipolar disorder [42].

In ten case-control studies, only one candidate gene was analyzed. In four studies, CLOCK rs1801260 was not associated with bipolar disorder

[48–51], whereas in one study in 260 patients with bipolar disorder and 350 controls it was the C-allele being the risk allele [61]. In addition, concerning the CLOCK gene, six tagging SNPs (including CLOCK rs1801260) in 149 patients with bipolar disorder and 795 controls [49] and three tagging SNPs (including CLOCK rs1801260) in 867 patients with bipolar disorder and 889 controls [51] were analyzed, and no association with bipolar disorder was found. In two studies, the NR1D1 gene has been on the focus, and there was no association with bipolar disorder both in the study by Kishi et al. [52] of three tagging SNPs in 147 patients with bipolar disorder and 360 controls and in the study by Severino et al. [62] of two tagging SNPs in 300 patients with bipolar disorder and 300 controls. One study of GSK3B rs334558 (SNP -50T/C) in 416 patients with bipolar disorder and 408 controls yielded that the C-allele was associated with bipolar type 2 disorder in a subsample of 57 women with bipolar type 2 disorder separate from the 183 patients with bipolar type 1 disorder and 254 controls [63]. Six tagging SNPs of the PER3 gene revealed no association in 105 patients with bipolar disorder and 104 controls [64]. The hypothesis-driven study by Sjöholm et al. [65] tested whether the four SNPs in the CRY2 gene that had been associated with winter depression [44] are associated with bipolar disorder. In a sample of 712 patients with bipolar disorder and 1,044 controls, two risk haplotypes and one protective haplotype in the CRY2 gene were identified for bipolar disorder with the feature of rapid cycling [65].

The study by Lavebratt et al. [44] demonstrated that CRY2 mRNA expression was decreased in depressed patients with bipolar disorder as compared with controls during sleep deprivation and that total sleep deprivation did not induce any increase in CRY2 mRNA levels in the patients opposite to the change seen in the controls. There was no difference in mRNA levels of NR1D1 or those of PER1 between patients with bipolar disorder (16 during a manic episode and 22 during a depressive episode) and controls [66]. However, the phase of expression was advanced for both genes during mania, as compared with that during depression which was close to that in controls [66]. Using skin fibroblasts from 19 patients with bipolar disorder and 19 controls, McCarthy et al. [67] revealed that lithium enhanced the resynchronization of blunted rhythms, gaining benefit especially to the cases. Further, skin fibroblasts from patients with bipolar disorder, being homozygous for the T-allele of GSK3B rs334558 (SNP -50T/C), did not benefit from lithium as far as the effects on the circadian period or amplitude were concerned [67].

In genome-wide association studies, the associations of ARNTL, GSK3B, and RORB gene variants with bipolar disorder have gained further support [68]. In addition, the RORB gene is among the top candidates that have been identified with data based on genome-wide association studies [68]. From their analysis of 21 core clock genes as well as 322 clock-controlled genes using the summary statistics for the genome-wide association studies of bipolar disorder, Byrne et al. [54] reported that 1 of the 80 SNPs of CRY2 (rs4132063) remained, after correcting the nominal threshold, significant with bipolar disorder in the analysis of 7,481 cases and 9,250 controls.

Moreover, there is a marked enrichment of core clock gene variants in bipolar disorder-related mental and behavioral illnesses [69]. In the analysis of an extended clock network, including 18 core clock genes, 343 clock modulator genes which affect the circadian period or amplitude, and 148 clock-controlled genes which are pervasively rhythmic in more than six tissues, it was found that the major influence on this enrichment originates within the core clock and downstream, arguing against a shared process upstream of both mood regulation and the circadian clock [69].

To balance the view, there are negative findings as well. A meta-analysis combining data from genome-wide association studies of mood disorders pointed out no association of the circadian clock gene variants with bipolar disorder or with major depressive disorder [70].

22.1.4 Seasonal Affective Disorder

Patients with winter depression are unique in their response to light exposure [71]. In the course of evolution, selection for or against the unique

response to light exposure may have resulted in difference in the circadian clock function. It may have affected the temporal organization of circadian rhythms and made the body clock more or less flexible to stimuli, respectively. The extent of the individual's responses to light exposure, however, seems to be controlled by a processor that is separate from, but may still be linked to, the principal circadian pacemaker. This phenotypic difference is to a major part genetically determined, since there is a marked degree of heritability or a substantial genetic effect on the phenotype, as shown in some animal species. Another characteristic of patients with winter depression is carbohydrate craving which is alleviated rapidly by treatment with scheduled light exposures, thereby providing a potential marker of treatment outcome. A key to the decoding of winter depression might therefore be the assessment and characterization of valid phenotypes, if any, of the disordered clockwork and responsiveness to light, which are endogenous by nature.

There are two case-control studies of seasonal affective disorder, or winter depression in specific, in which association with more than one candidate of the circadian clock genes has been analyzed. In the original study by Johansson et al. [20], four genes and one SNP in each were analyzed in 159 patients with winter depression and 159 controls being matched by their sex and age. NPAS2 rs11541353 (S471L) indicated a recessive effect of the A-allele on winter depression [20]. This was the first report of winter depression being associated with a SNP in a circadian clock gene, and subsequent haplotype analysis supported the initial finding. The polymorphism is not located in any of the conserved motif domains in the NPAS2 gene, and although a switch from serine to the less hydrophilic leucine may lead to changes in protein conformation, the biological effect, if any, is presently not known. In addition, carriers of the G-allele in the exon 15 (V647G) of the PER3 gene had more circadian preference to the morning hours in their daily activities among the patients and controls, it being more pronounced among the patients [20].

In the study by Partonen et al. [72], 13 SNPs of three genes were analyzed in 189 patients with winter depression and 189 controls being matched by their sex and age. PER2 rs56013859 (the so-called Spanagel–Albrecht SNP #10870) G-allele, ARNTL rs2290035 T-allele, and NPAS2 rs11541353 A-allele were associated with winter depression [72]. In addition, carriers of the PER2 rs56013859 G-allele had more circadian preference to the morning hours in their daily activities among the patients but not among the controls [72]. The rationale here was that these three genes (PER2, ARNTL, and NPAS2) and the encoded proteins PER2, ARNTL, and NPAS2 are interacting molecules in the core of the transcriptional circadian clock and form a functional triad where PER2 is a positive regulator of the ARNTL [73] and NPAS2 genes plus the principal regulator of NPAS2 [74], and PER2 stimulates the ARNTL-NPAS2 transcription complex [74]. In silico analysis of the biological effects of allelic differences provided an a priori hypothetical framework for the selection of target polymorphisms for each gene, and SNPs in each of the three genes were selected by assessing the potential functionality in silico, the linkage disequilibrium structure, and equal distribution along the genes, in order to identify combined gene effects [72]. Of these, PER2 rs56013859 might be an intronic transcriptional enhancer element, whose allelic constitution influences expression, as it is exceptionally rich in hypothetical allelic transcription factor binding site changes for SP1 (Sp1 transcription factor) and for MYB (v-myb myeloblastosis viral oncogene homologue [avian]) regarding the G-allele and for NFKB1 (nuclear factor of kappa light polypeptide gene enhancer in B-cells 1) regarding the A-allele. The rare risk allele of this SNP, i.e., the G-allele may create a binding site for SP1, which is not present in most of the normal population as indicated in the controls, increases transcript levels of the PER2 gene. Hampp et al. [75] analyzed later exactly the same set of genes (PER2, ARNTL, and NPAS2) and demonstrated that activation of the monoamine oxidase A gene was regulated by the proteins encoded by these three circadian clock genes. Furthermore, genetic deletion of the Per2 gene in mice decreased transcription and activity of the monoamine oxidase A enzyme in the mesolimbic

neurons, and it resulted in the increased levels of dopamine and altered neuronal activity in the striatum and a change in behavior [75].

The PER2 gene is known to be an E/E′-box transcriptional repressor of other circadian clock genes. Topological analysis of the complex transcriptional circuits in mammals suggests that regulation through E-boxes or prime E-boxes may well be the so-called Achilles' heel of the entire transcriptional network of circadian clocks, because it is the most highly connected node of the circuit diagram by Ueda et al. [76]. Furthermore, the rs2290035 in the ARNTL gene alters a SP1-binding site and may thereby regulate the circadian activities of the ARNTL protein [77], or possibly also those of the ARNTL2 protein [78], which as a partner of heterodimers with NPAS2 or CLOCK drives transcription from elements in promoters of the target genes such as the PER1, PER2, CRY1, and CRY2 genes. The negative feedback is thereafter carried to the ARNTL-CLOCK transcription complex through the actions of the CRY1 and CRY2 proteins during the night [32], to the ARNTL-NPAS2 transcription complex through the actions of the CRY1 and CRY2 proteins [79], and to the ARNTL2-CLOCK transcription complex through those of the PER2 protein [80]. In contrast, there is a positive feedback to the ARNTL-NPAS2 transcription complex through the actions of the PER2 protein [74]. Further, the activation of RORC increased mRNA levels of NR1D1 and CRY1 in brown adipocytes, and the genetic loss of RORC reduced those of NR1D1 and CRY1, suggesting that there is a reciprocal relationship between the regulation of RORC and two key clock genes [81]. Of further note here is that CRY2 was not tested in these experiments [81].

Concerning winter depression, there is one case-control study of one candidate gene. In the study by Lavebratt et al. [44], the CRY2 gene was on the focus. Four SNPs were analyzed in 204 patients with winter depression and 2,107 controls from two independent population-based samples, demonstrating that CRY2 rs10838524 A-allele was associated with winter depression [44]. The association of CRY2 genetic variants with winter depression gained further support from the analysis of CRY2 rs10838527 G-allele, CRY2 rs3824872 A-allele, and CRY2 rs7123390 G-allele in these populations [44].

Currently, there are no genome-wide association studies of winter depression. This is surprising, since winter depression is a clinically homogenous and relatively common condition, as approximately every tenth of recurrent depressive disorders follow a regular season-bound course and thereby fulfill, e.g., the criteria for seasonal pattern in the Diagnostic and Statistical Manual of Mental Disorders classification system [71]. In addition, in some patients with bipolar disorder, the clinical course is influenced by the fall in particular, as far as the likelihood of a first admission due to a depressive episode is concerned [82], but the majority of patients with bipolar disorder with seasonal pattern have bipolar type 2 disorder [83].

22.2 Potential Mechanisms in the Pathogenesis

Although two basic phases, daytime and nighttime, can be regenerated in addition to four phases near the subjective noon, dawn, dusk, and late night, from the three basic circadian phases, one basic circadian phase, the morning, escapes the transcriptional logic of mammalian clocks as analyzed using an in cellulo mammalian cell-culture system [84]. Hence, morning transcriptional regulation is still a "missing link" in the mammalian circadian system. Recently, it was demonstrated that a morning-specific repressor, once being degraded, permits evening-specific repressors to be active [85]. In the aforementioned study, the morning-specific repressor was CSP1 (conidial separation 1) whose closest human homologues are RCAN1 (regulator of calcineurin 1) and PRDM16 (PR domain containing 16), which are involved in actions with, e.g., GSK3B and PPARGC1A (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha), respectively, and thereby in the hormonal control of MYB and in control of brown adipose tissue activity, respectively. Both these control functions may be of importance to mood regulation. In the

following, I present a hypothesis on the circadian clock effects on mood regulation.

To start with, the stretch of DNA in which PER2 rs56013859 is located is exceptionally rich in hypothetical allelic transcription factor binding site changes for MYB, and the G-allele may increase expression of the PER2 gene [72]. Next, brown adipose tissue has been demonstrated to be overactive in the depressed who committed suicide [86], and its overactivity may induce deepening of a depressive episode through reducing warm tolerance to fluctuations in ambient temperature that are typically rapid and great during spring, i.e., when there typically are warm days but still cold nights [87].

Because a strong repressor during the evening seems indispensable to the morning phase of the circadian clock functioning [84], a plausible candidate is CRY1 or CRY2. The CRY1 gene in cells of the pars tuberalis reacts to the circulating melatonin concentrations, and a dusk-like response, i.e., a high expression of the CRY1 gene together with a low expression of the ARNTL, PER1, PER2, and NR1D1 genes, can be elicited by increasing melatonin levels occurring at any phase throughout the 24-h cycle [88]. It seems that it is a delay in expression of the CRY1 gene via clock-controlled DNA elements from the CRY1 promoter and a further delay via clock-controlled DNA elements from the CRY1 intron which keeps the amplitude of the circadian clocks as robust [22].

As the level of PER2 proteins in the correct phase is far more critical for the circadian synchronization than mere PER2 levels, the dimerization of PER2 with CRY1 or with CRY2 as a function of time may play a role in the pathogenesis [89]. It is of note that the phase angle difference tracks the interval between dawn and dusk and that the CRY1 and CRY2 proteins give a signal of the evening hours, whereas the PER1 and PER2 give a signal of the morning hours in day-active animals such as sheep [90] for the measurement of the phase angle.

It is not known whether this dynamics between PER and CRY proteins holds for humans as well. Here, if it does hold for humans, the model by Ye et al. [32] may provide a key to understanding of the pathogenesis in mood disorders. The model says that the CRY proteins are in a dynamic equilibrium with the ARNTL-CLOCK transcription complex on E-boxes, that in the presence of PER proteins at a sufficiently high concentration in the nucleus all CRY proteins become trapped by the dimerization, and that these dimers cannot bind to the ARNTL-CLOCK transcription complex on E-boxes [32].

In patients with winter depression, the melatonin signal [91], driving the phase angle difference, and the suppression of melatonin by light exposure are abnormal [92]. Furthermore, the phase angle difference between sleep and melatonin is abnormal [93], and the behavioral rest–activity cycles are elastic [94] in patients with winter depression, indicative of a reset error of the circadian pacemaker. Thus, at least concerning winter depression, a key to the pathogenesis may lie in abnormal signals of the evening that are due to the actions of the CRY1 and CRY2 proteins encoded by the CRY1 and CRY2 genes, respectively, whose genetic variants associate with major depressive disorder and winter depression, respectively.

The CRY2 gene is unique among the core circadian clock genes, since experimental findings of its effects on the circadian clockwork violate the predictions from a theory [27, 28, 95, 96]. CRY2 has a key role in balancing the expression of cryptochromes, as it not only acts as a general repressor, but also opposes in specific the actions of CRY1 and inhibits CRY1 from accessing to its targets too early [97]. However, these findings fit in, or not disagree with, the view that activation of the Per1 and Per2 genes occurs in the morning, whereas activation of the CRY1 and CRY2 genes occurs in the evening [90]. During the night, the CRY1 and CRY2 proteins give the negative feedback to the ARNTL-CLOCK transcription complex [32], but it is only CRY2 that can inhibit the activated forms of ARNTL while the CRY1 and PER proteins have no effect [30].

Further, if the findings from day-active animals can be generalized to concern humans as well, they suggest that activation of the PER1 and PER2 genes in the morning dominates the output from the principal circadian clock in winter, whereas activation of the CRY1 and CRY2 genes in the

evening dominates in summer [98]. From the seasonal point of view, it is intriguing that the CRY2 protein, being abundant in the retina, can function as a sensor of the Earth's magnetic field and does it in a light-dependent manner [4, 99]. Moreover, it is of note that great enough changes in the geomagnetic activity are routine phenomena in spring and fall and that they are the greater, the further away the location is from the equator. Geomagnetic activity tends to reduce melatonin concentrations in humans [100]. It is intriguing that the photoperiod induces changes in the dorsal raphe serotonergic neurons, including the programming of their firing rate, their responsiveness to noradrenergic stimulation, their intrinsic electrical properties, and depressive-like behavior of the individual being dependent on a melatonin receptor [101]. Furthermore, it affects the levels of serotonin and noradrenaline in the midbrain [101].

The CRY1 and CRY2 proteins block the increases in cAMP (cyclic adenosine monophosphate), which are driven by activation of G protein-coupled receptors and the subsequent cascades in the liver [102] and possibly universally in the organism [103]. It has been demonstrated that the increases in cAMP and the downstream activation of cAMP-responsive element-binding protein in the striatum lead to a depressive-like behavioral phenotype in mice [104]. Thereby, the CRY1 and CRY2 proteins are strategically positioned to modulate not only circadian rhythms of a cell or a tissue but also to influence emotional reactions and mood of the individual.

Both RORA and RORB are required for the maturation of photoreceptors in the retina [105]. Due to their action on core circadian clock genes and on metabolic target genes downstream and because of their functions as ligand-dependent transcription factors [106], the proteins encoded by the RORA and RORB genes may play a key role in the pathogenesis of mood disorders. However, the effect of their SNPs that have been associated with depressive disorder and bipolar disorder, respectively, on function of the encoded proteins has not been analyzed. Therefore, their influence is currently not known. Here, the PER2 protein may have a role, as its binding at the promoters of nuclear receptor target genes, such as the NR1D1 gene, oscillates and thereby propagates the circadian information to metabolic pathways [107].

NR1D1 is one of the so-called orphan nuclear receptors, while it seems to be a molecular link between the circadian clocks and mood regulation [108]. Chung et al. [108] demonstrated that genetic deletion of the Nr1d1 gene, or pharmacological inhibition of NR1D1 activity, in mice increased transcription and activity of the tyrosine hydroxylase enzyme in the mesolimbic neurons, and it resulted in the increased levels of dopamine and altered neuronal activity in the striatum and a change in behavior. These results are similar to those derived from another circadian clock gene target, i.e., those of genetic deletion of the Per2 gene as demonstrated by Hampp et al. [75]. Studies with Nr1d1-knockout mice agree with and support this finding, as there is upregulation of tyrosine hydroxylase in the hippocampus [109] and increased proliferation of hippocampal neurons [110] in these mice. In these experiments, their mood-related behaviors were manifested as less anxious and less depressive.

Among the circadian clock genes, NR1D1 is the only one that maintains its oscillation on time at the light–dark transitions as well as under constant darkness in organs throughout the body [111]. Therefore, NR1D1 seems to be the principal metronome of the body. NR1D1 regulates the transcription of "the long-day gene" TSHB (thyroid-stimulating hormone, beta), and through this action, NR1D1 is also a link between the effects of light and the seasonal variation in behavior [112]. Transcription of TSHB is induced to a greater extent about 14 h after dawn of the first long day in the spring by the increasing exposure to light [113].

Intriguingly, the circadian clock protein NR1D1 has recently been demonstrated to link the body's circadian and thermogenic networks through the regulation of the function of brown adipose tissue [114]. The physiological induction of uncoupling protein in the mitochondria by cold temperature is preceded by rapid downregulation of NR1D1 gene in brown adipose tissue, or in other words, the high levels of NR1D1 protein

must fall before cold ambient temperature can induce uncoupling protein 1 to start producing heat and warm up the body. This switching off of the NR1D1-dependent repression is a key to the acute thermogenic response to cold and to subsequent cold tolerance.

It is heat, or heat pulses, being exposed by ambient temperature changes [115] or generated by brown adipose tissue as I have hypothesized [116] that engages the cryptochrome proteins in the temperature entrainment of circadian clocks. If CRY1 or CRY2 or both were deficient, the feedback to the master ultradian and circadian clocks in the brain will be abnormal and mood will be affected.

It seems as if there were two simultaneous processes being compromised in the depressed: first, the thermogenic plasticity that is controlled by NR1D1 and, second, the temperature entrainment that is controlled by CRY1 or CRY2 or both. Switching the NR1D1-dependent repression on again after it has once been switched off, however, is challenged in the spring, when the days are already long but may still be cold. Combination of long light exposure together with cold ambient temperature gives a conflicting signal of seasonal mismatch to the body [116]. Having such conflict, the body is likely to continue producing heat and building up improvement in cold tolerance. If the activity of brown adipose tissue were not to be shut down as normal in the spring, it would easily become overactivated [117] and would produce excessive heat load that would give abnormal feedback from brown adipose tissue to the brain [118–120].

Conclusion

With the focus on the cryptochrome genes, earlier studies have indicated that CRY2 genetic variants associate with winter recurrent depressive disorder [44], with the rapid cycling of bipolar type 1 disorder where depressive episodes dominate the clinical course of illness [65], and with dysthymia which is characterized by a chronic course of illness where a depressive episode lasts for 2 years or longer and often deepens into a major depressive episode [40]. Furthermore, it has been previously observed that following the antidepressant sleep deprivation, the expression of CRY2 mRNA increased in controls, whereas no change or response to the treatment was observed in patients with bipolar disorder whose CRY2 mRNA levels were markedly lower than in controls [44]. A single CRY1 variant has been associated with major depressive disorder [38], and this finding has been replicated [39].

Circadian clock gene variants and encoded proteins are a fruitful target for studies on the pathogenesis of mood disorders. In detail, thus far, genetic association studies have suggested that variants of some, not all, circadian clock genes associate with mood disorders. Of them, genetic variants of CRY1 and RORA have been demonstrated to associate with depressive disorder; those of CRY2 with dysthymia; those of RORA, RORB, NR1D1, and TIMELESS with bipolar disorder; and those of NPAS2 and CRY2 with seasonal affective disorder.

Recent studies have thus far identified downstream targets of interest in mood disorders to include the neurotransmitters dopamine and serotonin whose levels are guided by activity of the clock-controlled key enzymes, including monoamine oxidase A and tyrosine hydroxylase. However, experimental studies elucidating the mechanisms of action by which the circadian clock proteins might contribute to mood disorders are missing. The loss of cryptochromes does change physiology, and dysfunction of cryptochromes may change mood. On the basis of the data presented above, CRY2 appears to be “a mood gene.” Success in the resetting after circadian disruption has been hypothesized to improve lowered mood in the depressed [121], whereas failure in the resetting may deepen a depressive episode any time of the year, especially in the spring. Here, “the missing link” might be the circadian clock protein NR1D1, but I hypothesize that its effects build as a function of time on the deficient CRY2.

Circadian clocks have a tight link not only to transcription of metabolic target genes

downstream in peripheral tissues but also to proteins that act as sensors of metabolic status as a function of time. Clinical data have demonstrated for long that there are abnormalities in the circadian rhythms and in the metabolic cascades in patients with mood disorder. Recent findings of molecular biology and molecular genetics have yielded the first insight into the targets of interest.

Conflict of Interest The author declares that he has no conflict of interest.

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Understanding the Biologically Adaptive Side of Mood Disorders: A Focus on Affective Temperaments

23

Xenia Gonda and Gustavo H. Vazquez

23.1 Introduction

One great paradox in psychiatry is the fact that psychiatric disorders have emerged during evolution, and their frequency is relatively stable in spite of the obvious reproductive and also survival disadvantages, and in several cases other illness associated with these conditions. One of the most straightforward solutions for this contradiction is supposing that psychiatric disorders, or the traits and characteristics encompassed by these disorders, are also associated with advantageous conditions enhancing fitness to balance the negative consequences. Such a hypothesis was first most widely entertained and investigated in the case of schizophrenia, with scientists putting forward the schizophrenia paradox arguing that genotype and also several phenotypical traits associated with schizophrenia are subject to balancing selection, with the benefits outbalancing and outweighing the disadvantages, including better resistance towards infections and allergies, increased fertility in females as well as different types of social advantages including increased artistic creativity [1, 22]. Other types of psychiatric disorders have subsequently also gained increasing interest from evolutionary psychiatrists and psychologists, and while some of the initially conceived advantages associated with mental illnesses have been proven to be only legends, or have been debated and dismissed, viewed from an evolutionary point of view, psychiatric illnesses, including affective disorders, carry a wide range of adaptive characteristics which, in their normal manifestation, enhance adaptive fitness and contribute to pathological conditions only when dysregulated.

Affective illnesses are a heterogenic group of psychiatric disorders having to do with dysregulation of mood and emotional states, manifested in various polarities, severities and durations, as well as showing differential association with the outer environment when it comes to seasonal patterning. Therefore different characteristics of

Xenia Gonda is a recipient of the Janos Bolyai Research Fellowship of the Hungarian Academy of Sciences.

X. Gonda (✉)
Department of Psychiatry and Psychotherapy, Semmelweis University, Budapest, Hungary

Laboratory of Suicide Research and Prevention, National Institute for Psychiatry and Addictology, Budapest, Hungary
e-mail: gonda.xenia@med.semmelweis-univ.hu

G.H. Vazquez
International Consortium for Bipolar and Psychotic Disorder Research, McLean Hospital, Belmont, MA, USA

Department of Neuroscience, Palermo University, Buenos Aires, Argentina

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affective illnesses may be both adaptive and advantageous from different aspects. The introduction of the affective and later the bipolar spectrum and understanding personality and temperamental components of affective illness as dimensions or spectra aid in understanding what different characteristics make up these affective disorders. The model of affective temperaments, introduced by Akiskal among others, does not only allow as to view affective illnesses as a given constellation of temperaments but also helped to extrapolate pathological temperamental conditions into the domain of mental health and to investigate their healthy, non-pathological, normal manifestations also in the general population as well as healthy, non-affected first-degree relatives of affective disorder patients who at least partially share the genetic background. However, each different level of affective phenomena, from illness through mood states to temperaments, has different prominent aspects when viewed from the perspective of adaptation and fitness.

23.2 Mood States from an Evolutionally Adaptive Point of View

23.2.1 Evolutionally Adaptive Aspects of Depressive States

Mood has been hypothesised to play a role in the regulation of investment strategies according to likelihood of payoffs, and in a situation where success is likely upregulation, while where it is unlikely, downregulation of efforts would be adaptive; thus mood plays a role in controlling allocation of resources and efforts [45]. Optimising activities and behaviours according to these contingencies, and this way low mood, can be adaptive in various situations for several functions.

Mood swings and sustained alteration of mood states leading to affective episodes have been viewed in the context of circadian and seasonal rhythms [38] postulating that rhythms, whether circadian or seasonal, have the function of organising the energy economy in such a way that its expenditure is enhanced on occasions where probability of success is high but it is conserved when probability of success is low [59]. Based on this, two genetically programmed strategies evolved to match threats to survival and energy homeostasis: engagement and withdrawal, corresponding to manic and depressive patterns of behaviour [59]. Each of these different poles of mood may have different evolutionary fitness-enhancing advantages viewed from the aspect of different theories, such as the conditions related to switching between them.

There are several studies supporting a connection between changes in light conditions and seasonality of mood [61]. In temperate climates the environmental conditions show sharp and marked circannual changes, posing different challenges in different seasons determining survival and reproduction, requiring different capacities and strategies as well as the capability of switching between them. One environmental factor showing also a marked circannual variation in association with seasonal changes and therefore corresponding to the availability of resources and the presence of challenges is the length of photoperiod. There are various neurobiological and chronobiological evidences for the influence of seasons and light conditions on human behaviour. As the length of the day and the light period decreases, this information through the nucleus suprachiasmaticus influences melatonin secretion leading to a decline in serotonin levels, while dopamine, more strongly associated with manic and hypomanic states, increases during summer [28, 44, 60].

In line with the above theory, in accordance with the seasonal changes, in those times of the year where energy resources were limited, therefore it was not effective to search for them, and in addition due to these limited resources, conservation of energy was also vital, and a decrease of exploratory behaviours decreased the risk of unnecessary energy spending. Lethargy and lack of motivation and initiative as characteristics of depression helped ensure that no activities linked to unnecessary energy spending occur [52]. Furthermore, lack of desire for social contact during depressive phases and also lack of desire for

food and sex decreased the likelihood of within-group conflicts and made living together under difficult and extreme circumstances possible [52].

However, beyond the availability of energy resources, there are several other circumstances when inactivity related to depressed mood increases likelihood of survival. In situations where any action would be dangerous or futile, a decrease in motivation and initiation is useful as this condition decreases the likelihood of any activity to be taken. From this aspect, depressive states can be seen as an adaptive means of inactivity not only by decreasing the chance of entering into dangerous situations but also by reducing energy expenditure in such conditions where success is unlikely [45].

Beyond the above, others hypothesise that the state of being depressed functions to help solve problems both on the social and psychological levels, considering depression as a time for rumination inducing cognitive changes which reallocate and enhance focus and capacities to solve key social problems [57, 63]. Furthermore, depression is also a signal for others that the depressed person needs help and thus shifts the burden to others [37, 63]. Depression, from a wider perspective, can also be seen as a plea for help, as a mean of communication to manipulate others for providing resources, and can also be considered as a failed attempt at adaptation due to the lack of help [45].

A further aspect of the potential adaptiveness of depressive states is related to affects regulating goal pursuit [30, 45] and that mood and emotions are also shaped by success and failure in the accomplishment of goals, with failure being a potent trigger of negative moods, and low mood in turn having the function of disengaging from unattainable goals and unproductive efforts [42, 45]. In this way, depression prevents and blocks down useless spending of energy on trying to reach goals which can't be accomplished, by decreasing initiative, motivation and also the self-esteem necessary for such activity. Depression may also serve to prevent the untimely and too early pursuit of new enterprises after failure [45]. Low mood has been linked not only to the inability to disengage from unattainable goals; it is also aroused by a mismatch between expectations and accomplishments [45] and was found to shift cognitive styles to more realistic and systematic [20, 45], therefore preventing useless energy expenditure before it occurs.

Another perspective from which depressive states can be considered to increase fitness and individual survival is that such states convey a submissive and yielding signal and behaviour towards those higher in hierarchy [48] thus avoiding and preventing attacks also by signalling to those higher in the hierarchy of the individual constituting no danger to them [53, 63]. Submissive and yielding behaviour associated with depressive states also promotes protective reactions from those members of the group which are more aggressive and more dominant [27, 31]. As noted above, depressive states by decreasing desire for social contact, sex or food also help to avoid the precipitation of conflicts [52].

Affective disorders can also be conceived in terms of position in the dominance hierarchy [48], since efficient communication of social ranks conserves energy [32] and helps avoid unnecessary dominance conflicts. From the aspect of social rank, depressive behaviour corresponds to omega members at the lower end of the hierarchy, while manic behaviour to alpha members at the top end [32]. While those higher in the social rank are freer to initiate interactions, have bigger access to commodities and greater freedom of action, those at the lower rank have a higher chance to experience deprivation, more constraints and higher stress [32]. Adapting these behaviours associated with the status of the individual communicates status positions and helps to avoid status conflicts [57]. A similar theory is related to selection pressure for prestige focusing on self-esteem alterations in different mood states, with depression characterised by low self-esteem and withdrawal while mania by inflated self-esteem and social pursuit [43]. Depressive states, by conveying submission, provide an alternative to ostracism from the group promoting survival and can be considered as exhibiting the reverse signs of prestige including symptoms of melancholia, such as avolition, inhibition,

withdrawal, reduced food demand and libido as well as psychomotor retardation [43].

Depression may also be a means of avoiding infection and reducing spread of infection [57]. Due to several immunological mechanisms, depression often arises as a result of infection [40]. Depressive state conserves metabolic resources for fighting infection and recuperating from them and reduces spreading by decreasing chances for transmitting through social interaction and also reduces chance of getting a new infection while vulnerable [57].

We have outlined several aspects of depressive moods and states increasing chances for survival and thus fitness. Some authors, however, argue that neither depressive nor manic states are adaptive, and it is not the affective states themselves, but the threat and promise associated with depression and mania, respectively, that enhances fitness [63]. From this aspect the threat and promise of depression and mania can be considered as incentives, with the possibility of depression motivating and incentivising behaviour away from those activities and outcomes which would reduce reproductive fitness while the promise of elation gears behaviour towards reproductive fitness-enhancing behaviours [57].

23.2.2 Gender Differences in the Adaptiveness of Depressive States

As mentioned above, during evolution in moderate climates, humans were subject to strong selective pressures due to fluctuating food and energy supplies in different seasons, and lack of energy leading to periodic starvation during winter contributed to significant weight loss which could stop ovulation and reproduction, meaning that selective pressures for winter depression were the most pronounced for reproductive-age women [52] leading to gorging before and depressive behaviours during winter with a decline of desire for activity, food, sex and social contact and minimal initiative to conserve energy [52] corresponding to symptoms of depression. Women were genetically programmed to be more prone to depressive states due to their role for carrying offspring and caring for them after birth [52]. Furthermore, this seasonal determination of behaviour being more pronounced in women also had an influence on time-adjusting chances of conception and pregnancy, and consequentially birth of the offspring, by increasing the likelihood of births in those periods, mainly during spring, where environmental conditions including availability of food were better adapted to aid survival of vulnerable offsprings [27]. The fact that depression is twice as frequent in females compared to males and that it is prominently associated with reproductive age in females also indicates its association with affiliative needs and gender roles related to childbearing and childrearing; therefore depressive states and traits may keep women away from danger and dangerous situations also irrespectively of seasonal variations, but these same traits are disadaptive in men who are considered providers and protectors [27], therefore avoiding, inactive, submissive behaviour decreasing not only their survival but also family members dependent on them. Similarly beyond the individual level, depressive states may be also adaptive at the group level, by promoting pair bonding providing longer and increased care for the offspring, which is also related to the observable gender differences in depression [27, 31].

23.2.3 Seasonal Affective Disorder

Seasonal affective disorder is a specific type of mood disorder where symptoms in many aspects resemble those of unipolar major depression, while certain symptoms are atypical, mainly hyperphagia and hypersomnia which are in sharp contrast to typical unipolar major depressive episodes more often characterised by insomnia and lack of appetite. Seasonal affective disorder patients are doing well during spring and summer in temperate latitudes or are even hypomanic, while the peak of depressive symptoms is in winter including hyperphagia, hypersomnia, weight gain, lethargy, decreased libido and social withdrawal [27]. Although some authors consider

seasonality a disadaptive by-product of evolution [51], it is associated with higher intellectual functioning and higher educational achievements. Seasonal affective disorder symptoms resemble those of hibernation seen in certain mammals [27] and may be a mean of energy conservation during winter periods. Furthermore winter depression may be advantageous in the context of procreational timing [27] by increasing the chance of births to happen during spring, between the end of winter and early summer corresponding to those periods when food supplies are more abundant and environmental conditions such as temperature also aid survival. For births in these periods, pregnancies need to be conceived during summer; therefore lessened activity during winter periods does not only conserve energy, but narrowed social interest and decreased libido associated with depressive phases also help to time procreation and pregnancy [27]. This is more important for females compared to males in accordance with the association of a higher incidence of depression in reproductive-aged females and is also in good agreement with the observation that in females mood also fluctuates during the menstrual cycle [46]. Viewed from this perspective, seasonality is aimed at managing changes in the outer environment and optimising energy intake, expenditure and metabolism and increasing the likelihood of births in the most appropriate time of the year [61]. Seasonal affective disorder also promotes the nuclear family [27] by enhancing pair bonding during pregnancy increasing chance for survival for both the female and the offspring [27].

23.2.4 Evolutionally Adaptive Aspects of Hypomanic States

As seen above, there are various adaptive aspects of depressive states depending on the theoretical background and also depending on which aspect of depressive symptomatology is emphasised. However, not only low moods but the other end of the mood spectrum can also viewed as adaptive; therefore hypomanic and manic phases can also be considered to enhance fitness.

While in winter due to restricted resources and necessity of energy conservation, depressive states were adaptive, after winter it was necessary to quickly adapt to changing conditions and the increasing abundance of energy supplies, especially since energy-abundant periods were short and also that lost energy resources and body weight had to be replenished as soon as possible. Therefore characteristics associated with hypomania including energy increases, optimism and self-confidence helped the restoration of activity as normal during spring. Also in this period, mating and socialising and executing goal-directed activities were also adaptive making hypomania a tool for survival [52]. Furthermore, independently of seasonal variation, incentiveness, initiatives and motivation associated with mania serve various adaptive purposes. While depression serves to discourage actions and behaviours when it would be dangerous or futile, elated mood encourages action and behaviour when resources are plentiful or likelihood of success is high [45].

23.2.5 Evolutionally Adaptive Aspects of Mania

While hypomania was adaptive as an increase in activity, exploration, motivation and goal-directed activity after the passing of the inactive period, mania could be more seen as adaptive by its capacity of sharp, swift and sudden change from an inactive phase to highly increased activity when unexpected challenges need to be faced. Mania is associated with tirelessness, unconditional self-confidence and self-esteem and decreased need for food or sleep aiding survival in harsh, extreme conditions [32]. Mania is a state characterised therefore as evolutionally fitness enhancing by enabling a swift change from depressive states, which could have been adaptive during extended periods of winter when the majority of the group was depressed yet had to face some unexpected outside danger [52]. One further notion underlying this hypothesis is that lack of sleep in several cases triggers mania, arguing for the manic behaviour-inducing capacity of disruption of winter sleep by unexpected dangers

[62]. Therefore, unlike hypomanic symptoms fit for quickly regaining energy and supplies and utilising resources during the short periods where they are available, mania is more associated with extreme emergencies requiring stamina and extended physical strength which may be exposed by natural catastrophes or attacks [52].

From another approach, explaining mood states being adaptive in terms of dominance hierarchy [48], manic behaviour corresponds to the alpha member of the group with excessive self-esteem, freedom for any type of activity as well as unlimited access to utilise resources and unlimited access to mating opportunities. Those higher in the social rank are freer to initiate interactions, have bigger access to commodities and greater freedom of action [32], characteristic also associated with manic phases. According to the prestige model of bipolar disorder, while depression is characterised by social disinvestment, mania is characterised by a frenzy of social engagement with the goal of increasing relational value, however, with a failure to recognise stabilised prestige and inducing a phase characterised by excessive self-marketing aimed at intersexual selection with affect display, linguistic expression, sociality, creativity and status [43].

In general, mania is associated with tirelessness, unconditional self-confidence and self-esteem and decreased need for food or sleep aiding survival in harsh extreme conditions as well as a rapid switch into this condition, and these characteristics are the reasons why bipolar disorder is frequently associated with good leadership skills [32], enhanced creativity and higher socioeconomic levels [21, 35, 50].

23.3 How Do Adaptive Aspects of Affective States Turn into Pathological Conditions?

There are several approaches to what extent affective states are still adaptive and at what level and due to what mechanisms they turn into pathological conditions. Adaptiveness of affective states and phases is the easiest to observe in case of hypomanic states as these conditions are associated with increased activity and energy and often also elated mood, leading to accomplishments but generally mild enough and thus causing no impairments [52]. Depressive and manic states, however, often lead to dysfunctional on non-functional states, and costs of low mood may be smaller than the costs related to inappropriately high mood states [45].

Depression may be a defence, a dysregulated malfunctioning defence or a defect [45]. Some authors postulate that normative depression is adaptive while severe manifestations of depression are not [19, 43]. Low mood may be useful in certain situation and becomes pathological only when excessive and prolonged [45]. Generally, states underlying the positive aspects of depression together with their neurobiological determinants were selected during evolution, and the pathological conditions arise when these states became independent and autonomous from their adaptive triggers. Considering the social hierarchy hypothesis of depression, it has been postulated and indicated in previous research that there are distinctive neuropsychological states paralleling behavioural patterns associated with these social positions, and in case of pathological conditions, this neurophysiological state is dissociated from the appropriate environment and is too easily triggered or too rigidly maintained, leading to the emergence of periods of depression or mania [32].

Bipolar disorder, which implies a periodical appearance of the two opposing mood phases, can thus in part be explained by the adaptiveness of each of these phases and their becoming autonomous from the appropriate trigger and becoming pathological instead of adaptive. As a consequence, these states are too easily triggered without appropriate stimulus and too rigidly maintained [32]. The social hierarchy model of bipolar disorder, as introduced above, explains the alteration of manic and depressive phases as extremes of social role [32]. While components of theses alpha and omega behavioural states are normal, they can be abnormal components of manic depression genetically transmitted as susceptibility factors, including excessive sensitivity to trigger and decreased sensitivity to offset stimuli contributing to an instability-rigidity complex [32].

23.4 The Model of Affective Temperaments: Decomposing Affective Disorders to Spectrum-Like Traits Reflecting Advantageous and Disadvantageous Characteristics

Each of the affective phases, including depressive, hypomanic and manic phases, can be considered to show different adaptive characteristics according to different hypotheses or theoretical considerations, and the same characteristics can be considered adaptive under some circumstances while disadaptive under others, especially when becoming excessive or dysregulated. Therefore instead of viewing affective disorders and even affective phases in their wholeness, it aids their understanding to decompose these complex manifestation illnesses to a number of dimensions, that is, traits or temperaments associated with the symptoms of these crippling conditions which also helps to reveal the adaptive characteristics of them, and beyond that, would make a more refined diagnosis, course prediction and treatment possible.

There have been several models of personality and temperaments proposed to aid dissecting human stable characteristics and methods of reacting into well-characterisable components; however, the majority of these models were drawn to describe the healthy personality and treat pathologies and pathological manifestations either as extremes of the given dimensions or as unlucky constellations of those dimensions. Furthermore, the majority of these models, while great tools in research and in mapping personality, have little utility in the clinical field. The model of affective temperaments, developed mainly by Akiskal, employs a reverse approach. Building on ancient and historical theories of temperament such as those of Aristotle and Kretschmer, but combining this knowledge with observations and research in clinical patients and their healthy, non-affected first-degree relatives, Akiskal distilled five dimensions of affective temperaments based on constantly observable patterns in mood disorder patients and also their healthy, psychiatrically non-affected first-degree relatives and extrapolated his findings to the domain of mental health. As a result the model includes five affective temperamental dimensions: hyperthymic, depressive, cyclothymic, irritable and anxious, each of which spans between mental health and mental pathology [5, 13]. The model postulates that while these affective temperaments are essential and healthy components of personality, when present in a marked or dominant form they constitute high-risk, subclinical and even prodromal states of affective disorders and different constellations of these affective temperaments are associated with different types of affective illness [10, 13, 15, 17]. Furthermore, affective temperaments help refine the bipolar spectrum and have in a large number of studies shown to play a pathoplastic role determining and influencing several characteristics of affective disorders including illness onset, course, response to therapy and endpoints including suicide [17].

23.4.1 The Five Affective Temperaments

Each of the affective temperaments has been described in a detailed way independently of its association with affective pathology.

The hyperthymic temperament is associated with lifelong exuberant, overenergetic and overconfident traits and has been described as expansive, extroverted and tireless while at the same time prone to verbal aggression, risk-taking and generosity [47].

Depressive temperament, on the other hand, has been described as showing low energy levels, being critical, negativistic, as well as introverted, who doesn't easily accommodate changes, explore new situations or meet new people. The depressive temperament is associated with self-denying and is prone to a depressive mood dominated by dejection, unhappiness and cheerlessness with a self-concept built on low self-esteem and feelings of worthlessness [11, 47].

Unlike the above two opposite temperamental types, cyclothymic temperament is an alternation

between hyperthymic and depressive moods, cognitions and behaviours also exhibiting a decreased need for sleep alternating with hypersomnia, instable self-esteem with lack of self-confidence alternating with a grandiose overconfidence and also fluctuating cognitive output with sharpened and creative periods alternating with those of mental confusion and apathy. These undulating levels of mood, activity and cognition are also paralleled by uneven quantity and quality of productivity, uninhibited people-seeking interchanging with introverted self-absorption and excessive pursuit of pleasurable activities without consideration or concern of the possible negative consequences alternating with reduced involvement in pleasurable activities as well as guilt over such past activities [11]. Those with a marked or dominant cyclothymic temperament switch between excessive optimism and pessimistic attitudes, between being overly talkative and joking to reduced talking and tearfulness, and exhibit frequent shifts in orientation in work, study, interests and plans as well as frequent changes in residence and employment, with a tendency for promiscuity, repeated romantic relationship failures and has a tendency to employ alcohol and drugs both to control mood and augment excitement [2].

Irritable temperament is another type of lifelong combination of hyperthymic and depressive temperaments, with characteristics of the two occurring not alternatingly but simultaneously. Thus the irritable temperament is characterised by depressive moods exhibiting periods of irritation, high but not sustained emotional implication in various activities, agitation and impulsiveness with inner tension and dysphoric restlessness, a proneness to criticism and complaining, and tendency for brooding [15].

In addition to the four original temperaments which also in many ways resemble the classical temperamental divisions of Aristotle and the German school by Kretschmer, the model of affective temperaments also integrates a fifth one, the anxious temperament [3] to include a predisposition for generalised anxiety disorder, which is characterised by the continuous presence of dependency, harm avoidance, shyness, insecurity and uncontrollable worry as well as hypervigilance, irritability and gastrointestinal distress [7].

Each of the above-described affective temperaments is a spectrum spanning from health to pathology, defining emotional reactivity. In their normal distribution, all of the affective temperaments and their manifestations are not considered pathological, making it possible to adapt successfully to the environment and enhancing fitness, while when they are expressed in a marked or dominant form, they can be considered high-risk or precursor states or even subaffective manifestations of different types of affective disorders. It is, however, in their normal distribution, as mentioned above, where their adaptive characteristics can be grabbed.

23.4.2 Evolutionally Adaptive Aspects of Affective Temperaments

Evolutionary theory postulates that organisms develop and exhibit favourable characteristics and traits that are advantageous and help them survive in a given environment. Genes encoding for these favourable characteristics increasing the chance of survival and reproduction have a higher chance of being passed on to the next generation via procreation, while disadvantageous traits and genes encoding for them have a tendency to become extinct. As there is a continuum between affective disorders and affective temperaments, it can also be hypothesised that affective temperaments are carried on through evolution from generation to generation because they may carry an adaptive component, and those genes, which basically encode for adaptive characteristics, in some extreme cases, contribute to the manifestation of psychiatric illness. In fact it is postulated that affective temperaments grab the evolutionally adaptive and socially positive aspects related to the genes in the background of affective disorders and may subserve key roles in emotional communication and survival [8].

Temperaments by definition have strong genetic and biological foundations; therefore it is straightforward to postulate that they have evolved

because they carry some evolutionary fitness-enhancing advantage increasing adaptation to environmental or social environments. Affective temperaments are considered the subaffective and subclinical manifestations of affective disorders, and, in line with this hypothesis and given their strong genetic background, a higher prevalence of a more marked manifestation of affective temperaments in the healthy, psychiatrically non-affected first-degree relatives of affective patients has also been observed [41, 54], along with higher levels of creativity compared to the general population [49] and also higher social and economic achievement [25]. These findings indicate that the same genetic and neurobiological background underlying affective disorders and affective temperaments may contribute either to the development of disorders or in other constellations to more advantageous adaptation. This would mean that those genes carrying adaptive mechanisms lead to the emergence of affective temperaments in most cases which are adaptive, while in some cases if expressed in a more marked form or in interaction with effects of other genes or the environment, they may lead to the appearance of major affective disorders. In other words, the chance of developing an affective pathology is the price to be paid for the chance of excellence, creativity or better adaptation. From these theoretical aspects, affective illnesses can be considered the genetic reservoirs for adaptive temperaments [14].

Evolutionary advantage of affective temperaments may be also manifested either at the individual or the group level, and it is very important to consider that any temperament can carry an evolutionary advantage for the group even if it is disadvantageous for the individual. So simply considering the individual level is not enough. Temperaments have a spectrum or continuum nature, and, in general, they are advantageous when expressed at their optimum level, while extreme deviations are more likely associated with psychopathology [24]. Affective temperaments, however, are hypothesised to be advantageous at least at the group level even when they are manifested in their extreme forms [10]. This is in line with the evolutionary theories concerning some psychiatric illnesses, such as affective disorder [52] and seasonal affective disorder [26, 27] and other types of mood disorders as discussed above. Although humanity and human culture and societies are not old enough to speak of evolution in its traditional sense, the aggregation of traits and temperaments more adaptive in a given society can already be observed leading to geographical differences in the relative distribution of more expressed forms of affective temperaments and their constellations.

The individual affective temperaments each have independent advantageous aspects. The key feature of the depressive temperament is sensitivity towards suffering; therefore promoting care for the young and helpless and thus favouring group selection on the one hand and on the other hand the depressive temperament also increase the tendency to conform with social rules and roles and contribute to self-denying devotion to others [9]. Furthermore, people with a marked depressive temperament tend to seek protection and security, look for harmony and social as well as professional bonds, which decrease tendency for exploration and experimentation [18], and, especially in women where this temperament is more prominent, it promotes family and marital bonding [29]. In men depressive temperament is hypothesised to promote work orientation [4]. It could therefore be postulated that, from an evolutionary point of view, the depressive temperament increases the likelihood of passing on genes to the next generation and in turn, by protecting the next generation, increases survival into adulthood and thus also the chance of successfully passing on genes to the subsequent generation. Translated into the social level, the depressive temperament may lay the foundation of cohesive family units based on trust and belonging, which are the basic units of our societies, and thus, besides social integrity and cohesion, it could also be an important element in passing on social and moral values to the next generation – again ensuring that the next generation will exhibit these adaptive temperamental traits as well.

The hyperthymic temperament is associated with exuberant, upbeat, overenergetic and overconfident behaviour, traits that could be stated as proper for leadership, territorial behaviour and

exploration and carry an advantage both on the individual and group level [12, 18]. Hyperthymic temperament's people-seeking and extroverted attitude, coupled with overconfidence and high energy and planning, ensures defending the territory from challenges both from within and outside of the social group [18], while the uninhibited, promiscuous and stimulus-seeking behaviour ensures exploration equally for new resources and for new mates, which not only increases the chance of passing on the genetic material but also increases the chance of combining it with a variability of genetic sets, making sure that at least one such intermixed variety will survive. This conceptualisation of the hyperthymic temperament is well reflected in its positive correlation with novelty seeking and negative correlation with harm avoidance [16].

The cyclothymic temperament is involved in romance and is associated with pursuit for love and lovemaking opportunities [18], obviously advantageous for the dissemination and transfer of genetic material, coupled with increased creativity [56], a generally adaptive feature [29].

The irritable temperament's evolutionary advantage is domination, particularly domination over the struggle for resources, as well as exposition of dominance and higher social rank in the form of ritualised fighting in order to avoid the risk of engaging in actual physical fight while maintaining social rank [18].

Finally anxious temperament may be the trait underlying altruistic anxiety, which subserves the survival of one's extended phenotype by promoting kin selection [6, 18], while on the individual level, it promotes individual survival through caution. Also, anxious temperament fosters dependence and favours the conjugal bond [6, 18].

In a recent study, it was reported that the distribution of dominant affective temperaments reflects and parallels important cultural characteristics as expressed in Hofstede's cultural indexes [34]. Comparing normative data on affective temperaments including the frequency of their dominant form in six large-scale national studies [34, 55], it was found that the order of countries according to the frequency of each dominant temperament reflects their order with respect to cultural indexes described by Hofstede [36, 39] in case of certain temperaments. A strong relationship was reported between individualism-collectivism and depressive temperament, uncertainty index and hyperthymic temperament and power distance index and irritable temperament. The striking similarity between each of these paired concepts of cultural indexes and affective temperaments, and their parallel distribution in the investigated national studies, points to and emphasises the evolutionary role of affective temperaments in social adaptation. The strongest relationship was found between depressive temperament and the collectivism endpoint of the individualism-collectivism spectrum. One additional very remarkable feature is that the 5-HTLPR s allele was found to be associated with the depressive temperament on the one hand [33], and the geographical frequency distribution of the s allele parallels geographical distribution of scores on the individualism-collectivism index [23, 58]. This genetic link between the two phenomena yields further strength to our findings and supports the evolutionary adaptive role of affective temperaments.

Conclusion

Thus several characteristics of affective disorders point to their adaptiveness and fitness-enhancing features both during evolution and also in our contemporary society, which helps understanding not only the nature of these illnesses but also sheds important light on their development, emergence, determinants and neurobiological and social background. Decomposing affective illnesses into affective temperaments is not only helpful for research but also for better understanding of these conditions, refining prevention, screening, diagnosing and also treating these illnesses both on the psychotherapeutic and pharmacotherapeutic levels, as well as within a community approach. Finally, understanding these disorders in their wholeness, from a wider scope, and conceiving them not as plagues or defects, but a necessary product of adaptation, may help alleviate the stigma associated with them.

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Sleep Abnormalities as a Diagnostic Tool for Major Depressive Disorder

24

Alan B. Douglass, Marcus Ward, Timothy Roerhrs, Cynthia L. Arfken, and Nash N. Boutrous

24.1 Introduction

The purposes of this chapter are to describe the REM sleep control system for researchers not primarily working in that area and to recount the history of its use in psychiatric research. For the uninitiated, there are many quirks and complexities in the psychiatric sleep literature which make it difficult to synthesize the findings of papers from different decades.

The usefulness of such a chapter in this book of this type hinges on two main points. First, many of the commonly used treatments for human major depressive disorder (MDD) cause easily identifiable changes in polysomnographic measures. Second, the neurophysiological systems controlling sleep, especially REM sleep, are primitive ones that are highly conserved across evolution. This allows the application of experimental findings from animals to the human situation. (In nonhuman species, REM sleep is usually called "paradoxical sleep" (PS), due to the paradox of a nearly awake cortex but with full paralysis of skeletal muscles.) Modern sleep recording-techniques make it possible to measure sleep accurately from skin surface electrophysiology; therefore, sleep can be used as a powerful dependent variable in modern pharmacological and behavioral experiments.

Human sleep research in psychiatry experienced a "golden age" in the period 1970–1995, during which time several sleep variables were found to be abnormal in depressed patients. These included a shortened *REM latency* (time interval between sleep onset and the onset of REM sleep, "RL"), decreased amount of *deep sleep* (slow-wave EEG), and increased *REM density* (the number of eye movements per minute in REM sleep, "RD"). While the latter was easily visible upon visual inspection of the paper sleep recording, it proved to be difficult to quantify in detail with the technologies available at the time.

Since 1995, there has been some disappointment in the field of sleep research in depression, mainly due to the fact that the supposedly specific relationship between short REM latency and major depression was also found to occur in some

A.B. Douglass, MD (✉) • M. Ward, MSc
Royal Ottawa Mental Health Center, University of Ottawa Institute of Mental Health Research, Ottawa, ON, Canada
e-mail: alan.douglass@theroyal.ca

T. Roerhrs, PhD
Henry Ford Sleep Disorders and Research Center, Henry Ford Health System, Department of Psychiatry and Behavioral Neurosciences, Wayne State University, Detroit, MI, USA

C.L. Arfken, PhD
Department of Psychiatry and Behavioral Neurosciences, Wayne State University, Detroit, MI, USA

N.N. Boutrous, MD
Department of Psychiatry, University of Missouri – Kansas City, Kansas City, MO, USA

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patients with schizophrenia [1], alcoholism [2], borderline personality disorder [3], and other diagnoses. In addition, even the diagnostic specificity of short RL in major depression was found to be age dependent [4] – it was fairly reliable at separating normals from MDD groups older than age 45, but not so clear in the younger age groups. The physiological reason for this has not been elucidated, although RL is known to gradually shorten in everyone over the lifespan.

A philosophical question also arose about the relationship between REM sleep and deep sleep: is the short RL that is observed in depressed patients due to a stronger pressure for REM sleep, or is it due to a deficit in their production of deep sleep, which usually occupies the time interval known as RL? To some degree, this was clarified by the creation of scoring rules for RL that involved subtracting from the total RL any brief periods of awake time occurring in the RL interval, thereby producing the new variable "REM latency minus awake" (RLMA). While this new variable was more stable, in that it had a lower variance and was more repeatable night to night [5], the philosophical argument remains.

Finally, it must be noted that various research sleep laboratories around the world have habitually used slightly different or even idiosyncratic ways of scoring and tabulating sleep variables, resulting in less international collaboration than might otherwise have been the case. For example, there are at least three different definitions of sleep onset, one of which requires the entry into Stage 2 sleep, whereas many labs would accept three 30-s pages of Stage 1 sleep. Since the 1990s, happily, the rise of clinical sleep laboratories for the purpose of diagnosing sleep apnea has resulted in international standards for most of the common measurements [6]. However, this is certainly not the case for RD, which continues to have the most definitions and scoring algorithms.

As will be described below, it is possible that more information about depression is encoded in RD than had been appreciated during the "golden age." So much so that RD analysis might be the way forward for research into the sleep of depression. A short historical review of the science of REM sleep is necessary for a background.

24.2 Discovery of REM (PS) Sleep Physiology

REM sleep was first observed by Aserinsky et al. [7, 8] in humans and then subsequently by Jouvet in the cat [9]. Jouvet's "pontine cat" preparation was the result of a midbrain transection just rostral to the pons, which left REM sleep features intact caudal to the cut and abolished them rostral to the cut. This suggested that the major control center for REM sleep resided in the lower brainstem and began lengthy investigations to pinpoint the structures involved. Much of the early work was done on cats and led to the identification of the main REM sleep initiation area. This proved to be the nucleus subcoeruleus (SubC), a region ventral to the main group of noradrenergic neurons in the locus coeruleus (LC) and dorsal to the gigantocellular tegmental field (FTG). In the rat and mouse, the functionally equivalent region is called the sublaterodorsal nucleus (SLD), roughly the rostral part of the SubC.

In both humans and animals, paradoxical sleep (PS) is characterized by low-amplitude, relatively fast EEG rhythms, as well as by skeletal muscular atonia and episodic bursts of ocular saccades – the individual rapid eye movements (*rems*) that occur during this stage of sleep. Based on other physiology linked to the occurrence of *rems* during PS, Moruzzi [10] proposed to distinguish tonic versus phasic components of PS. Briefly, those portions of PS with bursts of *rems* constitute the phasic component and are typically associated with cardiorespiratory variability [11]. Other parts of PS are nearly devoid of *rems* and constitute the tonic component; these are associated with penile tumescence [12] and rare isolated *rems*.

Phasic PS has also been closely associated with the occurrence of ponto-geniculo-occipital (PGO) waves [13]. PGO waves are prominent phasic bioelectrical potentials, first identified in the lateral geniculate nucleus [14] as spikes occurring in isolation or in bursts. Although PGO waves can be observed in many parts of the brain [15], they are most easily recorded in the pons, the lateral geniculate bodies, and the occipital cortex, hence their name. Some authors have suggested the existence in humans of a process similar

to animal PGO waves [16–18], but they are not recordable from scalp electrodes in humans and so remain of theoretical interest only.

Animal data suggest that PGO waves could be implicated in spontaneous visual activity during PS [19] as well as in central nervous system maturation [20]. Furthermore, they may play a role in learning and memory consolidation in the rat [21] and have been proposed to reactivate memory traces during REM sleep [22]. Indeed, prolonged learning periods have been shown to be accompanied by an increased RD in subsequent PS [23]. In humans, more active or more emotionally intense dreams have been reported during the appearance of *rems* [24]. More recent studies have shown a relationship between the frequency of *rems* during PS (i.e., REM density) and visual imagery in PS [25].

All of this early work was integrated in 1975 into the *reciprocal interaction model of REM sleep* [26, 27]. It was found that neurons localized in the FTG showed an increased discharge rate 5 min before and during REM sleep. Noradrenergic neurons in the LC decreased their rate of discharge moderately during the transition from wakefulness to NREM sleep, but then more sharply during the transition from non-REM sleep into REM sleep. These authors proposed that "REM-off" LC cells exert an inhibiting effect on "REM-on" cells in the FTG, which gradually wanes from sleep onset until the REM-on cells escape that inhibition, leading to a sudden increase in REM-on activity and REM sleep onset. As REM-on activity increases during REM sleep, it has an excitatory effect on the REM-off LC neurons, whose inhibitory influence on the REM-on cells then ends REM sleep. These authors further speculated that serotonergic neurons in the dorsal raphe nucleus (DRN) also play an inhibitory role in REM sleep control [26].

24.3 Modern Neurophysiology of REM Sleep Generation

Subsequent revisions of the reciprocal interaction theory of REM sleep were made possible by advances in histochemistry and immunology [28–30]. REM-on neurons were identified as cholinergic and residing in the laterodorsal- and pedunculo-pontine tegmental (LDT/PPT) nuclei, situated at the pons-midbrain junction. These project both to glutamatergic effector neurons in the medial pontine reticular formation (mPRF) and to many distant brain areas which control the various aspects of REM sleep, such as low-voltage fast EEG activity, *rems*, and muscle atonia [31]. Mutual positive feedback between cholinergic LDT/PPT and glutamatergic mPRF neurons [32, 33] helps to stabilize individual REM periods, which in humans can last for over an hour.

Monoaminergic REM-off neurons in the DRN (serotonin) and the LC (norepinephrine) tonically inhibit REM-on neurons during NREM sleep but also inhibit themselves in a negative feedback loop, causing a gradual waning of REM-off activity to the point that REM-on neurons are released to initiate REM sleep. During REM sleep, REM-on neurons are gradually excitatory to the DRN and LC REM-off neurons, which then eventually inhibit the REM-on neurons, terminating the REM period [31]. This circuit allows the cyclic pattern of REM sleep that is observed across the night, producing a REM period approximately every 90 min in humans.

GABA has been a relatively recent addition to models of REM sleep control. This is partly because it is a ubiquitous inhibitory neurotransmitter, so it was difficult to identify its local effects precisely [34]. However, there is evidence that LDT/PPT neurons exert an inhibitory effect on GABAergic mPRF interneurons, whose job it is to inhibit glutamatergic mPRF neurons (REM-on cells); this connectivity disinhibits glutamatergic mPRF neurons just before and during REM sleep [30]. In a microdialysis study in the cat, the muscarinic agonist carbachol induced REM sleep and decreased local GABA concentrations when applied to the mPRF [35]. As well, there are data suggesting that the concentrations of GABA in the mPRF of the cat and the rat [36] are decreased during REM sleep versus wakefulness. There is also evidence that GABA is involved in the inhibition of REM-off neurons in the DRN [37, 38] and the LC [39].

In an elegant series of papers, Datta laid out an overall theory of REM sleep regulation called the "Cellular-Molecular-Network (CMN) model," showing how various brain areas contribute to the production of REM sleep – all driven by the cholinergic LDT/PPT:

> muscle atonia is executed by the activation of neurons in the locus coeruleus alpha (LCα), rapid eye movements result from the activation of neurons in the peri-abducens reticular formation (PAb), PGO waves emerge by the activation of neurons in the caudo-lateral peribrachial area (C-PBL) of predator mammals and in the dorsal part of the nucleus subcoeruleus (Sub C) of prey mammals, hippocampal theta rhythm is produced via the activation of neurons in the pontis oralis (PO), muscle twitches appear with the activation of neurons in the nucleus gigantocellularis . . . and increased brain temperature and cardio-respiratory fluctuations occur via the activation of neurons in the parabrachial nucleus (PBN). The cortical EEG activation sign of REM sleep, however, is executed jointly by the activation of neurons in the mesencephalic reticular formation (MRF) and rostrally-projecting bulbar reticular formation (. . . medullary magnocellular nucleus). [40]

Another paper [41] detailed the way in which glutamatergic projections from the mPRF to REM-on neurons in the cholinergic LDT/PPT nuclei are the major trigger for *rems*. These neurons display both glutamatergic (kainate) and GABA-B receptors. Physiological release of glutamate onto these cells acts as an agonist at the kainate receptor, a calcium ion channel. Calcium flux through this receptor then acts intracellularly via adenylate cyclase and protein kinase A (PKA) to cause the release of acetylcholine (ACh) from the cell's axon to distant regions (as listed above), which together produce the various physiology of REM sleep. In contrast, the release of endogenous GABA onto these same LDT/PPT cells activates their GABA-B receptors and suppresses their release of ACh, thereby terminating the REM period [42]. The CMN model can therefore be described in terms of the interactions of GABA, glutamate, ACh, serotonin, and norepinephrine. Of note, the mPRF glutamatergic cells must innervate the LDT/PPT cholinergic cells in order to trigger the above cascade of downstream events that we recognize as the physiology of REM sleep.

Focusing only on the eye movements of REM sleep, he noted [40] how LDT/PPT innervation of the peri-abducens area triggers discharges that produce action potentials in the abducens motor neurons – thereby moving the eyes in the horizontal saccades that are the *rems* of REM sleep. These neurons are not involved in waking saccadic or smooth pursuit eye movements nor in the slow-rolling eye movements that are seen in Stage 1 sleep. Therefore, the *rems* that are recorded in the REM sleep stage of a clinical sleep study are directly due to cholinergic LDT/PPT activity, which in turn is directly driven by glutamatergic (kainate receptor) signaling from the mPRF.

Obviously, the complex patterns of *rems* observed can be said to reflect complex brainstem neurophysiology. In addition, the substantially elevated number of *rems* observed in depressed patients suggests cholinergic and/or glutamatergic system abnormalities in these patients. While RD, a single number, is a simple description of this activity, there is much more information that can be extracted from the patterns in which *rems* occur. Attempts to extract this deeper information are detailed below.

24.4 Clinical Studies of Sleep in Depression

Meta-analyses of Sleep Measures in Depression

In her meta-analysis of over 1,000 papers, Benca [43] concluded that REM sleep has shown the largest and most consistent differences between normals and psychiatric patients. This was also the conclusion of her subsequent review [44]. The physiological basis for this finding is as yet unknown. A more recent literature review [45] also found that REM sleep is the most promising stage of sleep for research in depression and described some of the underlying theories. Several of us published a recent meta-analysis [46] in which four sleep variables that had been proposed as diagnostic of depression (RL, RD, sleep efficiency, and slow-wave sleep) were compared using the best available papers in the world literature. The conclusions are summarized below as effect sizes (Cohen's "d").

Twenty-eight studies examined the effect size of RL, the most commonly reported variable in research on MDD (Fig. 24.1.). The mean for the patient group was 60.7 min compared to 81.4 min for the control group. Cohen's d was −0.63 (95 % CI −0.81 to −0.45). However, Q statistic was large (79.30) indicating substantial heterogeneity.

For RD, there were 18 studies, all of which were reported using the Kupfer method in which RD was calculated from visually scored RA divided by RT (Fig. 24.2). There were no papers meeting criteria for the meta-analysis that used computer detection of individual eye movements or any of the burst detection algorithms. The mean for the patient group was 1.94 compared to 1.63 for the control group. Cohen's d was 0.51 (95 % CI 0.25–0.75). However, Q statistic was large (64.46) indicating substantial heterogeneity.

For sleep efficiency (SE), the duration of all sleep stages divided by the total time in bed, there were 23 studies. The mean for the patient group was 83.0 % compared to 89.9 % for the control group. Cohen's d was −0.68 (95 % CI −0.81 to −0.55). Q statistic was small (28.6) and nonsignificant.

For slow-wave sleep (SWS), there were 17 studies. The mean for the patient group was 9.6 % compared to 12.5 % for the control group. Cohen's d was −0.26. The Q statistic was small (18.76) and nonsignificant.

In summary, there were moderate effect sizes for all of these measures except for slow-wave sleep, which had a small effect size. We now review the aspects of REM time (RT), REM activity (RA), and REM density (RD) as they relate to MDD.

Studies of RD Considerable sleep research since the 1960s has focused on abnormalities in the sleep of patients with mental illness, especially RL and RD. All definitions of RD seek to express

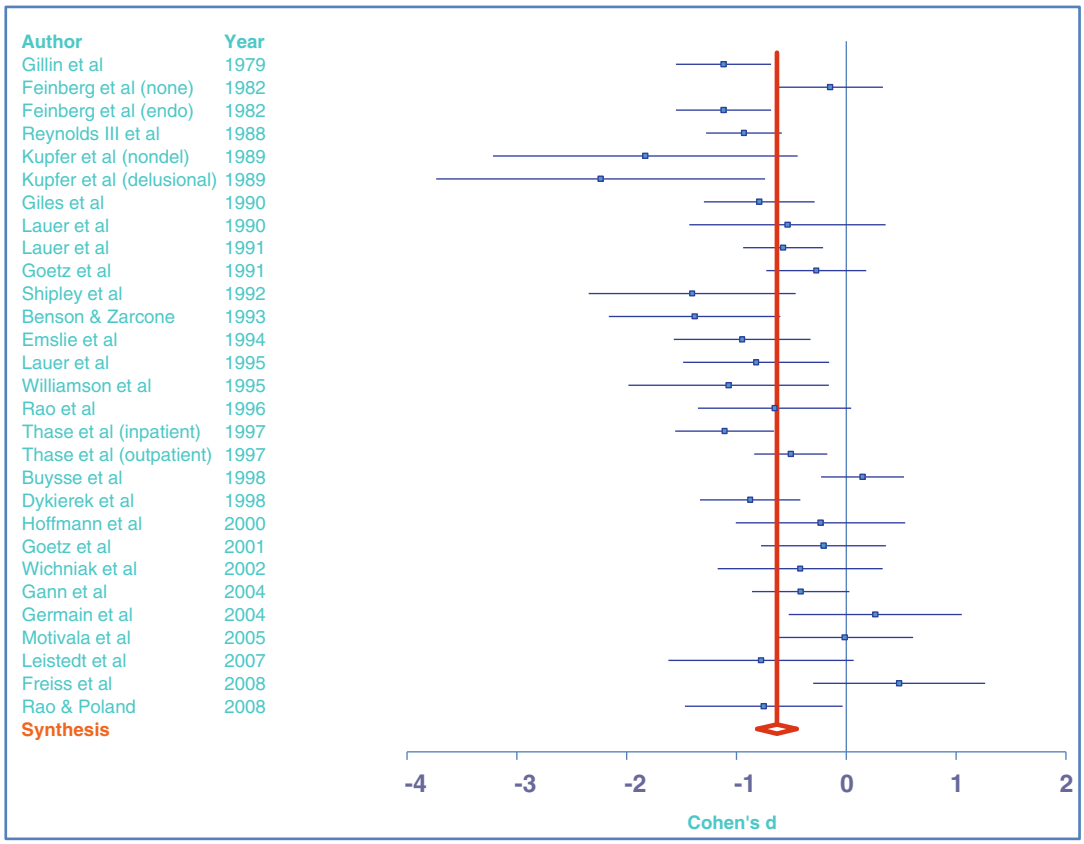

Fig. 24.1 Meta-analysis of REM latency in depression (From Arfken [46], used by permission of J. Aff. Disorders)

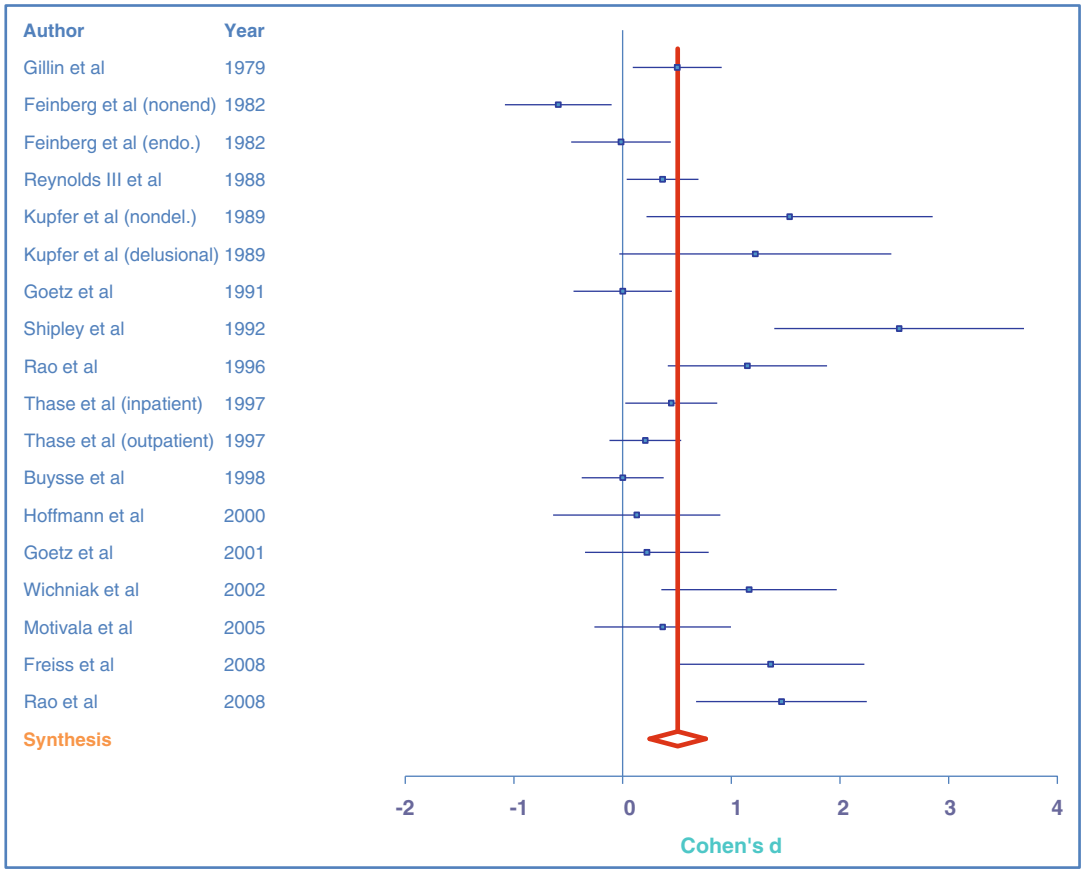

Fig. 24.2 Meta-analysis of REM density in depression (From Arfken [46], used by permission of J. Aff. Disorders)

in some way the number of *rems* per unit time of REM sleep, i.e., they are chiefly a measure of phasic REM sleep. While the definition of RT has been agreed upon for some time [47], the definition of RD has varied greatly, which makes it difficult to compare studies. RD also has a reputation for having high variability.

In the 1970s, Foster [48] found that patients with primary depression could be distinguished from those with depression secondary to serious medical illness by their phasic REM activity ("bursts" of *rems*). He concluded that phasic RA and RD could be considered as "objective indicators of central nervous system impairment," to indicate the "organicity" of depression. Soon after, Sitaram [49] found that injections of the anticholinesterase physostigmine shortened RL and increased RD in bipolar affective disorder patients in remission. He later demonstrated a similar effect with an intravenous cholinergic agonist, arecoline [50]. These cholinergic agents caused a greater effect in patients than in controls, suggesting that cholinergic systems may be oversensitive in depression and that short RL and elevated RD might amount to a biological marker for depression.

Lauer [4] found that depressed patients demonstrated significantly higher RD across all adult age groups when compared to age-matched controls, whereas there were no group differences in slow-wave sleep. RL shortened with age in all subjects, but depressed patients did not differ from controls until the fourth decade of life. Another study by these authors [51] found that RD was elevated in the first REM period in high-risk probands who had never been depressed, whereas RT and RL did not differentiate the groups.

Holsboer's group [52] showed that elevated RD at 18 years of age in the adult children of depressed patients is more predictive than the other REM measures of new-onset depression. A review of this area by Staner [53] concludes that abnormal RD is reflective of a cholinergic-aminergic imbalance. Another German group [54] emphasized the primacy of RD in depression research, "REM density seems to be the most reliable marker for sleep in depression [p. 48]." This is in contrast to the focus on RL (a tonic REM measure) that occupied the field in the period 1970–1995. The reason that RD has been underreported is likely due to the difficulty in detecting individual *rems*.

Methods of Reporting RD and Individual Eye Movements Over the Years While the original sleep-scoring manual by Rechtschaffen and Kales [47] specified electrode placement, amplifier gain, and filter settings, their suggested electrode placement for eye movement detection was more sensitive to lateral *rems* (i.e., left to right) than to vertical or oblique *rems*. This standard is still used in the majority of sleep laboratories. Fortunately, it has been shown [55] that about 56 % of *rems* are exactly lateral, whereas only 13 % are vertical. The remaining 30 % are near enough to lateral to be partially detected by the Rechtschaffen and Kales montage. Therefore, one can assume that most sleep studies in mental illness reported to date have adequately recorded individual *rems*.

The most straightforward method of determining RD is to count visually each *rem* in a REM period (REM activity (RA)) then divide the total by RT; however visual counting is difficult because there can be hundreds or even thousands of *rems* per night. This approach also discards valuable information about the time intervals between subsequent *rems*. We will hereafter call this type of RD "crude" or "scalar" RD.

To facilitate the process of manual REM detection, Kupfer and the Pittsburgh group designed a method to score RD in a somewhat subjective manner from the raw paper polysomnogram that has been described as "an integrative approximate measure of the frequency of rapid eye movements during sleep (each minute of REM sleep is scored 0–8)" [56]. This was done by estimating, for each REM minute, how many eighths of the minute included *any* pen excursions on the eye movement channels. Note that the exact number of pen excursions was not counted. This score for each minute was then summed over the REM period to create RA. RD was then defined as RA divided by RT. Although this is obviously a subjective approximation, it has been by far the commonest way in which RD has been reported in the literature to date (Fig. 24.2).

A slightly different approach [57] was to count the number of 1-s time intervals in a REM period that contain at least one *rem* (scored as "1") or no *rems* (scored as "0"). These values were then expressed either as a type of RD (i.e., number of epochs with "1 s" divided by the total number of epochs, expressed as a percentage) or analyzed as time-series process [58].

The Munich method of defining RD is similar: ". . . the ratio of 3-s mini-epochs of REM sleep including [at least one] *rem* to the total amount of 3-s mini-epochs of REM sleep of the total night," which is then expressed as a percentage [52]. This method is also used elsewhere in Germany [54].

Of course, none of the above methods solve the problem of detecting individual *rems* in the first place. With the change from paper recording of polysomnograms to digital computer recording, it became possible to write software that automatically detects individual *rems*. While a few computing pioneers did so in the late 1970s [59], commercially available software using published algorithms has only recently become available [60–62].

Another result of modern detection methods is the easy availability of the exact time of occurrence of each *rem* and therefore the exact time interval between *rems* ("inter-*rem* interval" or IRI). Such time intervals are intrinsic to the definition of "bursts" of eye movements.

"Burst" Versus "Isolated" *Rems* Researchers long ago observed that *rems* were not randomly distributed over time – closely spaced "bursts" of *rems* were separated from each other by long periods of rarely occurring "isolated" *rems*.

Unfortunately, it was shown [63, 64] that the "stationarity" assumptions of common time-series statistics were violated by this statistical distribution of *rems*, thereby invalidating the use of Fourier analysis, period analysis, and autocorrelation. But bursts were easier to count manually than individual *rems*, and very few of the early authors measured IRIs manually. One exception was Spreng [65], who laboriously did so in a study of the correlation between autonomic activity and bursts of rems. They plotted a probability distribution of IRIs and found that these did not match the expected random distribution due to a substantial excess of IRIs <3.0 s. They therefore defined bursts as any sequence of *rems* with a separation (IRI) of <3.0 s.

While there has since been little agreement on exactly what constitutes a burst, their identification served both a practical and a theoretical purpose. For example, the amount of REM time spent in bursts was shown to be under the control of serotonergic mechanisms [66], depressed patients having more *rems* per burst than normal controls. De La Pena [67] defined bursts as "a sequence of at least two consecutive 2.5 s mini-epochs" with at least two *rems* in each mini-epoch. They used 2.5 s for the length of their mini-epochs because, "fortuitously . . . [the polygraph paper] . . . contained prominent bold vertical lines already printed at convenient 2.5 s intervals." Zarcone [68] defined a "burst rem" as one found within 2 s of a neighboring one; a burst was defined as the interval occupied by all such consecutive *rems*. Burst time was the amount of time elapsed between the first and last *rem* in a burst, summed over the whole REM period. Petre-Quadens [69] considered burst *rems* to be those within 1 s of each other, then defined an "ocular-motor index" – the number of burst IRIs divided by the number of isolated IRIs.

After an analysis of the statistical distribution of IRIs, Boukadoum and Ktonas [70] defined bursts as *rems* occurring within 5 s of one another. They partitioned each REM period into "burst" and "isolated" states, which were then submitted to a Markovian analysis. A subsequent paper by one of them [71], used a threshold of 2 s in order to define a burst. In fact, the very concept of dividing IRIs into burst and isolated states implies the existence of Markovian transition probabilities between the states. Briefly, a Markov process is one where there are distinct categories of events or processes that follow each other repetitively over time; therefore, a specific probability of transition from one state to another exists. A detailed numerical example of this method using psychiatric patients has been published [72].

Conclusion

Sleep measures in MDD have certainly proved to be scientifically valid, despite the heterogeneity of the condition. An extensive literature on the basic science of REM sleep control has emerged since the 1990s, which implicates nearly all of the neurotransmitters and receptors targeted by psychiatric drugs used to treat depression. The REM system is well conserved across species, so that pharmacological animal studies can make strong predictions about human responses to similar interventions.

The measure with the most promise for future research appears to be RD, especially since the Munich group showed that elevated RD might mark a vulnerability trait for depression. In addition, modern digital technology allows the detection of individual *rems* and the time intervals between them; this allows for much more complex and fine-scale mathematical analysis than was possible with the simple scalar RD reported in most of the clinical studies noted above. Potentially, this new knowledge could allow researchers to "drill down" from abnormalities observed in clinical sleep studies of depressed patients to discrete brain areas and neurotransmitters that might be at fault. It may also allow better correlation of sleep measures with diagnostic subgroups and treatment response.

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Depression: Correlations with Thyroid Hormones in Major Depressive Disorder

25

Dominika Berent

25.1 Introduction

Major depressive disorder (MDD) is a leading contributor to the global burden of diseases. It has been estimated that, until the 2020, depression will be a second cause of disability among reproductive population [1]. Regarding to the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders*, MDD contains recurrent episodes of depressed mood or a loss of interest or pleasure in daily activities for more than 2 weeks, which impairs one's social, occupational, and educational functions [2].

MDD is a heterogenous disorder that results from a complex interplay of immunological, genetic, neuroendocrine, and psychosocial factors. This review explores current knowledge on the role of thyroid hormones (THs) in etiology and course of MDD. Theoretically, elevated THs are considered to be connected with an elevated mood, and lowered THs – with diminished mood. However, as it was previously said, MDD results from an interplay of many factors, and thus studies on only one isolated factor, including THs, may provide contradictory results. Thyroid function can be disturbed in an overt (both, thyrotropin (TSH) and free thyroid hormone levels disturbed) or subclinical (only TSH levels disturbed) manner. Maes et al. (1993) indicated that basal morning TSH, free triiodothyronine (fT3), and free thyroxine (fT4) plasma levels fell within the normal, euthyroid range in 96.8 % of depressed patients [3]. Also, Ordas and Labatte (1995) found overt thyroid disease rare among depressed inpatients [4]. They reviewed thyroid function tests obtained on 277 consecutive first-time admitted patients with MDD or dysthymia and found TSH outside the normal range in 17 patients (6.5 %). Of these, there were two cases (0.4 %) suggestive of hyperthyroidism and no overt cases of hypothyroidism. Eight patients had subclinical hypothyroidism (elevated TSH, normal fT4). Similarly, further studies, including own clinical observation [5] (Fig. 25.1a, b), show that it is common in depression to note slight (but still euthyroid) free thyroid hormone disturbances with concomitant normal TSH. Nonetheless Sintzel et al. (2004) estimated the prevalence of subclinical hypothyroidism in population of resistant depression and simple depression as 52 % and 8–17 %, respectively [6]. To compare, the prevalence of an overt (elevated TSH levels and low levels of fT3 and fT4) and subclinical (elevated TSH levels only) hypothyroidism in adult general population is 9 % and 0.4 %, respectively [7].

D. Berent, MD, PhD
Department of Psychiatry,
Medical University of Warsaw,
Kondratowicza 8, Warszawa 03-242, Poland
e-mail: dominikaberent@poczta.fm

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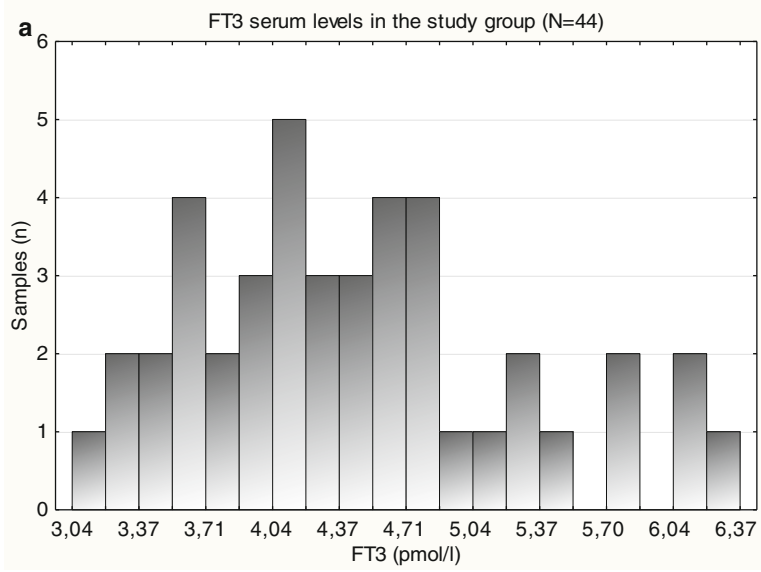
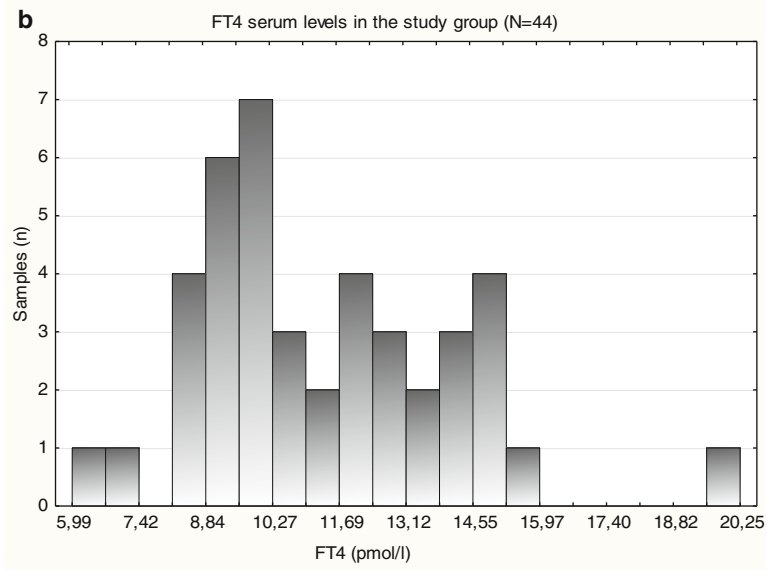


- FT3 was assessed in 43 patients (97.73%, 24 males, 19 females), missed in one woman.
- The mean level (±SD) was 4.45 (± 0,81) pmol/l (reference values: 4.00 – 8.3pmol/l).
- 30 patients (68.18%, 19 males, 11 females) were in norm. Majority of them was in the lower range of reference values, ≤5 pmol/l (21 patients).
- Thirteen patients (29.55%, 5 males and 8 females) were below the norm.



- FT4 was assessed in 42 patients (95.45%, 23 males, 19 females), missed in one woman and one man.
- The mean level (±SD) was 11.43 (± 2.69) pmol/l (FT4 reference values: 10.6-19.40pmol/l).
- 35 patients (77.27%, 20 males, 14 females) were within the norm.
- One woman had elevated FT4 level of 20.25 pmol/l with normal TSH and FT3.
- Seven patients (15.91%, 3 males and 4 females) were under the lower limit of FT4 reference values.

Fig. 25.1 (**a**) FT3 concentrations in the study group of 44 MDD patients with slight to very severe depression episode (From Berent et al. [5]). (**b**) FT4 concentrations in the study group of 44 MDD patients with slight to very severe depression episode (From Berent et al. [5])

It is possible to assess hypothalamus-pituitary-thyroid axis (HPT axis) hormonal indices both in urine and blood. However, the usefulness of testing the thyroid hormone excretion in 24 h urine remains questionable. Wiersinga et al. (2007) described two women with a diagnosis of hypothyroidism based on urine test which was further excluded after the measurement of thyroid hormone blood levels [8]. Li et al. (2012) found monitoring urinary iodine but not thyroid hormones useful in pregnant women urine remains questionable [9].

It is necessary to add that the measurement of basal TSH, fT3, and fT4 plasma levels was indicated to be more proper than thyrotropin-releasing hormone (TRH) assessment for evaluating HPT axis function [10].

25.2 Thyroid Hormones (THs), Thyroid Transporters, and Thyroid Receptors (TRs)

THs (fT4 and fT3) are critical to the normal development of the human central nervous system and adult brain functioning. They play an important role in brain development, and then in the adulthood, they influence structure, perfusion, and function of the central nervous system [3]. The actions they display in the central nervous system include the regulation of glucose utilization, synaptic activity, oligodendrocyte differentiation, axonal and dendritic outgrowth, and differentiation of the retina and auditory system [11].

Thyroid gland secretes predominantly fT4, and, to a lesser extent, fT3. fT3 is the most active form of THs, since it has a higher affinity by the nuclear thyroid hormone receptors (TRs). Its predominant amount in the bloodstream derives from fT4 deiodination. It is observed that in healthy subjects, thyroid indices show substantial interindividual variability, whereas the intraindividual variability is within a narrow range [12]. This interindividual variability results from environmental factors, such as food, smoking, or iodine intake, and genetic factors. The intraindividual variability remains one's characteristic, suggesting of being a result of individual genetically warranted THs gene expression and THs metabolism.

Briefly, the mechanisms of THs action in the brain cells are warranted by the availability of free hormone, activity of thyroid hormone transporters and receptors, and activity of deiodinases. It is necessary to underline that there are few mechanisms that regulate THs action and should be taken into consideration while discussing results of the studies on THs role in depression and other disorders:

- Binding with thyroxine-binding globulin, transthyretin, or serum albumin in the circulation
- Expression on the cell membrane and activity of thyroid transporters
- Transport of thyroid hormone across the cell membrane via thyroid transporters
- Expression on the nuclear membrane and activity of thyroid receptors
- Conversion of fT4 to fT3 in target tissues catalyzed by deiodinases

Heuer (2007) summarized the physiological process of THs' entry and action in the central nervous system (CNS) [13]. To act in the brain, first, THs have to cross the blood-brain barrier or the choroid plexus-cerebrospinal fluid (CSF) barrier. Entering via the blood-brain barrier seems to be the main and preferred form of the overall distribution. Entering via the CSF barrier is an additional way to provide circumventricular areas with sufficient amount of THs. Heuer (2007) concluded that thyroid hormone transporters must exist and act in the brain capillary endothelial cells, in the choroid plexus endothelial cells, and in ependymal cells of the ventricles [13]. After passing one of these barriers, fT4 has to be taken up by astrocytes for further activation. Finally, fT3 – either released by astrocytes or directly taken up from the circulation – has to enter neuronal cells, which not only express thyroid receptors (TRs) but also participate in the inactivation of fT4 and fT3 by expressing type 3 deiodinase (D3). It is often assumed that fT4 – but not fT3 – predominantly enters the CNS. The local conversion of fT4 to fT3 by type 2 deiodinase (D2) in astrocytes was estimated to produce as much as 80 % of the fT3 bound to the nuclear receptors in the adult rat brain, indicating that

astrocytes are critically involved in regulating the amount of fT3 available for neuronal uptake [14].

THs are liposoluble and thus it was previously suspected that THs transport goes via diffusion across the lipid bilayer of the plasma membrane. However it was further estimated that THs enter cells via a few families of THs transporters located in cellular membranes. Van der Deuer et al. (2007) reviewed the current knowledge about genetic variations in four families of THs transporters: the Na+/taurocholate cotransporting polypeptides (NTCPs), the heterodimeric amino acid transporters (HATs), the organic anion-transporting polypeptides (OATPs), and monocarboxylates (MCTs) [15]. The first three families are capable of THs transport and also bile salts, bromosulfophthalein (BSP), steroid hormones, and numerous drugs. The last one, MCT family, is probably involved in brain and muscle energy metabolism. MCT8 and MCT10 have been shown to be active iodothyronine transporters [16, 17]. Van Deure et al. (2007) analyzed studies on polymorphisms in the *SLC16A2* for MCT8 and *SLC16A10* for MCT10 and generally concluded that they are not associated with serum thyroid parameters. The polymorphism *SLC16A2*-Ser33Pro was not associated with serum TH levels in two analyzed studies. The minor allele of polymorphism *SLC16A2*-G/T intron 5 was associated with lower fT4 in a group of 97 men. However this finding was not replicated in a group of 55 women of the same sample, and further studies on larger population are needed. Individuals with minor allele of the polymorphism *SLC16A10*-C2201A were found to have lower fT3 and lower TSH levels. However this finding was not confirmed in larger population [15].

After the THs enter the cell, they act via thyroid receptors (TRs). TRs are predominantly present in the cerebral cortex, amygdala, plexus choroideus, and structures of adult neurogenesis: the hippocampus and olfactory bulb [18]. TRs are encoded by two genes: *THRA* and *THRB*, located on human chromosomes 17 and 3, respectively. Primary transcripts of these genes undergo alternative processing generating several protein isoforms that have distinct tissue distributions and biological functions [19]. The main binding isoforms are TRa1, TRb1, and TRb2. TRa1 protein is encoded by *THRA* and TRb1 by *THRB*. They are expressed at different times in the development. TRa1, being a key regulator of cardiac output, is expressed first during fetal development and is widely expressed in adult tissue. TRb1 is one of the factors controlling liver metabolism, appears later in development, and is expressed in the adult liver, kidney, and lung [20, 21]. Of the three TR isoforms, only TRb2, which is produced via alternative splicing of *THRB*, is significant for MDD etiopathology. TRb2 is restricted largely to the hypothalamus, pituitary, cone cells of the eye, and the sensory hair cells in the cochlea [22]. Moreover, TRs rely on a diverse group of coregulatory factors known as coactivators and corepressors. As we can see, thyroid hormone signaling is provided by a net of proteins and may be impaired at any level [23]. THs act via genomic and nongenomic pathways. The genomic (nuclear) actions of THs are initiated when fT3 binds to its nuclear receptors (TRa, TRb). The fT3 receptor usually forms a heterodimer with the retinoid X receptor (RXR), which binds to T3-responsive elements (TREs) in the promoter region of TH-responsive genes. TRs are bimodal in their action and can either repress or activate genes. The nongenomic (extranuclear) actions of TH are not TRE-mediated and have only recently been recognized. They contain rapid actions on cardiovascular system, i.e., ion fluxes across plasma membrane channels and the phosphatidylinositol 3-kinase/protein kinase Akt pathway [24].

25.3 Deiiodinases

As it was previously said, fT4 is the thyroid principal secretory product but essential metabolic and developmental effects are all mediated by fT3, which is produced from the prohormone by 5-prime-deiodination. Deiodinases are membrane proteins with their active center located in the cytoplasm, although one study describes an extracellular location of the active site of type 3 deiodinase [25]. There are three types of deiiodinases characterized in human: 1, 2, and 3 deiodinases (D1, D2, and D3). The type I deiodinase (D1) catalyzes both outer and inner ring deiodinations (ORD, IRD), the type II deiodinase (D2) is capable of ORD, and the

type III deiodinase (D3) exclusively catalyzes IRD. D1 is expressed in the liver, kidney, and thyroid. D2 and D3 are expressed in the brain and several other organs. D1 produces serum fT3 and clears serum reverse T3 (rT3). D2 catalyzes the outer ring deiodination of fT4 to fT3. D3 catalyzes the inner ring deiodination of fT4 to rT3 and of T3 to 3,3′-T2. Types 2 and 3 deiiodinases create a safety mechanism providing the brain with a proper amount of fT3 in the state of both hypo- and hyperthyroidism. The activity of D2 and D3 depends on the current demands of the human brain. In the presence of low levels of circulating thyroid hormone, D2 activities are increased to produce more active fT3, and D3 activities are decreased to avoid thyroid hormone inactivation. This mechanism protects the brain in conditions of hypothyroidism as long as circulatory fT4 is available. To some extent, it remains also effective to protect the brain from detrimental hyperthyroidism [13]. The enzymatic activity of D3 is observed to be especially high during fetal and neonatal development, suggesting that it might act as a scavenger to prevent a premature thyroid-hormone-induced differentiation of neural cells [13].

Kirkegaard and Faber (1998) reviewed that in depressed patients fT3 levels were reduced, while fT4 levels were elevated [26]. It may indicate that low serum levels of active hormone with concomitant good availability of prohormone may be due to altered prohormone metabolism, i.e., D2 low activity [5]. There are only few studies evaluating individual disturbances in deiodinase activity in mood disorders [27–29].

D1 is encoded by type 1 thyroxine deiodinase gene (*DIO1*), mapped to chromosome 1p33-p32 [30]; D2 – by type 2 iodothyronine deiodinase gene (*DIO2)*, located on chromosome 14q24.2-q24.3 [31]; and D3 – by type 3 iodothyronine deiodinase (*DIO3*), mapped to chromosome 14q32 [32]. Emerging data support the notion that iodothyronine deiodinase gene polymorphisms may impact in health and disease. Studies on genetically based variability in thyroid function may provide strategies on personalized treatment in MD patients.

T allele of the *DIO1* SNP rs11206244 was confirmed to be associated with increased fT4 levels but not TSH levels [27, 33, 34]. Cooper-Kazaz et al. (2009) assessed a population of 35 patients treated with sertraline plus triiodothyronine and 29 patients treated with sertraline and placebo for 8 weeks [28]. The sertraline doses were 50 mg/day for 1 week and then 100 mg/day if tolerated, and triiodothyronine – 20 mcg/day for 1 week and then 40 mcg/day if tolerated. *DIO1*-C785T was associated with efficacy of triiodothyronine but not placebo supplementation. The *DIO1*-785T allele is associated with lower DIO1 activity. Patients with *DIO1*-785T allele have less active DIO1 and lower fT3. Thus, it is possible that they benefit more from triiodothyronine supplementation [28].

25.4 THs in Depression Studies

Many data on THs impact on serotoninergic transmission indicate a possible clinical benefit from THs supplementation. It was shown that reduced 5-HT responsiveness in patients with hypothyroidism is reversible with thyroid replacement therapy [35, 36]. It was also shown that triiodothyronine supplementation results in increase cortical serotonergic neurotransmission due to a loss of autoinhibitory serotoninergic receptor type 1A (5-HT1A) receptor sensitivity [37] and by increasing cortical serotoninergic receptor type 2 (5-HT2) receptor sensitivity [38].

Cooper-Kazaz et al. (2009) showed that depressed patients with low fT3 on admission benefit more from triiodothyronine supplementation [28]. It may be more efficient to augment and accelerate the treatment of MDD with triiodothyronine instead of levothyroxine because of possible intraindividual differences in thyroid hormone metabolism [5]. However it should be done carefully in elderly patients. Gussekloo et al. (2004) found in individuals aged 85 years and older that subclinical hypothyroidism may be related to prolonged survival [39]. In my opinion this condition may mirror general age-related diminished metabolism, and excessive supplementation can disturb this physiological balance.

Own analysis (2014) [5] of TSH, fT3, and fT4 measurements in 44 MDD inpatients was done, and further assessment of hormonal items association with depression severity and

improvement was conducted. The study comprised 44 MDD inpatients with slight to very severe depression episode (20 women, 24 men) of a mean age of 51.93 ± 11.54 years. All the assessed TSH indices were within normal ranges. TH levels are depicted in Fig. 25.1a, b. Lower fT3 and fT4 concentrations on admission were significant predictors of worse clinical improvement. Male patients did not differ from female patients regarding fT4 concentrations but they presented significantly higher fT3 serum levels and improved their depressive symptoms significantly better than women during the treatment with selective serotonin reuptake inhibitors (SSRIs). There were no significant differences between male and female patients regarding basic clinical characteristics (current age, age at depression onset, disease duration, number of hospitalization, number of suicide attempts). It was hypothesized, sex related differences in fT3 concentrations may be connected with different THs metabolism i.e., higher D2 activity [5].

Tsuru et al. (2013) delivered interesting data on HPT axis dysregulation during the major depression episode [40]. They recruited 25 patients (16 women and 9 men, 48.1 ± 11.4 years of age, range 22–84) with MDD. Patients who recurred within 10 years after remission exhibited significantly higher TSH responses to TRH at the time of admission when compared to those who did not recur. Researchers stated, it may suggest that the free thyroid hormone decline at the time of depression recurs which makes the pituitary gland more vulnerable to TRH stimulation [40]. Here also, all the studied patients were treated with SSRIs, so there were no possible differences in influencing final clinical outcome between different antidepressant groups [40].

25.5 Summary

THs are essential for brain development and functioning. There are still new data incoming on their complicated signaling, transport, and metabolism. It is observed that in healthy subjects, serum thyroid parameters show substantial interindividual variability, whereas the intraindividual variability is within a narrow range [12]. This interindividual variability results from environmental factors, such as food, smoking, or iodine intake, and genetic factors. Considering this variability in the current studies, may provide strategies on more efficient, personalized treatment in MDD patients.

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History of the Discovery of Antidepressant Drugs

26

Francisco López-Muñoz and Cecilio Álamo

26.1 Introduction

During the 1950s, a veritable revolution took place in the fields of psychopharmacology and psychiatry, with the clinical introduction of the main groups of psychoactive drugs still used today (see [83]). Therapeutic approaches to affective disorders, from the perspective of current scientific pharmacology, also date from the 1950s, with the clinical introduction of imipramine and iproniazid (see [68]). Although it is clear that in these early phases of psychopharmacology serendipity played an important role in the discovery of the majority of psychotropic drugs [9, 11, 12, 52, 85], what was truly important were the final results of these research processes. In this sense, we could classify the serendipitous discovery as the discovery of something not sought, independent from the systematic process that led to the accidental observation. Thus, our group has proposed an operational definition of serendipity (with four patterns) to classify discoveries as serendipitous and non-serendipitous [11, 82, 85]. A pattern includes those initial serendipitous discoveries (frequently in laboratory animals) that led secondarily to non-serendipitous discoveries. This category comprises many of the most important findings of the decade of the 1950s, among them the discovery of the antidepressive effects of iproniazid and imipramine [84]. Other pattern includes the purely non-serendipitous discoveries that arose from systematic research programmes that were designed specifically to develop effective medications for various psychiatric disorders. In this group we include the selective serotonin reuptake inhibitor (SSRI) antidepressants such as fluoxetine.

When we speak of a "revolution" in the area of psychoactive drugs during the 1950s [73], our intention is to highlight the crucial importance of the introduction of truly effective therapeutic tools for treating the different psychiatric disorders. And this is the case of iproniazid and above all of imipramine, two drugs which not only marked a new era in the treatment of depression

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F. López-Muñoz (✉)
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal
e-mail: francisco.lopez.munoz@gmail.com; flopez@ucjc.edu

C. Álamo
Department of Biomedical Sciences (Pharmacology Area), Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain

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Table 26.1 Classification of monoaminergic antidepressants according to action mechanism and historical perspective on their clinical introduction

Family	Acronym	Prototype substance	Period
5-HT and NA reuptake inhibitors with blocking action of diverse receptors	TCA	Imipramine	1957–1980
Irreversible MAO inhibitors	MAOI	Phenelzine	1960–1965
NA reuptake inhibitors with blocking action of diverse receptors		Maprotiline	1970–1980
Antagonists of α_2 autoreceptors		Mianserin	1970–1980
Selective DA reuptake inhibitors	SDRI	Bupropion	1980–1990
Selective 5-HT reuptake inhibitors	SSRI	Fluoxetine	1980–1990
Reversible MAO inhibitors	RIMA	Moclobemide	1980–1995
5-HT reuptake inhibitors and blockers of 5-HT_2 receptors		Nefazodone	1985–1995
Antagonists of α_2 auto- and heteroreceptors and 5-HT_2 and 5-HT_3 receptors	NaSSA	Mirtazapine	1975–2000
NA and 5-HT reuptake inhibitors	NSRI	Venlafaxine	1975–2000
Selective NA reuptake inhibitors	SNRI	Reboxetine	1980–2000
Agonists of MT_1 and MT_2 melatonin receptors		Agomelatine	1990–2010

[70, 80]. Even so, the clinical introduction of these drugs had many critics, since psychoanalytic currents, doctrinally dominant in the psychiatry of the time, considered depression as a symptomatological manifestation of certain internal personality conflicts. In our opinion, iproniazid and imipramine made two fundamental contributions to the development of psychiatry: one of a social-health nature, consisting in an authentic change in the psychiatric care of depressive patients; and the other of a purely pharmacological nature, since these agents have constituted an indispensable research tool for neurobiology and psychopharmacology, permitting, among other things, the postulation of the first aetiopathogenic hypotheses of depressive disorders [68].

On the other hand, the clinical introduction of SSRIs, in the late 1980s, once again revolutionized therapy for depression [68, 70], opening the way for new families of antidepressants (see Table 26.1). Nevertheless, they all continue to employ the same action mechanism as the classical drugs, that is, the modulation of monoaminergic neurotransmission at a synaptic level.

In the present chapter, we analyzed the clinical introduction (and its implications) of these three agents that have marked the history of antidepressant drug therapy: iproniazid, imipramine and fluoxetine.

26.2 The Discovery of the Antidepressant Effects of Iproniazid

This family of antidepressant drugs have their origins in the hydrazines and the research carried out by Emil Fischer in the 1870s. This father of organic chemistry discovered phenylhydrazine in 1874, accidentally, while he was working in the laboratory of Adolf von Baeyer in Strasbourg [65]. From hydrazine hydrate, a powerful reducing agent, Hans Meyer and Josef Malley, of the German Charles-Ferdinand University (Prague), synthesized isonicotinyl hydrazine in 1912, as part of the work for their doctoral thesis [89]. However, forgotten for almost 40 years, it would not be until the early 1950s that it was resynthesized and that it was discovered, by chance, that the compound, at any experimental level, had powerful antitubercular properties [13].

Prior to the discovery of the antitubercular properties of hydrazines, Mary L. Hare (Mary Bernheim), a researcher at the University of Cambridge, described for the first time in 1928 how an enzyme (which she called tyramine oxidase) was capable of bringing about oxidative disamination of the biogenic amines [43]. This enzyme was identified in 1937 by the groups led by Hermann Blaschko and Derek Richter, at the

Physiology Department of Cambridge University [15], and by those of Caecilia E. Pugh and Juda H. Quastel, at the Biochemical Laboratory of Cardiff City Mental Hospital [97], being given the name monoamine oxidase (MAO). Blaschko's [15] group showed that MAO, isolated from cells of the liver, kidney and small intestine, was capable of metabolizing adrenaline through oxidation, a process that could be inhibited by ephedrine. For their part, Quastel and Pugh also identified this enzyme in the brain, though its significance remained unclear [97]. It would later be definitively demonstrated that MAO constitutes a flavoprotein enzymatic system located at the level of the mitochondrial membrane and whose function is to produce oxidative disamination not only of the biogenic amines (serotonin and catecholamines), but of other sympathomimetic amines (tyramine, benzylamine, β-phenylethylamine, etc.).

The discovery of the antitubercular effects of this hydrazine derivative, which took place in 1951, is due to the research work carried out, independently, by two scientific teams, led by Herbert Hyman Fox of Hoffmann-La Roche Laboratories (Nutley, New Jersey) and Harry L. Yale of the Squibb Institute for Medical Research (Princeton, New Jersey), respectively [83, 108]. In the framework of a research programme on anti-infectious drugs at the Squibb Institute in the 1940s, under the direction of Frederick Wiselogel, hundreds of compounds were synthesized and studied in mice previously infected with *Mycobacterium tuberculosis*. In 1951, one of the chemists in the group, Harry Yale, synthesized from a hydrazide intermediary (isonicotinyl hydrazine), isonicotinyl-aldehyde-thiosemicarbazone, since researchers at the University of Indiana had already shown the tuberculostatic efficacy of thiosemicarbazones. However, there was enormous surprise when the synthetic intermediate employed was found to be much more active in the animal model than the final product. Almost at the same time, 2 weeks after the publication of the first Squibb report (10 January, 1952), Fox's team at Hoffmann-La Roche also announced the antitubercular properties of isonicotinyl hydrazine [41]. Their research line started out from knowledge of the tuberculostatic effects of nicotinamide, a group B vitamin, so that their idea was to combine a pyridine derivative of this group with a thiosemicarbazone. As occurred with Yale, Fox's group found that the synthetic intermediate used, isonicotinyl hydrazine, showed greater antitubercular power than the final product, isonicotinyl-aldehyde-thiosemicarbazone [108]. Clinical trials were carried out with this compound almost immediately in different hospitals in New York State; its antitubercular effectiveness was demonstrated, and it became known as isoniazid. Subsequently, this line of research was continued by Herbert H. Fox himself and John T. Gibas, at the Hoffmann-La Roche Laboratories, and among different derivatives of isoniazid, these two scientists synthesized an isopropyl derivative called iproniazid (1-isonicotinyl-2-isopropyl hydrazine), whose tuberculostatic activity was similar to that of isoniazid in lab animals, but superior in humans [37].

In 1952, the team led by Ernst Albert Zeller at Northwestern University Medical School (Chicago, Illinois) observed for the first time that iproniazid (and not isoniazid) was capable of inhibiting MAO [118]. Subsequent studies found that serotonin in the brain was converted into 5-hydroxyindoleacetic acid by MAO; for a long period, indeed, this process was thought to be the only path for the metabolization of serotonin. In 1957, Sidney Udenfriend and colleagues, at the National Institutes of Health (NIH) (Bethesda, Maryland), observed that administration of iproniazid to experimental animals produced a rapid increase in brain levels of serotonin, similar to those resulting from the administration of 5-hydroxytryptophan, a substance capable of crossing the blood-brain barrier and producing serotonin through decarboxylation [111]. Simultaneously, Bernard B. Brodie and his colleagues at the Laboratory of Chemical Pharmacology of the National Heart Institute (a division of the NIH), Parkhurst Shore and Alfred Pletscher (the latter being Director of Research at Hoffmann-La Roche in Switzerland), confirmed that reserpine and other compounds produced a release of serotonin in the brain, platelets and

intestine [18, 95]. All such works opened up an interesting research line on brain functions, in the framework of which are the contributions of Charles Scott at Warner-Lambert Research Laboratories (Morris Plains, New Jersey), who carried out certain animal experimentation studies that would be of great interest for the future characterization of these hydrazide drugs as antidepressants [71, 78]. Scott believed that the tranquillizing effects observed in animals given reserpine, an alkaloid of *Rauvolfia serpentina*, were due to the release of serotonin caused by the reserpine. With this hypothesis, and knowing the results of Zeller's work, Scott administered iproniazid with the aim of limiting the enzymatic destruction of serotonin. However, the pretreatment with iproniazid carried out by Scott before administering the reserpine had the opposite result to that expected by the researcher: a stimulant effect, rather than the predicted tranquillizing effect [29]. Similar results were obtained by Brodie's team at the NIH. In 1956, Scott's group described this effect of experimental alertization with iproniazid, which he called "marsilization", in reference to the tradename of this agent [29].

From the clinical perspective, the origin of the first specifically antidepressant drugs, the MAO inhibitors (MAOIs), lies in the antitubercular hydrazide agents that had been used since the early 1950s [70, 71, 78, 80]. It was precisely in 1952 that studies began, at the Sea View Hospital on Staten Island (New York), on the clinical effects of iproniazid, carried out by Irving J. Selikoff and Edward Robitzek, who observed that this drug, compared to isoniazid, possessed greater power to stimulate the central nervous system (CNS), an effect initially interpreted as a side effect [103]. The psychological changes observed in tuberculosis patients treated with iproniazid were especially striking [44, 70, 78, 80, 100]: these patients showed greater vitality, to the point in some cases of wanting to leave hospital, and a gradual increase in their social activity. A photograph by *Associated Press* from 1953 immortalized the effects of iproniazid. It shows several patients at the Sea View Hospital in party mood, even dancing. Under the picture it says: "A few months ago, the only sound here was the sound of victims of tuberculosis, coughing up their lives". Other authors reported that patients were "dancing in the halls tho' there were holes in their lungs" [100]. Similar psychostimulant effects were also observed in patients with other chronic illnesses, such as rheumatoid arthritis or cancer, treated with iproniazid [94].

However, the results of the first clinical trials with iproniazid indicated a safety profile, in the treatment of tuberculosis, inferior to that of isoniazid, so that it was practically abandoned, except in particular cases. For example, David M. Bosworth, Director of Orthopedics at St. Luke's and Polyclinic Hospital in New York, insisted on the superiority of iproniazid for treating bone tuberculosis [16]. But at this point in the story, serendipity came into play [11, 12, 84] when a few very wise clinicians saw in the psychostimulant "side effect" – which had emerged by chance – a potential "primary effect" that could be useful in other types of patient, basically those of a psychiatric nature. These inspired individuals included Jackson A. Smith, of Baylor University (Waco, Texas), who in his analysis of iproniazid as a "tranquillizer" observed some improvement in two depressed patients from a group of 11 treated over a period of 2 weeks (increased appetite, weight gain, increased vitality and improved sleep) [107]; Gordon R. Kamman, of the University of Minnesota (Twin Cities) [53]; and Carlos Castilla del Pino, of the University of Córdoba (Spain), who described the euphoric and mood-raising effects of hydrazide therapy in tuberculosis patients [22]. Some studies were even published that assessed the mood-raising effect of isoniazid in psychiatric patients [30, 31, 99]. Indeed, it may even have been one of these authors, Max Lurie (a psychiatrist with a private practice in Cincinnati), who coined the term "antidepressant" to refer to the effect of isoniazid in depressed patients [45].

Finally, the year 1957 would be a key for the future of hydrazide drugs as antidepressant agents, since it was at a meeting of the American Psychiatric Association (APA) in April of that year, in Syracuse, that the first data on the effects of iproniazid on depression were presented.

Although it was much less widely used than isoniazid, George Crane, of New York's Montefiore Hospital, reported improved mood in 11 out of 20 tuberculosis patients with concomitant depression [27] treated with iproniazid, while Frank Ayrd, an assistant at the Taylor Manor Hospital in Baltimore [6], reported similar results. Nevertheless, these researchers never referred to iproniazid as an "antidepressant". On the other hand, Nathan S. Kline (Fig. 26.1) and colleagues (Harry P. Loomer and John C. Saunders), of Rockland State Hospital (Orangeburg, New York), who knew about Scott's research, especially the capacity of iproniazid for preventing the immobility in mice induced by reserpine [29], were the first psychiatrists to assess the efficacy of iproniazid in non-tuberculosis depressed patients (chronic psychotic depression), carrying out the same procedures with humans as Scott did with animals. For their study, they recruited 17 highly inhibited subjects with severe schizophrenia and 7 with depression, all patients at Kline's private surgery; participants were given a 50-mg dose of iproniazid three times a day. Their results, also reported at the Syracuse meeting but not published until the following year, indicated that iproniazid had a stimulant effect on depressed patients and that 70% of the patients who received iproniazid had undergone a substantial improvement (raised mood, weight gain, better interpersonal capacity, increased interest in their surroundings and themselves, etc.) [67]. Such was the impact of the new drug that, in November of that same year, the Hoffmann-La Roche company sponsored the Symposium on the Biochemical and Clinical Aspects of Marsilid and Other Monoamine Oxidase Inhibitors, which discussed its effectiveness not only for depression but also for other pathologies, such as hypertension or angina. Within the framework of this symposium, eight studies were presented, covering a total of some 300 patients affected by different mental disorders, mainly depression.

In 1957, Kline, who combined his work in clinical research with the post of Assistant Professor of Psychiatry at the University of Columbia, published a report on the first neuropsychiatric experiences with iproniazid (previously presented at the annual meeting of the APA in Syracuse), during a Congress of the Committee on Appropriations of the United States Senate, held in May [57], proposing the term "psychic energizer" to refer to the drug's action [66]. Even 2 years later, at a symposium held in Montreal, Werner Janzarik proposed using the term "thymeretics", that is, substances that act through an increase in the stimulative action of the impulse, to cover all the drugs that presented a spectrum of action similar to that of the incipient MAOIs. However, Kline's group ran into considerable difficulties for pushing further with the study of the antidepressant effect of iproniazid: in early 1957, when their clinical research project was already under way, they lost the explicit support of the doctors in charge at Hoffmann-La Roche, who judged the indication of their drug as an antidepressant to be subject to an uncertain and inadequate market [44]. Without losing hope, they managed, according to Kline [57] himself, to arrange a secret meeting with L. David Barney, President of the pharmaceutical company, at Theodore's Restaurant in New York. During the course of this meal, the researchers from

Fig. 26.1 Nathan S. Kline (1916–1983), one of the great pioneers of psychopharmacology. Kline, at Rockland Psychiatric Center (later called Rockland State Hospital) in Orangeburg (New York, USA), was responsible for the clinical introduction of iproniazid in psychiatry

Rockland State Hospital managed to interest Barney in their project, which was thus able to continue. Incidentally, the problems continued just after the APA meeting of April 1957, as the three members of the research group became involved in a serious conflict among themselves, carried on first through the pages of medical journals (*Journal of the American Medical Association*, 1965), and later even in the courts (Appellate Division of the Supreme Court, First Judicial County of New York, Index No. 7770, April 15, 1980; New York Court of Appeals, Decision of the Appellate Division of the Supreme Court, Upheld in Saunders vs. Kline, No. 141, March 26, 1981), over the attribution of the discovery of the antidepressant effect of iproniazid [100].

One year after the Syracuse meeting, and despite the fact that iproniazid was only marketed as an antitubercular agent, under the tradename Marsilid®, more than 400,000 patients affected by depression had been treated with the drug [108], which opened the way for the first group of specifically antidepressant drugs, later known as MAOIs. This great initial success of iproniazid was due, in part, to two important factors [70, 78, 80]: the good previous results obtained in the treatment of tuberculosis patients and the lack at the time of truly useful therapeutic tools in the treatment of depression, so that the demand was enormous.

Iproniazid soon gave way to other agents with much greater power to inhibit MAO [51], such as isocarboxazid (Hoffmann-La Roche), tranylcypromine (Smith, Kline and French) [38, 86] and phenelzine (Warner-Lambert) [98], as well as other hydrazine derivatives (nialamide, mebanazine and pheniprazine) or indole derivatives (etryptamine) [8]. But the story for MAOIs was as mayfly, and the decline in the use of these drugs was as rapid. Indeed, the commercial life of iproniazid was really quite short, since it was withdrawn from the US market in 1961 after accusations of its having induced a series of cases of jaundice and nephrotoxicity. The imputability of these adverse effects was fiercely challenged, since no specific immunological studies were carried out to determine whether the small number of jaundice cases observed were induced by the drug or were simply cases of viral hepatitis [57]. These hepatotoxic adverse effects of iproniazid were also described for the rest of the MAOI drugs from the hydrazine group, such as nialamide, isocarboxazid and phenelzine. As regards the last of these, more than 50 % of deaths during treatment were attributed to hepatic cell damage, and this led to the withdrawal from the market of the majority of MAOIs at an international level [78, 80]. As occurred with iproniazid, tranylcypromine was withdrawn from the US market, though for different safety reasons, in 1964, after reports of an increase in the number of hypertensive crises related to the drug. These hypertensive crises, associated with intense headaches and in some cases with subarachnoid intracranial haemorrhages, were described from the very moment the drug was approved, in 1961, and according to Barry Blackwell, at Maudsley Hospital in London, it was traced to the ingestion of certain cheeses, so that these crises became referred to as the "cheese effect" [14]. A.M. Asatoor and colleagues [4], at the Westminster Medical School in London, showed that after the ingestion of cheese, there was a considerable increase in the excretion of metabolites of tyramine. Later, it was confirmed that many other foods (products made with yeast, chicken liver, snails, pickled herrings, red wines, some types of beer, tinned figs, broad beans, chocolate, products including cream and so on) contained amines with indirect action (chiefly tyramine), which could also provoke hypertensive episodes in patients treated with MAOIs.

These problems attributed to MAOIs, together with the results of some clinical trials, as sponsored by the British Medical Research Council [17] (strongly questioned later on), that confirmed their lower efficacy for major depression versus tricyclic antidepressants, considerably limited their therapeutic use, especially in European countries [92], and favoured a generalized loss of prestige. In fact, today, MAOIs are always considered a second-choice drug, potentially of great help in cases of intolerance or lack of response to other antidepressants (refractory depression) [8].

26.3 The Discovery and Clinical Introduction of Imipramine

The history of tricyclic antidepressants (TCAs) began in 1883, with the synthesis of the first phenothiazine by Heinrich August Bernthsen, a 28-year-old laboratory chief at the Badische Anilin und Soda Fabrik (BASF) in the German city of Mannheim [68, 77], who was commissioned to experiment with chemical dyes, particularly methylene blue [44, 54]. In 1883 Bernthsen synthesized for the first time a phenothiazine that would serve as the basis for the subsequent synthesis, in 1899, of iminodibenzyl, by J. Thiele and O. Holzinger [110]. However, at the time, no use was found for this agent as a dye for the textile industry, so that it ended up gathering dust, so to speak, in a Basle warehouse, though remaining on the files of Swiss chemical company J.R. Geigy AG, which had used it at the end of the nineteenth century in the preparation of the Sky Blue dye [44].

Half a century later, the director of Geigy's Pharmacology Section, Robert Domenjoz, later to become director of the Pharmacology Institute at the University of Bonn, was impressed by the data from the Paris company Rhône-Poulenc, which had developed, in close collaboration with the Institut Pasteur, that some antihistamines promised to be commercially successful as hypnotics or sedatives. Prof. Domenjoz encouraged his team to look into the effects of the phenothiazines, for which no important application had been found at that time, in the hope of their being useful as sedatives. In 1948, Geigy's chemists F. Häflinger and W. Schindler used iminodibenzyl as the basis for synthesizing 42 derivatives [44, 102]. The pharmacological tests carried out on these compounds revealed that the majority of them had, to a greater or lesser extent, antihistaminic effects, in addition to their sedative, analgesic and antispasmodic properties, and that the differences between them were related to the chemical structure of their lateral chain. Moreover, the basic toxicological and lethal dose 50 (DL_{50}) studies carried out in mice revealed no significant adverse effects [109].

After these experiments in laboratory animals, and even some involving self-administration, these chemists contacted hospitals who might be interested in clinical research on these products, at a time when there were practically no bureaucratic restrictions on the implementation of these types of study. One of these substances – known internally as G-22150 – was sent to Roland Kuhn (Fig. 26.2), assistant medical director at the Thurgauische Heil- und Pflegeanstalt in Münsterlingen (close to Lake Constance) and pupil of Jakob Klaesi (who introduced the famous sleep cures), to see whether it could serve as a hypnotic. Kuhn discarded the possibility of using the substance in "pills for sleeping", due to its irregular and rather unreliable results, but observed "a rather peculiar positive effect" [62]. However, interest in continuing the clinical development of this substance fell away.

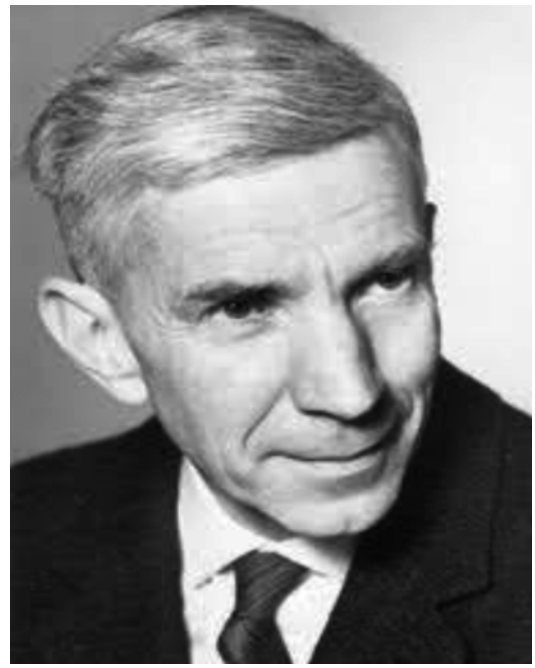

Fig. 26.2 Roland Kuhn (1912–2005), Medical Director at the Psychiatric Clinic of Thurgau Canton, in Münsterlingen, near Basel (Switzerland). Kuhn was other of the great pioneers of psychopharmacological research and the discoverer of the antidepressant properties of imipramine

Subsequently, in 1952, came the news that Pierre Deniker and Jean Delay had made an important discovery while testing a phenothiazine called chlorpromazine at the Saint-Anne University Hospital in Paris [74, 77], with 38 psychotic patients showing spectacular improvements. These findings spurred an intensification of the search for substances with similar properties by the pharmaceutical companies, concerned that Rhône-Poulenc might corner the new market that was opening up. The result was that some long-forgotten antihistamines filed away by the Geigy company were dusted off, in the hope that they might prove useful to psychiatry [34, 44, 62, 105].

At this time, the prevailing doctrine on the aetiology of depression was that it was the result of psychodynamic processes, despite the fact that the therapeutic effects of electroconvulsive therapy were obvious. Consequently, the thesis that drugs could have some effects on the symptoms of mood states but would not modify the course of the illness itself enjoyed the support of a majority of specialists. Even Roland Kuhn himself was scientifically committed in this philosophical field of psychoanalysis, despite being considered as one of the pioneers of the new generation of "biologicist psychiatrists" by virtue of his extensive knowledge of organic chemistry and biochemistry [105], especially after February 1954, when he contacted Geigy (or vice versa, according to some authors) to study the possible antipsychotic effects of G-22150, which he had tested years before. Kuhn had to interrupt some treatments with this substance due to problems of tolerance [62, 102], but asked Geigy to send him another phenothiazine, in the hope of finding another potent antipsychotic agent for his patients and avoiding a shortage of drugs at the Münsterlingen clinic. In early 1956, Kuhn received a compound called G-22355, a substance with the same lateral chain as chlorpromazine, which had been synthesized by Häflinger and Schindler, in 1948, from promethazine, replacing the sulphate bridge of phenothiazine with an ethylene bridge [34, 80]. This substance had been registered in 1951 with US licence number 2554736 [36].

The extensive clinical research that took place in 1956 at the Kantonsspital Münsterlingen, near Basle, soon made it clear that agent G-22355 lacked any appreciable neuroleptic effect. Indeed, in some patients who had previously been treated with chlorpromazine, their schizophrenic condition worsened, giving way to a state of agitation that gave cause for clinical concern. As a result, the clinic's image was tarnished and relations between its directors and the pharmaceutical company became strained [109]. However, Kuhn observed that three patients diagnosed with depressive psychosis showed a marked improvement in their general state within just a few weeks. Consequently, in a letter to Geigy dated 4 February 1956 [9], Kuhn raised for the first time the possibility that this substance might have an antidepressant therapeutic effect. Subsequently, another 37 depressive patients were given this drug, thus, demonstrating its special efficacy in the treatment of depressive disorders [59, 60, 62]. The antidepressant effect of imipramine was, therefore, totally unexpected, and its discovery completely accidental. Although Kuhn found G-22355 was a failure in treating the psychotic symptoms of schizophrenics, he chanced to notice mood elevation in these patients. This serendipitous finding led to his successful study of depressive patients in 1956 [59]. Kuhn had the sagacity to recognize an antidepressant drug while searching for an antipsychotic one [11, 82, 84].

Kuhn's impressions of the initial results, in a total of 40 depressive patients, were presented on 6 September 1957, at the Second World Congress of Psychiatry in Zurich, to an audience of scarcely more than a dozen [59]: "The patients appear, in general, more animated; their voices, previously weak and depressed, now sound louder; they are more communicative, the lamentations and sobbing have disappeared. The depression, which had manifested itself through sadness, irritation and a sensation of dissatisfaction, now gave way to friendly, joyous and accessible feelings". These results were published for the first time on 31 August 1957, in German, in the journal *Schweizerische Medizinische Wochenschrift* ("*Über die Behandlung depressiver Zustände mit*

einem Iminodibenzyl derivat (*G 22355*)") [59]. Moreover, during the Zurich Congress, Paul Schmidlin introduced the term "thymoleptic" to describe the new substances whose action was similar to that of Geigy's compound [80].

Kuhn's reports of the efficacy of this compound, now formally called imipramine, were received, as the author would later confess [61], with some scepticism by the medical community, since the view emerging from the numerous conferences on pharmaceutical and pharmacological disciplines held between 1953 and 1958 was based on the assumption that there could never exist a truly effective antidepressant substance, which went beyond reducing the symptoms of depression. The most widespread hypothesis at this time, as referred to above, was that depression as such emerged from intrapsychic conditions and conflicts [50], leading to the conviction that chemical tools merely masked the true symptoms of depressive conditions. Despite this clear opinion among experts, the antidepressant effects of imipramine were confirmed by such prestigious specialists as Paul Kielholz and Raymond Battegay, and imipramine was put onto the Swiss market by Geigy at the end of 1957, under the tradename Tofranil®. The drug came onto the market in the rest of Europe in the spring of 1958 [36, 44] and represented a giant step in the treatment of depression, as the first example of a new family of drugs, known as imipraminic or tricyclic antidepressants. As Kuhn [61] put it: "We have achieved a specific treatment of depressive states, not the ideal already going far in this direction. I emphasize 'specific', because the drug largely or completely restores what the illness has impaired –namely, the mental functions and capacity and what is of prime importance, the power to experience."

In September 1958, at the First Congress of the recently founded Collegium Internationale Neuro-Psychopharmacologicum (CINP), held in Rome, an audience made up primarily of psychiatrists began to become aware of the positive effects of the new drug, though more than through the work of Kuhn, through that of other research teams. In fact, the year 1958 saw the publication of two new studies on imipramine in the *American Journal of Psychiatry*. One of these reproduced the lecture given by Roland Kuhn at Galesburg State Hospital in May of that year and was published in the November issue. Although the article added nothing new with respect to what had appeared previously in the *Schweizerische Medizinische Wochenschrift*, it did have greater international repercussions. In it, Kuhn described at length the pharmacological effects of imipramine, reported its efficacy data and adverse effects and offered recommendations for its clinical use and dosage and the duration of treatment. He recounted how "the patients got up in the morning voluntarily, they spoke in louder voices, with greater fluency, and their facial expression became more lively. They began to do some individual activities, they once more sought to make contact with other people, they began to train on their own, to participate in games, to become happier and to recover their ability to laugh" [60]. His observations were confirmed later through studies with larger samples [61]. The following year, 1959, saw the publication of more than 60 studies assessing the therapeutic effects and adverse reactions of imipramine in different groups of patients. The countries in which there was most expectation in relation to the new drug were Italy, France and Canada, though positive results were also reported in Russia, Poland, Sweden and South Africa.

The introduction of imipramine in North America was due largely to the work of one of the great pioneers of psychopharmacology and the man who had previously introduced chlorpromazine there [74, 76], Heinz E. Lehmann, a psychiatrist from Berlin who had fled Nazi Germany and was working at the Verdun Protestant Hospital in Montreal (now the Douglas Hospital). At the Second World Congress of Psychiatry in Zurich, Lehmann heard Kuhn's lecture and immediately began treating groups of Canadian patients [44]. Lehmann designed and implemented a study on the efficacy of imipramine in a sample of 48 depressive patients, thus permitting the drug to be marketed in the United States [64]. The fact that Lehmann, an expert of great international prestige, put his faith in

imipramine was of enormous importance for its acceptance worldwide [68].

The first imipramine-placebo-controlled clinical trial was performed in 1959 by Ball and Kiloh [7], demonstrating the efficacy of this substance, especially in so-called endogenous depressions and in psychotic depressions. In March of that same year, at the McGill Conference on Depression and Allied States, an international event held in Montreal, all the data on imipramine accumulated up to that time from North American and European studies were presented. However, it would be another 6 years before Gerald L. Klerman and Jonathan O. Cole demonstrated that imipramine was significantly superior to placebo in the treatment of depression, thanks to an analysis with data from 23 published studies and a total of 1,000 patients treated with imipramine (550 patients) or with placebo (459 patients). The results of this analysis confirmed the rate of improvement at 65 % in the imipramine group, as against 31 % in the placebo group [56]. Indeed, imipramine has maintained its status as one of the most effective antidepressants up to the present day.

Despite the great success of imipramine, it was not until 1961 that a second TCA came onto the market: On 7 April 1961, amitriptyline (Merck and Co. Pharmaceutical) was approved as an antidepressant by the Food and Drugs Administration (FDA), under the tradename Elavil®. During the 1960s, a whole series of TCAs were developed [34]: nortriptyline (1963), desipramine (1964), trimipramine and protriptyline (1966), iprindole (1967), dothiepin and doxepin (1969) and clomipramine (1975).

The efficacy of TCAs in the treatment of depression was indisputable by the end of the 1950s [68, 72]. However, their action mechanism was not well understood. In 1959, Robert Domenjoz and W. Theobald confirmed that imipramine produced an antagonism of the effects of reserpine [32]. Two years later, Bernard Brodie's team at the NIH demonstrated the physiopathological role of biogenic amines in depression, on finding, in studies with laboratory animals, that imipramine inhibited the absorption of noradrenaline. Another NIH team, led by Julius Axelrod, demonstrated a reduction in the uptake of noradrenaline in the synaptic nerve endings during treatment with TCAs [87]. But the fruits of this between Brodie and Axelrod finally served at least as the basis for the subsequent work of William Bunney Jr. and Joseph Schildkraut, who in 1965 proposed the catecholamine-deficit hypothesis of the aetiology of depression [79].

26.4 The Discovery and Clinical Introduction of Fluoxetine

As already mentioned, the incorporation of TCAs and MAOIs into the antidepressant arsenal was the fruit of coincidence and of the observational skills of some researchers, both basic and clinical. However, the scientific value of these drugs, in the framework of the pharmacology of mental disorders, is of crucial importance, since they provide highly relevant data on the action mechanisms of pharmacological agents at the synaptic transmission level and, by extension, throw light on the aetiology of the antidepressant effect, opening the way for the development of new, much more specific drugs.

In contrast to the case of the way these substances were introduced into the clinical context, the SSRIs constitute the first family of psychoactive drugs developed in line with a procedure of rational and directed design [96], that is, following a strategy planned in advance, which involved seeking a drug capable of acting on a specific *locus* of action (the serotonin reuptake pump, in this case), avoiding, moreover, other nonessential *loci* (e.g. different neuroreceptors) and the associated potential undesirable effects. So that the discovery of fluoxetine and the other SSRIs was the non-serendipitous outcome of a programmatic and theory-driven research effort [11, 85].

Fluoxetine was the first SSRI to be synthesized and developed, by the US firm Eli Lilly (Indianapolis, Indiana), and is considered the prototypical molecule of this family of antidepressants, becoming, indeed, the world's most widely prescribed antidepressant. The first publication on fluoxetine dates from 1974. The 15 August issue of the prestigious journal *Life*

Sciences included the article by Wong et al. entitled "A selective inhibitor of serotonin uptake: Lilly 110140, 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine", which described the actions of the new molecule on amine reuptake systems and postulated its potential utility for the study of the serotonergic function and of certain mental disorders [115]. Twelve years later, the drug came onto the pharmaceutical market in Belgium, and in 1987 (12 December) it was approved for sale as an antidepressant by the FDA. Fluoxetine is, moreover, the most written-about drug – together with chlorpromazine – in the history of psychopharmacology, being the subject of well over 25,000 scientific publications [68].

The story of the synthesis and development of fluoxetine [33] begins with the studies carried out in the 1960s on the action mechanisms of TCAs. At the end of that decade, the serotonergic hypothesis of depression began gaining momentum among researchers after studies demonstrated the powerful inhibition of cerebral reuptake of serotonin exercised by imipramine and other tertiary derivatives [19] – an inhibition that was much more powerful in the case of clomipramine (a molecule with a chloride group added to the triple ring of imipramine). Clinical evidence also supported this serotonergic hypothesis, such as a decrease in levels of serotonin and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the rhombencephalon of depressive patients who had committed suicide [104] and reduced concentration of 5-HIAA in the cerebrospinal fluid of depressive patients [5]. Moreover, treatments with precursors of serotonin, such as tryptophan or 5-hydroxytryptophan, showed antidepressant effects [25], while the incorporation of new technologies, such as preparations of rat brain synaptosomes, enabled Lilly Research Laboratories, among other things, to determine the high-affinity kinetics in the uptake of serotonin [114].

The development of fluoxetine, from a historical perspective, can be dated from 1971, when a prestigious pharmacologist with experience in serotonin research, Ray W. Fuller, joined Lilly. Fuller soon began trying to make the directors of the company aware of the importance of these new neurotransmitters in the genesis of affective disorders, though there was some initial reluctance from Lilly to embark upon research in this direction. That same year, Solomon H. Snyder from Johns Hopkins University – one of the founding fathers of modern biological psychiatry – was honoured by Lilly Research Laboratories and invited to give a lecture. The theme he chose was neurotransmission, and his lecture highlighted the great utility for biological research of so-called brain synaptosomes, procedure that he himself developed, which would subsequently be applied in the development of fluoxetine [33]. The insistence of Fuller, supported by the biochemist David T. Wong (Fig. 26.3), resulted in the formation of a "serotonin-depression study team", made up of Fuller, Wong, the organic chemist Bryan B. Molloy and Robert Rathbun, and which would be the driving force behind the development of the new antidepressant [70, 75].

Fig. 26.3 David T. Wong (1936–), biochemist at Lilly Research Laboratories (Indianapolis, USA) and leader of the research group that developed and introduced fluoxetine

This group, led by Wong, devoted its research efforts in the early 1970s to obtaining molecules capable of selectively inhibiting the reuptake of serotonin, as potential antidepressant agents, and which would lack the cardiotoxicity and anticholinergic properties of TCAs. The work of Molloy and Rathbun was pioneering in this regard. Having observed that diphenhydramine and other antihistamines were capable of inhibiting the reuptake of monoamines [20] and of blocking, to the same extent as imipramine and amitriptyline, the ptosis induced by tetrabenazine in mice [10] – a standard test of antidepressant activity, Molloy synthesized a series of phenoxyphenylpropylamines as analogues of diphenhydramine. One of these substances, LY-14939 (later known as nisoxetine), was studied by Rathbun and Richard Kattau, who observed that it was as powerful as TCAs in reversing hypothermia induced by apomorphine in mice. Moreover, both nisoxetine and desipramine antagonized the hypothermia induced by reserpine in mice [106] and were powerful inhibitors of noradrenaline reuptake in brain synaptosomes, though nisoxetine scarcely blocked the reuptake of serotonin and dopamine [116]. Wong considered that small chemical modifications of the phenoxyphenylpropylamine compounds could provide selective serotonin reuptake inhibitors and hence chose 55 derivatives of this series and 2 naphthalene oxide analogues to test the power of reuptake inhibition in the three monoamines in vitro. On 8 May 1972, a member of Wong's team, Jon-Sin Horng, tested fluoxetine oxalate (LY-82816), and on 24 July of that same year, it was found that fluoxetine chlorhydrate (LY-110140) was the most powerful and selective inhibitor of serotonin uptake in the entire series [115], with a potency for inhibiting the uptake of serotonin six times greater than N-methyl-phenoxyphenylpropylamine, the father compound of the series, while its potency was 100 times smaller in the uptake of noradrenaline. Precisely, the p-trifluoromethyl radical of the phenoxyl ring of fluoxetine chlorhydrate was confirmed as the key element in the great potency and selectiveness of this drug for serotonin reuptake. Moreover, the affinity of fluoxetine for different neuroreceptors was seen to be very low [117], which explained the scarcity of adverse effects associated with it, especially compared to the range of side effects typical of TCAs (constipation, urine retention, blurred vision, orthostatic hypotension, sedation, memory disorders, dizziness, etc.).

In relation to the clinical development of fluoxetine, it should be mentioned that after the synthesis of the SSRIs and the corresponding experimental safety studies, there began in Indianapolis and Chicago a series of clinical trials which, despite promising results, were never published, perhaps because of the company's commercial policies. Moreover, the first clinical publication on this drug was of a negative nature, since it reported the appearance of a severe dystonic condition in a patient treated with this antidepressant [88].

Nevertheless, in 1980, Lilly decided to commit themselves definitively to the new molecule, entrusting the clinical research task to John Feighner, who carried out his studies at his private psychiatric clinic in La Mesa (California). In 1983, the first positive results began to appear: fluoxetine was as effective an antidepressant as the classical tricyclic drugs and, moreover, showed far fewer adverse effects [75]. Feighner [35] began talking of a "new generation of antidepressants". Between 1984 and 1987 clinical trials with fluoxetine multiplied, and finally, in December 1987, the FDA definitively approved its clinical use, under the tradename Prozac®.

Numerous clinical trials subsequently confirmed that the antidepressive efficacy of fluoxetine was similar to that of TCAs in outpatient major depression and superior to that of placebo [90]. The efficacy of fluoxetine was also confirmed in patients with major depression and melancholia [46], dysthymias [47] and different degrees of inpatient depression [23]. All such conditions were included, in the early 1990s, by two researchers at the McLean Hospital, in a category called "Disorders of the Affective Sphere" [49]. Thus, perspectives for the use of fluoxetine were highly promising. Indeed, growth of fluoxetine use has been the most rapid in the history of psychotropic drugs: in 1990, 3 years after its introduction in the USA, it was already the most

widely prescribed drug by North American psychiatrists, and in 1994, it sold more than any other drug worldwide, except Zantac® [105]. Furthermore, as occurred with Miltown®, Prozac® became established within the cultural scene of the 1990s, making the front pages of popular newspapers and periodicals, such as the *New York Times* (With Millions Taking Prozac, A Legal Drug Culture Arises, 1993), or *Newsweek* (How A Treatment For Depression Became As Familiar As Kleenex And As Socially Acceptable As Spring Water, 1994), and becoming the main protagonist of a bestseller (*Listening to Prozac*, 1993) by Peter D. Kramer [58], Professor of Psychiatry at Brown University.

In the wake of the article by Wong et al. [115], a large quantity of SSRIs, of highly diverse chemical origin, began appearing in the scientific literature. Six of them, including fluoxetine (duloxetine, atomoxetine, nisoxetine, dapoxetine, fluoxetine and norfluoxetine), owed their existence to the research efforts of Wong's group [33]. The first SSRI to be marketed was zimelidine, in 1982, by Astra Pharmaceuticals (Södertälje, Sweden). However, this drug was rapidly withdrawn from the market (just the following year) due to problems of hypersensitivity (fever, myalgias, increased levels of aminotransferases and, above all, various cases of neurological complications) [91]. Subsequently, and within a relatively short period, four more drugs from this family were made available to physicians, each one developed by a different pharmaceutical company: citalopram (Lundbeck), fluvoxamine (Solvay), paroxetine (AS Ferrosan, Novo Nordisk) and sertraline (Pfizer). Fluvoxamine was first marketed in 1983 in Switzerland, citalopram in 1989 in Denmark, sertraline in 1990 in the UK and paroxetine in 1991 in Sweden.

26.5 Epilogue

In the area of psychopharmacology, purely serendipitous discoveries, in contrast to what has been postulated, are rather rare. The majority, as in the field of antidepressants, presents a mixed pattern, a combination of serendipitous and non-serendipitous finds, and they generally follow a consistent pattern that begins with an initial serendipitous observation. In any case, it seems clear that serendipity had a fundamental role in the development of modern psychopharmacology during the 1950s, notwithstanding, of course, the systematic and rational search for results, a phenomenon inherent to scientific investigation that was consolidated in the following decades [11, 82].

The clinical introduction of psychotropic drugs in the 1950s constitutes one of the great medical advances of the twentieth century, and the importance of the event has been compared with the discovery of antibiotics and vaccines. J. Allan Hobson, neurophysiologist at the Harvard University, remarks in his book *The Chemistry of Conscious States*, in reference to the introduction of psychoactive drugs in the 1950s, that "… the development of drugs that interacted with the brain's chemical systems is the most important advance in the history of modern psychiatry" [48]. Moreover, from the clinical and public health points of view, the net result of this introduction was a "powerful social change in the conceptualisation and acceptance of mental illness, including through the media, in the organization of mental health services, in the development of the specialty and nosology of psychiatry, in the dynamics of high technology economies" [55].

Iproniazid, in spite of its short psychiatric life, and imipramine deserve a privileged place in the history of psychopharmacology and psychiatry, since not only did they open the door to the specific treatment of mood disorders – lending great prestige to the psychiatry of the time, improving everyday clinical practice and increasing patients' quality of life – they also made it possible to develop the first serious hypotheses on the biological nature of these affective disorders [70–72, 79, 80, 93, 94], the monoaminergic theories of depression, around which scientific discussion revolved in the specialized journals in the 1960s and 1970s [112] and which postulated a functional deficiency of noradrenergic or serotonergic neurotransmission (see [69]) in certain brain areas as a primary cause of

these pathologies [25]. The "catecholaminergic hypothesis" was the first to be postulated, based on the observations mentioned above about the effects of the antidepressants recently discovered: the inhibitory action of iproniazid on MAO [118] and the blocking of synaptic reuptake of noradrenaline by imipramine [40]. This hypothesis on the biological mechanism of depression, presented by Joseph J. Schildkraut of the Massachusetts Mental Health Center (Boston) in a classic work published in 1965, suggested that this pathology was due to a fall in noradrenaline level in the inter-synaptic cleft: "some depressions, if not all, are associated with an absolute or relative deficit of catecholamines, particularly noradrenaline, in important adrenergic receptors in the brain. Contrariwise, elation may be associated with an excess of such amines" [101]. To date, Schildkraut's article is still the most cited in the entire history of the *American Journal of Psychiatry*. For their part, the team led by Alec J. Coppen of the Neuropsychiatric Research Institute, which belonged to the Medical Research Council of London, demonstrated that the administration of tryptophan, a precursor of serotonin, to depressed animals boosted the therapeutic effects of MAOIs [26]. Another important group was that of Dutch psychiatrist Herman M. van Praag, of the Department of Biological Psychiatry at University of Groningen. Van Praag, working initially with the biochemist Bart Leijnse, concluded that there were reasons to acknowledge a relationship between MAO inhibition and antidepressant action, and between serotonergic dysfunctions and the appearance of certain types of depression [113]. However, this serotonergic hypothesis was postulated without a clear demonstration of neurobiochemical correlates at a central level [25], until in 1968, Arvid Carlsson and colleagues at the University of Gothenburg (Sweden) described for the first time how TCAs blocked the reuptake of serotonin at a brain level [21], permitting Izyaslav P. Lapin and Gregory F. Oxenkrug to postulate, in 1970, the serotonergic theory of depression, as opposed to the catecholaminergic hypothesis, based on a deficit of serotonin at an inter-synaptic level in certain brain regions [63]. In vivo neurochemical studies also confirmed the specificity of fluoxetine in the inhibition of serotonin reuptake, thus reinforcing the serotonergic hypothesis of depression: "[fluoxetine] has represented a valuable pharmacological instrument for the study of serotonergic transmission mechanisms and the physiological functions of serotonergic brain neurones" [39].

Psychoactive drugs in general, and iproniazid and imipramine in the particular case that concerns us here (and later fluoxetine), thus permitted the gradual definition of the neurochemical process underlying mental illness and the generation of a physiopathological theory on it, permitting the advent of so-called biological psychiatry [81]. Thus, support began to grow among the scientific community for the notion that these drugs might be correcting a kind of specific "chemical imbalance", the underlying cause of the illness [55]. We are talking, then, about an approach that could be considered "pharmacocentric" [24], with far-reaching heuristic implications for psychiatry [9]. This situation emerged as unique in the history of medicine, since a large quantity of aetiological hypotheses were based on the action of a series of drugs whose application to psychiatric pathologies resulted, in many cases, as today, from the intervention of chance. With iproniazid and imipramine, depression ceased to be an illness of the mind (an "alteration of the soul") to become an illness of the brain [79, 80].

Despite the considerable clinical advantages of SSRIs, based largely on their better tolerance and safety profile and greater convenience, there are a series of problems encountered in antidepressant therapy that have yet to be resolved. It suffices to mention the delay in the onset of antidepressant response or the percentage, estimated at around 30 %, of patients who do not respond to treatment [42]. Such problems, among others, have justified research efforts in the quest for new antidepressants and which have led, in the last 20 years, to the clinical introduction of new drugs, with different pharmacodynamic properties. Notable among these are venlafaxine, duloxetine, nefazodone, mirtazapine and reboxetine. However, these drugs, despite belonging to four new families of antidepressants [28], continue to

share an action mechanism that revolves around the enhancement of aminergic functioning: inhibitors of noradrenaline and serotonin reuptake (venlafaxine, duloxetine, milnacipran), antagonists of serotonergic 5-HT_2 receptors (nefazodone), specific noradrenergic and serotonergic antidepressants (NaSSAs) (mirtazapine) and selective inhibitors of noradrenaline reuptake (reboxetine, atomoxetine) [68]. Only recently, it has introduced in clinical setting the first antidepressant with proven clinical efficacy that act via the extraaminergic mechanisms, agomelatine. This agent's primary mechanism is its agonistic action at MT_1 and MT_2 melatonin receptors, but it may also indirectly increase monoamine presence [1, 2] (see Table 26.1). In any case, the scientific evidence accumulated over the last decade appears to confirm that there is no unitary biochemical mechanism to explain the antidepressant effect of all the substances currently available, hence the movement of research on the action mechanism of antidepressants in other directions, as well as efforts to find new *loci* of antidepressant action. Thus, research is in progress on the role of neurotrophic factors (neurotrophins and BDNF), of corticotropin-releasing hormone (CRH) and glucocorticoids, of excitatory amino acids and of the NMDA (N-methyl-D-aspartate) receptor, as well as on other possible targets (opioid system, interleukins, P substance, somatostatin, neuropeptide Y, melatonin, nitric oxide and so on).

However, it should be borne in mind that one of the most important challenges facing research today, in the field of Neurosciences, is an understanding of the molecular mechanisms responsible for intraneuronal communication and those through which cells manifest their phenotype in response to environmental signals. Detailed knowledge of these mechanisms could provide us, in the future, with much more specific and safer pharmacological tools for treating different neurological and psychiatric conditions, among the depressive disorders. Possibly, the psychopharmacology of this new millennium will cease to be a superficial and synaptic psychopharmacology and happen to be an intracellular psychopharmacology [3].

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Neurocognitive Deficit in Bipolar Disorders

27

Dimos Dimelis, Xenia Gonda, and Konstantinos N. Fountoulakis

27.1 Introduction

Major mood disorders and especially bipolar disorders are among the most aggravating causes of disability. Of particular importance is their role in neurocognitive functioning. Their co-occurring neurocognitive dysfunction, for many years, has been in the center of a debate. It was not clear whether mood switching could explain the observed neurocognitive shortage or this could be attributed to either iatrogenic or alcohol and/or drug abuse effects. Furthermore, it was unclear whether this observed weakening was a "trait" or "state" phenomenon. On one hand if the principal reason for these deficits is an underlying neurodevelopmental structural brain change, then this could be considered a "trait" phenomenon, while on the other hand if the major causal factor is the mood dysregulation, then this might represent a state phenomenon. Literature suggests that that these deficits should be considered a rather primary characteristic of mood disorders rather than a by-product of either mood swings or medication side effects [127]. Nevertheless, the majority of mood disorder patients, especially those suffering from psychotic symptoms (57.7 % of psychotic BD patients and 58.3 % of psychotic major depressive ones) [286], report neurocognitive deficits.

Although bipolar disorder (BD) patients are observed to show increased creativity and exhibit a higher average IQ [12–14, 187, 188], recent reports describe a noteworthy neurocognitive deficit which seems to precede the first mood symptoms, while it shows a rather persistent nature during the different phases and also as the illness progresses a deterioration has been described [20, 50, 54, 223, 247, 292]. A decline in neurocognitive functioning has been detected in one, two, or three or more domains in 40 %, 30 %, and 22 % of BD patients, respectively [162, 257]. This shortfall in neurocognitive functioning

Prof. Xenia Gonda is recipient of the Janos Bolyai Research Fellowship of the Hungarian Academy of Sciences.

D. Dimelis
Department of Psychiatry, 424 Military Hospital, Thessaloniki, Greece

X. Gonda (✉)
Department of Psychiatry and Psychotherapy, Semmelweis University, Budapest, Hungary

Laboratory of Suicide Research and Prevention, National Institute for Psychiatry and Addictology, Budapest, Hungary
e-mail: gonda.xenia@med.semmelweis-univ.hu

K.N. Fountoulakis
Division of Neurosciences, 3rd Department of Psychiatry, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

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may represent a trait feature in BD, not affected by alterations in the mood state, and it differs substantially from the one that is observed in schizophrenia being, among others, less pronounced [80, 107, 244, 285, 313, 355]. Furthermore, several factors that might affect neurocognitive performance have been recognized: gender and age, phase of the illness (patients suffering from severe exacerbations of either mania or depression cannot be questioned), and medication effects.

On the other hand, the research in this field is significantly limited by the fact that the borders between neurocognitive routes are more or less unclear and any test might be influenced by more than one process. The situation is further perplexed by the several different approaches to the categorization of neurocognitive processes, and the field of executive functions represents, maybe, the most heavily affected domain.

27.2 General Neurocognitive and Intellectual Functioning in Bipolar Disorders

It is suggested by the literature that average IQ and general intellectual functioning are either above [98, 211, 259, 281, 382, 391, 392] or at least similar in BD patients when compared to healthy controls [142, 151, 287, 317, 400], although it should be noted that functioning at a higher level, belonging to a higher social class, and preserving substantially the neurocognitive performance might turn the diagnosis toward the direction of a mood rather than at the direction of schizophrenia [7, 84, 87, 111, 122, 140, 142, 150, 192, 268, 317, 326, 335, 391]. A more complex relationship between BD and premorbid IQ has also been reported, with the lowest and highest IQ (especially verbal or practical ability) in men being associated with the greatest risk for pure BD (without comorbidity) [137], while data on the possible relation between premorbid IQ and psychotic BD are inconclusive [25, 147].

In general, the literature reports that in BD patients, general neurocognitive functioning is moderately reduced, regardless of the phase of the illness, as this is reflected in their IQ scores and also their performance in neuropsychological batteries [84, 114, 122, 145, 376]. Furthermore, these shortfalls, although milder, might be of similar quality to the deficits detected in schizophrenia patients [91, 313]. This impairment worsens when psychotic features are present [318], and they are similar to that seen in schizophrenia, during the acute psychotic manic phase [93, 108, 178, 399].

The full IQ is a composition of two subscores: the verbal IQ (VQ) and the nonverbal or performance IQ (PQ). In case of the deficit during acute manic phases, IQ, PQ, and VQ show effect sizes of 0.47, 0.06, and 0.28, respectively [93, 178]. On the other hand, there is a lack in studies during the depressive phase. The accurate IQ deterioration cannot be assessed accurately, and it might be underestimated, as it is a possibility that the premorbid IQ of BD patients is higher in comparison to that of healthy controls, and in the studies the decline is being compared to the norms of general population.

BD patients tend to show higher VQ in comparison to PQ scores [208, 263]. This reflects, actually, the fact that all PQ subtest scores are uniformly reduced while the accompanying VQ scores are being preserved. The slowing of the mental speed could not be responsible for this reported discrepancy because four PQ subtests and only one VQ subtest are timed, while the deficit persists during euthymic periods, and targeted research confirms its existence [302]. Although there are data suggesting that this characteristic pattern exists from the initial stages of BD [266], latest data dispute this [334]. It has also been reported that the VQ-PQ discrepancy may diminish because there is a positive correlation between the severity of the depression and the VQ scores reduction [152]. It is noteworthy that this divergence does not exist in patients suffering from unipolar depression [153]. It has been proposed that the VQ-PQ discrepancy might be the result of how BD affects the "fluid intelligence," while "crystallized intelligence" is being preserved. Nevertheless, any effort for a deeper understanding of this deficit is not only risky but also premature [152].

Taking into account all phases of the disorder, the effect sizes for current IQ reduction range from 0.36 to 070 [152, 213]. For patients in remission, the reported effect sizes for IQ range from 0.40 to 0.50 [52, 152] to lower and within the normal range, which is 0.11–0.16 [20, 247]. Finally, the effect size of premorbid IQ alterations in euthymic BD patients is reported to be low and insignificant (0.04–0.20) [52, 365].

27.3 Neurocognitive Deficits in Bipolar Disorders

27.3.1 Psychomotor and Mental Speed in Bipolar Disorders

Mental speed and psychomotor activation are clearly different concepts although they might overlap in areas like reaction time, cognitive and motor speed, and manual dexterity. Moreover, the majority of the neuropsychological tools that are available for the assessment of psychomotor and mental speed also evaluate other neurocognitive functions.

In bipolar depression the reaction time seems to be prolonged [258], but euthymic patients, too, exhibit prolonged reaction times and more error rates in a visual backward masking test. Overall, burden of illness seems to prolong reaction time, and this prolongation has been found to be related especially to past history of depressions and not to current medication [242].

The speed of mental processing in BD patients is slower, and this finding is unrelated to the phase of illness and the severity of the symptomatology [16, 33, 74, 84, 110, 111, 116, 122, 139, 176, 181, 228, 250, 254, 262, 263, 269, 274, 317, 338, 346, 380, 393], but it is less affected in comparison to schizophrenia [140, 150, 174, 266], although there are some data suggesting the presence of a similar degree of impairment between patients with BD and patients with schizophrenia [177]. Instead, regarding psychomotor speed, it has been reported that no significant differences exist between depressed or euthymic BD patients and healthy controls [163]. Another study suggested that patients with BD in the first year after resolution of their initial manic episode displayed a linear improvement in processing speed [367]. Depressive BD patients show a slowed mental speed in comparison to manic patients and unipolar depressives. Distraction seems to improve the mental speed of BD depressives while it negatively influences the speed of the other two groups [43].

The deficit could be manifested even during the early stages of the disorder [212]. The magnitude of impairment regarding mental speed in BD patients corresponds to an effect size of 0.82–1.08 [33, 47, 145]. In euthymic state, a meta-analysis reported an effect size (with the use of the TMT-A) of 0.52, while using the DSST, it was 0.59 [292]. Effect sizes for mental speed impairment of 0.60–0.84 were reported by various studies [20, 52, 247, 365].

The deficit in the processing speed might be a significant confounder on the performance in almost all neurocognitive testing. So controlling between-group difference in various neurocognitive domains for the processing speed might make them disappear [15]. Additionally, global functional impairment is connected to poor performance on a cognitive measure of processing speed (e.g., WAIS Digit Symbol or the TMT) [64, 254].

27.3.2 Attention in Bipolar Disorder Patients

Attention incorporates a number of processes, including working memory (the ability to keep a limited number of mental objects in awareness for a limited duration of time, often classified as part of executive functions), vigilance (the capacity to identify a specific target among several other stimuli), freedom from distraction or interference, and the ability to split or rapidly shift attention. Concentration is the ability to sustain attention over prolonged periods of time. The existing tests assess the previously mentioned processes, and beyond attention, they are also influenced from other processes.

While current symptomatology and the phase of the illness do not affect the magnitude of the

attentional impairment, in the literature significant variability of data can be found [5, 22, 74, 81, 82, 118, 122, 124, 145, 169, 170, 176, 236, 243, 246, 300, 305, 317, 340, 354, 367, 380, 387, 402]. Studies report that attention is impaired already during the early stages of the disorder [212], but the deficit is less pronounced than the one seen in patients with schizophrenia [1, 150, 279], although some studies report that the magnitude of impairment is similar between schizophrenics and BD patients [161, 177, 350]. Performance in divided attention (DA) varies considerably over time within patients, while a significant quadratic relationship between manic symptoms and DA performance has been found. Mild hypomanic symptoms seem to have a positive influence on DA scores; however, moderate to severe manic symptoms have a negative influence. On the other hand, depressive symptoms were not associated with DA performance [210]. The magnitude of effect sizes ranges from 0.36 to 0.82 [21, 47, 145] depending on the domain assessed and the composition of the study sample.

Another meta-analysis reported that attention is impaired during the acute manic/mixed state, with effect sizes ranging from 0.79 to 0.90, during the acute depressed state with an effect size up to 0.80, but also during euthymia with effect size from 0.41 to 0.65 [223]. Other meta-analyses reported effects sized between 0.37 and 0.8 for attentional deficits measured by different tests during different testing conditions in euthymic phases in BD [20, 52, 292, 365].

There is also a limited number of studies which indicate no impairment in attention [31, 154, 277, 351, 393], and this concerns especially euthymic patients [345]. One study has found that depressed BD patients, euthymic BD patients, and healthy controls had no significant differences in attention [163].

27.3.3 Learning and Memory in Bipolar Disorder Patients

Learning refers to the ability to acquire and store new information, while memory is the mental process that allows individuals to retrieve the new information at a later time. Learning and memory involve a number of processes including attention and concentration, encoding, and allocation of effort. These processes are distinct from one another but interrelated and interdependent. Moreover, there are different strategies and processes involved, depending on whether a short- or a long-term effect is desirable and also depending on the quality and nature of the information and the frame it is presented in.

Due to the fact that much of research on memory is focused on "depression" and does not distinguish between unipolar and bipolar depression, the results and the conclusions from these studies should be received with reservation because it is uncertain whether they apply specifically to BD and to which extent. It has been suggested that there is a deficit in working memory [30, 33, 61, 110, 111, 139, 147, 176, 181, 250, 327, 380, 386] and specifically in the visuospatial working memory [151, 157, 158, 183, 261, 357]. Psychotic patients might have worse performance [136, 145]. The impairment is probably present already since the early stages of the illness [24]. Some studies have shown that the impairment in working memory affects only patients with acute mania [157, 158, 178]. Compared to patients with schizophrenia, the impairment in visuospatial working memory is less pronounced [279].

Additionally, there are studies indicating a deficit in declarative memory [36] and specifically in semantic [73] and episodic memory [100, 145, 246, 273] and verbal learning [17, 23, 45, 63, 69, 110, 111, 139, 195, 253, 376] in both BD-I and BD-II patients [56, 183]. A deficit is also present concerning verbal memory [5, 7, 17, 23, 69, 125, 150, 181, 252, 254, 305, 374, 380] and also during periods of euthymia [32]. One study, however, has shown that depressed BD patients showed greater impairments in verbal memory than euthymic BD patients [163]. It has been suggested that the magnitude of the effect size of the verbal memory deficit is 0.7–0.9 [7, 100], and it is less pronounced in comparison to that seen in schizophrenia [67, 379] and cannot be explained by the attentional deficit [154]. Patients at early stages of BD have better performance in the total immediate free recall and in

delayed free recall compared to patients at a later stage and to patients with schizophrenia. Additionally, concerning the ability to retain learned words, BD patients at a later stage and chronic patients with schizophrenia were more impaired than BD patients at early stage and patients with recent onset schizophrenia [90]. It has also been indicated that delayed free recall is worse in patients with BD compared to healthy controls [306].

There are impairments in total learning as well as short- and long-delay verbal recall, recognition, discriminability and learning slope [70], associative learning [58], implicit motor learning [79], immediate memory [183], delayed memory [33, 47, 110, 111, 164, 176, 250, 317, 402], nonverbal memory [99], visual memory [5, 84, 122, 150, 228, 305, 393, 402], and autobiographical [205, 316] and prospective memory [71]. It has been shown that prospective memory deficits [401] and short-term non-affective memory [34] are also present in remitted BD patients suggesting that they constitute a trait deficit. Moreover, it has been shown that BD patients manifest a deficit in incidental contextual memory in the absence of a binding cue at encoding. There was no difference found between the groups for contextual memory even under incidental encoding with the binding cue. One study has indicated that the impairment in the contextual memory was reduced by providing cognitive support at encoding [86].

There are also negative studies concerning the presence of impairment in working memory [113, 229, 356], in spatial working memory [283], and in verbal learning [73] and verbal [199, 227, 277] and visual memory [199] in bipolar patients. One study has shown that the ability to learn is maintained both by BD and schizophrenic patients [90], and another study has reported that there were no differences between BD patients and healthy controls in terms of their slope of learning, retrieval index, retention percentage, semantic or serial clustering, errors, or level of retrieval [375]. It was suggested that most memory impairments are due to the presence of confounding variables except maybe for verbal recall [243]. The possibility that difficulties in semantic clustering or other strategic processing deficits are the cause for the verbal memory impairment has both positive [100, 164] and negative data [35, 36, 99, 100].

In meta-analytic studies, when all phases of the illness are taken into consideration, the magnitude of the effect size is 0.60 for working memory, 0.43 for immediate verbal memory and 0.34 for delayed, and 0.26 for immediate visual memory and 0.51 for delayed [213]. The meta-analysis of short-term memory studies revealed an effect size of 0.58 when span tasks were utilized, without any difference between auditory or visual tasks. When verbal learning tasks were used, the effect size was 0.91 [152].

During the acute manic/mixed state, the magnitudes of the effect sizes were 1.43 for verbal learning and 1.05 for delayed free verbal recall. Additionally, concerning the acute depressed state, the effect size for verbal memory was 1.20, while during euthymia, the domains impaired were working memory (0.65), verbal learning (0.81), long-delay verbal free recall (0.78), immediate nonverbal memory (0.73), and delayed nonverbal recall (0.80) [223].

The average effect size for episodic memory in euthymic BD patients is reported to be 0.62 and for working memory 0.60 [247]. Again in euthymic BD patients, a large effect size is reported for verbal learning and working memory (0.98) and somewhat lower effect sizes for aspects of immediate (0.73) and delayed (0.71) verbal memory [292] with further meta-analyses reporting effect sizes of similar magnitudes [20, 50, 52, 365]. It seems there is a publication bias especially concerning verbal learning, and after correction for this, the effect size is attenuated (from 0.85 down to 0.66) [50].

Overall the literature suggests that BD, irrespective of illness phase, is characterized by a severe deficit in the acquisition of new information, but not in the retention [7, 35, 99, 152, 373]. In spite of some opposing data, the most probable interpretation which derives from empirical studies is that the attention and concentration deficits impair the acquisition of information and learning, by disrupting the engagement of effortful processing which results in a shallow rather than

deeper level of processing (e.g., acoustic rather than semantic) [35, 36, 76, 101, 117, 165–167, 172, 297, 384, 385].

27.3.4 Verbal Skills in Bipolar Disorder

The evaluation of verbal skills includes mostly the evaluation of verbal fluency. Although the literature has reported that verbal skills are impaired during all phases of BD [23, 34, 97, 113, 217, 250, 252, 327, 343, 380, 402], there are some studies reporting that this impairment is not present during euthymia [7, 399] or during the first mood episode [31]. One study has indicated that there are errors in speech during the acute manic state, and these errors are independent from the coexistence of an attentional deficit [354].

Concerning the magnitude of the effect size, it has been reported that it is small [113] and even smaller in comparison to the effect size seen in schizophrenia [266]; however, patients with psychotic BD have a higher effect size (0.68–1.73) [47, 161, 319]. Even smaller (below 0.50) effect sizes have been reported [152], while when all phases of the illness are taken into consideration, an effect size equal to 0.63 emerges [213]. Both letter fluency and semantic fluency are impaired during the acute manic/mixed state, and the effect sizes are equal to 0.51 and 0.59, respectively. The phonemic fluency is impaired during the acute depressed state with an effect size equal to 0.93, while during euthymia both phonemic fluency (0.51) and semantic verbal fluency (0.75) are impaired [223]. In euthymic BD patients, the average effect size for verbal fluency ranges between 0.56 and 0.90 [247, 365], but it is smaller (0.34) for verbal fluency by letter [292].

27.3.5 Visuospatial Skills in Bipolar Disorder

The evaluation of visuospatial skills is usually made with the use of the complex Rey figure or with the WAIS-R block design. Patients with BD and their unaffected relatives show impairment in the visuospatial/constructional abilities [23, 111, 307] and in visual learning and memory [121, 307]. It is interesting that euthymic BD patients and patients with schizophrenia have similar impairment when the results are controlled for possible confounding factors [2]. Also, one study has shown that BD patients were significantly impaired on all three object location memory processes (positional memory, object location binding, and a combined process), with the largest effect found in exact positional memory ($d = 1.18$) [138]. Unaffected relatives demonstrated an intermediate level of performance in comparison to patients with BD and to normal controls [134]. Contrawise, one study suggested that the visual motion integration is intact in BD patients [75]. Some authors suggest that the impairment in visuospatial skills is restricted in the acute phase, while these skills might not be affected during remission [7, 152]. One study has reported that the overall effect size is equal to 0.65 [152], and other studies have reported that in remitted patients, the effect size is 0.22–0.57 [20, 247].

27.3.6 Executive Functions in Bipolar Disorder Patients

The executive system is considered to be involved in planning, decision-making, error correction, and troubleshooting in situations where responses are not well rehearsed or contain novel sequences of actions, are dangerous, or constitute technically difficult situations or situations requiring the overcoming of a strong habitual response or resisting temptation. In other words, "controlling of mental and neurocognitive processes" seems to be the key phrase describing the role of executive functions. In patients with BD, reasoning should be considered separately from the rest executive functions due to the fact that it seems to rely heavily on verbal and linguistic skills [152].

A severe impairment in executive functions except reasoning has been suggested during all phases of BD [5, 16, 17, 31, 33, 47, 55, 59, 84, 94, 96, 110, 111, 118, 122, 132, 134, 139, 145, 154, 163, 181, 243, 246, 248, 252–254, 268, 269,

296, 299, 305, 309, 317, 319, 327, 351, 359, 361, 380, 381, 390, 402] and early during the course of BD [212], but the deficit is less pronounced in comparison to schizophrenia [140, 150, 177, 266, 278, 350, 351]. However, at least a subgroup of patients is as severely impaired as patients with schizophrenia [19, 379, 397]. One study indicates that depressed BD patients showed greater impairments in executive functions comparing to euthymic BD patients [163]. It is suggested that this impairment might be particularly severe concerning interference and inhibitory control [28, 44, 121, 198, 229, 262, 319, 338, 376, 402]. Additionally, patients with BD might make more risky [3] or erratic choices [72, 395] especially when a history of alcohol abuse exists [194].

However, not all data are straightforward. Normal overall executive function has been suggested by one study [69], while another reported only prolonged time to complete the test [299]. Also a bimodal distribution of the Wisconsin Card Sorting Test (WCST) scores in patients with BD is reported, with some patients at near-control levels and others significantly impaired [7]. Some authors argue that there is no difference between BD patients and controls in executive functions [97, 123, 135, 274, 277, 356], while others argue that the deficit is present only in the more severe and chronic cases [97]. It has been also indicated that BD patients show an improvement in the executive functions in the first year after resolution of their initial manic episode [367].

The deficit in executive functions might be overall independent from illness phase; however, there are data suggesting that some aspects of the deficit are related to affective lability [8], duration of illness, residual mood symptoms, and current antipsychotic treatment [132] and history of psychosis [145]. One study found that the impairment in executive function was related to the severity of general psychopathology as it is measured by the PANSS [248], and another one correlated impaired insight with impaired executive functions [106].

A meta-analysis suggested that when taking all phases of the illness together, the effect size of this impairment is equal to 0.79 when reasoning (which was reported to be intact) is excluded [152]. Moreover, another meta-analysis reported an effect size of 0.34 for concept formation and of 0.55 for executive control, with no significant effect for current clinical condition [213]. During the acute manic/mixed state, the general executive function and the speeded set shifting are impaired with the magnitude of effect sizes to be equal to 0.72 and to 0.64, respectively. During the acute depressed state, speeded set shifting is impaired (0.64), while during euthymia problem-solving tasks (0.54), set-switching tasks (0.73), and verbal interference (0.75) are impaired [223]. Three recent meta-analytic studies in euthymic patients reported that the effect sizes for executive functioning were equal to 0.80 for the TMT-B, 0.56 for the WCST, and 0.80 for the Stroop test [247], and other meta-analyses reported effect sizes of similar magnitudes [20, 50, 52, 292, 365].

27.3.7 Social Cognition and Theory of Mind (ToM) in Bipolar Disorder

The term "social cognition" constitutes a psychological domain with several dimensions. It refers not only to the ability of the person to assume that other people have minds similar to his/her own and to interpret but also to understand and predict the emotions, desires, intentions, behaviors, and speech of others (including nonverbal elements). Social cognition shapes communication and interaction with others and in this way enabling adaptive social adaptation. It involves a complex set of processes including the representation of internal somatic states, knowledge about the self, perception of others, and interpersonal motivations [9].

The broad theory of mind (ToM) includes three main processes (a narrow definition of ToM, emotion processing, and affective decision-making). The narrow definition of ToM (mentalizing or mindreading) refers to the ability to attribute mental states (e.g., beliefs, desires, and intents) to oneself and to others. Emotion processing is the ability to identify and discriminate basic emotions. Affective decision-making is

crucial for an appropriate social behavior and concerns weighing up choices in association with reward and punishment [304].

The tests which are used to evaluate these domains are both verbal (scenarios) and nonverbal (pictures). They demand the subject to identify and comprehend the situation, the roles, and the interactions and to make appropriate planning. So far, empirical data has confirmed the universality of facial emotions. This means that the specific ability to process and identify facial emotions is a substantial feature of human communication and social interaction, which is independent of culture. It has been found even in ecological tests that mimic real-life scenarios that patients with BD showed less impairment on neurocognitive performance compared to patients with schizophrenia in this domain [67].

27.3.7.1 Theory of Mind (ToM)

It has been shown that there is a robust deficit concerning ToM and social cognition during all phases of BD [3, 46, 49, 88, 89, 115, 182, 225, 226, 255, 267, 274, 316, 321, 390], although there are also studies indicating subtle deficit [115] as well as negative studies [29, 298, 308]. It is possible that after controlling for medication and other confounding factors, the performance of patients is the same with the performance of controls [255]. There is also evidence that the impairment in ToM is restricted to the acute phases of the illness even when memory was controlled for [183], and thus there is no impairment during remission [197]. Moreover, patients with BD with psychotic features and patients with schizophrenia were found to be equally impaired in their scores for ToM stories, although patients with schizophrenia manifested a worse performance [67, 295]. Another study reported no impairment in the accuracy of responses but only the presence of a prolonged latency time for the response [204]. Probably the impairment in ToM in BD is associated with mood symptoms, and it might reflect more fundamental underlying neurocognitive deficits rather than representing a specific trait marker of the disorder [183].

27.3.7.2 Emotion Processing

There are inconclusive data concerning the recognition of emotions in BD patients. On one hand there are studies indicating an impairment in emotion recognition and in the identification and discrimination of emotions even during remission [2, 40, 57, 60, 96, 104, 115, 141, 226, 231, 232, 238, 255, 298, 398]; on the other hand, however, there are studies reporting no impairment in these domains, especially after controlling for medication and other confounding variables [39, 221, 245, 255, 267, 274, 290, 321, 344, 377].

Patients with BD-I seem to be more impaired in comparison to controls on face emotional recognition (FER) fear subtests, happiness, surprise test, and FER total scores [96]. It has been also reported that patients with BD do not have any impairment in face recognition in general, but the impairment exists specifically in the facial affect labeling, even during euthymia [141, 378]. Maybe specific phases affect specific emotions. For instance, in acute mania, the recognition of fear and disgust is reported to be impaired [231], while during euthymia, patients recognize disgust better [171]. Reduced biases in the emotion recognition are related with acute bipolar depression, while on the contrary increased biases in emotion recognition are related with acute mania [95]. This mood-congruent bias is state rather than trait and contributes as a core characteristic [6, 232, 378]. Younger BD participants performed worse than expected relative to healthy comparison participants of similar age. The deficits were found both concerning child and adult faces and were particularly strong for angry child faces, which were most often undertaken as sad. The results of this particular study were not influenced by medications, comorbidities/substance use, or mood state/global functioning [383].

27.3.7.3 Emotional Decision-Making

The literature reports that there is little or no difference between patients with BD and controls on the emotional decision-making component [3, 72, 255, 299].

27.3.7.4 Reviews and Meta-analyses Regarding Social Cognition in Bipolar Disorder

One meta-analysis reported an effect size concerning ToM equal to 0.75–0.86 and concerning emotion processing equal to 0.35. The same analysis found that there was no difference between patients with BD and healthy controls concerning the emotional decision-making. That specific meta-analysis suggested that the performance in ToM and in emotion recognition was not associated with years of education, age, sex, duration of illness, and medication [304].

The significant heterogeneity in the results may be due to the differences among studies. These differences concern the neuropsychological tools used and the study samples. The specific tests might play a significant role since the performance of BD patients might be similar to controls in some aspects of emotion recognition, but other aspects could be impaired or not, depending on the clinical state. For example, it has been found that stable BD patients might exhibit impaired facial emotion discrimination [2], while on the contrary the depressed BD patients' performance could be similar to healthy controls in the perception of chimeric faces. In contrast manic patients might perceive all chimeric faces as positive [235]. It is interesting that patients might outperform normal controls in specific domains (e.g., euthymic BD patients in the recognition of disgust) [171]. Depressed BD patients might show impairment only in the most difficult tasks [238]. It is important to be noted, however, that the effect of the clinical state is controversial since it is not consistent across studies [3, 390]. An additional problem is the fact that these are highly sophisticated neurocognitive functions and depend on the intact performance of lower ones; for example, the recognition of facial affect requires a compilation of attentional, executive, and emotional abilities. Thus, it is difficult to determine the exact location of the impairment identified within these mechanisms [398].

In this frame it is interesting that it has been found not only that the impairment in ToM is independent of other neurocognitive dysfunctions [57, 390] but also that this impairment is unrelated to a history of psychotic symptoms [225]. Of course there are data supporting the opposite opinion as well, which seems more reasonable. Even in euthymic BD, executive dysfunction and some other neurocognitive impairments like basic emotion recognition might be at least partially responsible for the impairment in ToM and social cognition tests [49].

A number of factors have been identified as contributing to or confounding the deficit. Low level of education and family history of BD might predict this impairment [3]. Moreover, the perception of emotion may be affected by the use of psychotropic medication and particularly by the use of benzodiazepines and both use and nonuse of antidepressants. In healthy subjects, benzodiazepines impair the recognition of anger, citalopram and reboxetine reduce the perception of negative expressions, and propranolol increases the reaction time to recognize sadness [3, 378].

Conclusively, although the available data suffer from significant methodological drawbacks, the literature suggests that there is an impairment in ToM in patients with BD [51]. Additionally, the theory that the deficit in emotional recognition occurs due to an impairment located in the right hemisphere does not seem to be strong or sufficient [37] and any neurobiological dysfunction is likely to be state dependent [293].

27.4 Clinical Correlates of Neurocognitive Deficits in Bipolar Disorder

Neuropsychological dysfunction in BD may also be related to the clinical symptom pattern and severity, and it has been correlated with age, earlier age at onset, and medication status, as well as with idiosyncratic factors affecting the long-term course.

27.4.1 The Effect of Medication

Medication constitutes an important confounding variable when comparing the different phases of

BD. Some acutely ill patients might be medication-free during testing; however, this is not the case with patients in remission. As a result, medication status not only constitutes a confounding variable which is difficult to control for but also might introduce a bias toward the detection of a deficit, especially in patients in remission. On the other hand, however, patients with severe mania or severe depression cannot be tested and are rarely off medication. Medication could be a possible reason why patients with BD have poor performance on certain neurocognitive tasks. This is in accord with the traditional concept that BD is considered to belong to the "functional psychoses." According to this approach, the attentional impairment is considered to be the core neurocognitive deficit and the cause of all other deficits in neurocognition.

Overall, medication is considered to be an important factor, especially given also the possible neuroprotective or neurotoxic effect of several agents. For example, while most authors argue that lithium is neuroprotective, there is a possible neurotoxic effect in the long term, even at therapeutic levels, especially when it is prescribed in combination with antipsychotics [131].

It is of prime importance to differentiate the neurocognitive deficit caused by the illness itself and the deficit which could be medication induced. This differentiation determines not only the long-term therapeutic design but also the overall outcome. Moreover, such a differentiation requires a comprehensive assessment, on the basis of knowledge of those neurocognitive domains which are most affected by specific medication agents [149].

Patients under lithium often report that lithium inhibits their productivity and creativity [322]. There is no specific reason for this although it has been shown that lithium reduces unusual associations, and this could be the possible underlying mechanism [322]. On the other hand, however, it is not clear whether this constitutes a true deficit or it reflects a subjective feeling as a consequence of the transition from the manic/hypomanic to the euthymic state. It should be noted that this loss of creativity might be specifically related to lithium and not to divalproex [339].

Additionally it seems that lithium has a negative impact on neurocognition especially on memory and psychomotor functioning [180, 200, 209, 239, 336], but fortunately the insult does not seem to be cumulative [119]. More specifically, it has been found that lithium impairs both mental and motor speed, short-term memory, and verbal or associative fluency, but the impairment is reversible when lithium is withdrawn and reestablishes when lithium is re-administered [148, 209, 323]. Lithium also causes a deficit in the long-term recall (retrieval) without having an effect on attention or on encoding [193, 219, 289, 323, 336]. This impairment might especially concern verbal memory [47, 320]. Fortunately, it has been shown that cognitive complaints do not seem to be significant predictors of discontinuation of lithium treatment [85, 241].

There are limited data which disagree with the suggestion that lithium causes a significant neurocognitive impairment [186]. These data especially argue that lithium has no adverse effect on the reaction time [242] and executive functions [135]. Moreover, a longitudinal study found no evidence for a neurocognitive deterioration over a 6-year period in a sample of BD patients treated with lithium [119].

Overall it seems that the effect size of the neurocognitive deficit related to lithium treatment is small and equal to 0.30 [20]. This is especially true concerning immediate verbal learning and memory (0.24), creativity (0.33), and psychomotor speed (0.62). Delayed verbal memory, visual memory, attention, and executive function might not be affected at all [388].

The data on the possible deleterious effect of antipsychotics and antiepileptics on neurocognition are rare and conflicting [149, 179, 276]. Valproate and carbamazepine might cause an impairment in attention [358]. Topiramate, an agent which is not used in the treatment of BD per se, but is often administered in patients in order to treat a comorbid substance abuse disorder or to lose weight, impairs verbal memory and attention, causes psychomotor slowing, and impairs word-finding even at very low dosages (25–50 mg/day). This deficit is reversible after discontinuation of the drug [148, 303].

In general, neuroleptics cause impairment in sustained attention and in visuomotor speed [206]. Moreover, even after controlling for clinical features, current antipsychotic treatment is related to worse performance across all executive function tests as well as in verbal learning and recognition memory and in semantic fluency in BD patients [7, 132, 190]. One study did not find any adverse effects concerning risperidone [288], While another one, suggested that years of exposure to antipsychotic medication, was related to the impairment in executive functions [402]. It is unclear whether this deficit constitutes a true medication adverse effect or it is the consequence of the manifestation of psychotic symptoms, for the treatment of which, antipsychotics were prescribed.

Overall, it has been shown that medications have a limited adverse effect on neurocognitive function [240] if any at all [155, 237, 294]. On the contrary there seems to be a close relationship between poor treatment adherence and neurocognitive impairment, but the causal inferences of these findings are uncertain. It is unclear whether it is the poor treatment adherence which leads to a worse neurocognitive performance through worsening of the overall course of BD, or, on the contrary, it is the neurocognitive impairment which causes poor treatment adherence and reflects a more severe form of the illness [249].

It is known that patients with BD are often treated with benzodiazepines which interfere with memory [337]. Also, some patients are treated with complex combinations of lithium, antipsychotics, antiepileptics, antidepressants, and benzodiazepines, and the combinatorial effects of these drugs on neurocognition are a matter of speculation rather than research.

Medication probably causes some degree of neurocognitive deficit particularly in sustained attention and in psychomotor speed [50]. It is difficult to differentiate this impairment from the impairment caused by the illness per se. It has been reported by studies comparing euthymic patients with or without medication that there are little effects of medication on neurocognitive test performance [191, 340]. It has also been mentioned that patients which were assessed during their first episode, thus before any exposure to medication drugs, also showed a neurocognitive impairment that was more or less similar to the impairment observed in chronically medicated patients [271]. However, there is one study which reported that medicated BD-II patients performed worse in sustained attention than unmedicated BD-II patients [179].

In those individuals with full inter-episode remission doing well off medication, this adverse effect might be obvious; however, it is also well known that staying off medication will adversely affect the overall course of the illness. It is unfortunate that no currently known pharmacotherapy improves neurocognition in BD substantially. Preliminary findings suggest some potential value for adjunctive stimulants such as modafinil and novel experimental agents [149].

27.4.2 The Effect of Psychotic Symptoms

It has been shown that the presence of psychotic symptoms is strongly related to a worse overall neurocognitive performance [4, 47, 92, 144, 145, 147, 225, 233, 236, 250, 311, 319, 324, 327, 346, 402], and in psychotic BD patients, various aspects of the neurocognitive impairment are similar in magnitude to those observed in patients with schizophrenia [92, 176, 261]. Some negative studies exist as well [33, 52, 225, 319], and they suggest that the deficit in BD is overall less pronounced in comparison with schizophrenia. However, in several neurocognitive domains like working memory and executive function, the deficit is similar to that seen in schizophrenia although as a general profile the neurocognitive deficit in psychotic BD patients is similar to that seen in unipolar psychotic depression [52].

A meta-analysis reported that patients with BD perform better than patients with schizophrenia, and the effect sizes of the difference varied between 0.26 and 0.63 for IQ, mental speed, verbal working memory, immediate visual memory, verbal fluency (with the largest effect size equal to 0.63), executive control, and concept formation, but without any difference concerning the

rest of domains [213]. It is important to note that there were only quantitative, and not qualitative, differences. Significant heterogeneity in effect sizes was present between studies, and this was partially due to the methodological issues and the size and clinical characteristics of the study samples [92].

Even in the absence of current psychotic symptoms, a history of psychotic features is also strongly related to a worse neurocognitive performance [97, 327], especially concerning measures of executive functioning and verbal and spatial working memory [145, 250]. However, it does not seem to exist a complete categorical distinction between psychotic and nonpsychotic BD since this effect is modest [53]. The presence of family history correlates with a worse visuomotor attention in psychotic BD patients [348]; however, this load is less severe in comparison with schizophrenia since the offspring of mothers with BD have less neurocognitive impairment in comparison to the offspring of mothers with schizophrenia [314]. Probably depending on the study sample (proportion of BD-I and BD-II patients) and the definition of psychosis, the results vary and are suggestive of a nosological continuum between psychotic and nonpsychotic bipolar cases. It is interesting that such a history of psychotic symptoms is inversely related to the neurocognitive function in the patients' relatives [184].

Another meta-analysis reported that between patients with and without a history of psychotic symptoms, there were no differences concerning attention and visual memory. The observed differences in global IQ, mental speed, working memory, planning and reasoning, and executive functions are small, and only after excluding one outlier study [225] the effect size in the executive functions increases to 0.55 [53].

It is possible that the neurocognitive differences between psychotic and nonpsychotic BD patients are in fact the result of an earlier onset of illness or medication use rather than a result of psychosis per se. In accord with this, one meta-analysis of cases suggested that psychotic BD patients in the above studies had a younger age of illness onset and more hospital admissions, and a larger proportion of them were using antipsychotics. Also they had lower education [53].

Schizoaffective patients perform poorer in a global way, maybe because current psychotic symptoms or history of psychosis is related to more severe neurocognitive impairment no matter the specific diagnosis [327, 346, 362]. Euthymic and stabilized schizoaffective patients are reported to perform worse than BD patients in attention, concentration, declarative memory, executive function, and perceptuomotor function [341, 362].

27.4.3 The Effect of Mood Symptoms

Data are not available for very severely manic or depressed patients because most of these patients cannot be tested. Additionally, extrapolating conclusions on these patients from the study of less severe cases is problematic.

The overall severity of mood symptoms might not affect memory performance at least in those patients whose neurocognitive functions can be assessed [35]. There are studies showing no effect of either acute phase on the neurocognition of patients with BD [63, 74]. However, the bulk of the literature suggests that acute mania is associated to impulse control [301] and executive function impairments [112]. Acute bipolar depression is related with an attentional bias [179] with lowering of mental speed and impaired attention in general [236, 371]. There is also a verbal fluency [74], verbal recall, and fine motor skills deficit [243]. It has been found impaired performance on theory of mind tests in both acutely depressed and manic patients, even when memory was controlled for [197]. The fluctuations in both manic and depressive symptoms have an appreciable impact on neurocognitive functioning [223]. While moderate changes in affective symptoms did not covary with neurocognitive ability [103] when changes are more severe or when rapid-cycling emerges, there is neurocognitive dysfunction [264].

It has been shown by a number of research studies and meta-analyses that in many domains there is a severe neurocognitive deficit even

during remission [20, 50, 52, 92, 189], although there are also studies which keep some reservations [159, 197]. One meta-analysis showed that the illness phase had no effect on the short-term memory deficit [152], and some authors indicate that the consequence of antipsychotic therapy alone might be responsible for the impairment observed during the euthymic phase [190].

The data concerning the impairment in executive functions are inconclusive since there are studies suggesting that the deficit is independent from illness phase; however, there are reports indicating that some aspects of it are related to affective lability [8], duration of illness, residual mood symptoms, current antipsychotic treatment [132], and history of psychosis [145]. A relationship between the impairment in executive function with the severity of general psychopathology as it is measured by the PANSS [248] and with impaired insight has been reported [106]. Finally, differences in the neurocognitive performance could be the result of differences in somatic comorbidity (and co-medication) between BD patients and normal controls [272].

An important methodological problem is that the definition criteria for euthymia differ among studies, and as a result, conclusions are suspect. Maybe even subsyndromal conditions might affect verbal memory [154], and also the presence of residual mood symptoms, regardless of polarity, might have a negative impact, on measures of attentional interference [132]. The impairment in ideation fluency is associated with residual mania, but not depression [186]. Our picture concerning the presence of the neurocognitive deficit during euthymia might change after controlling for residual symptoms in stabilized euthymic patients [81, 121, 355].

One study reported that apart from current symptomatology, the number of previous hospitalizations and family history of mood disorder are associated with the impairment in memory in patients with BD [36]. Particularly, the impairment in verbal memory might relate to the presence of subsyndromal mood symptoms, the duration of illness, and the numbers of previous manic episodes, suicide attempts, and hospitalizations [69, 154, 252].

The first follow-up study found that from patients without any neurocognitive impairment at first episode, one third had significant deficits after 5–7 years [105]. The overall picture could be more complex since during the first episode of the illness, in nonpsychotic patients, there might be no neurocognitive deficits at all, while patients with psychotic features manifest a deficit comparable to that seen in patients with schizophrenia [4]. After the first episode, the time to recover was associated with executive function and possibly with verbal fluency [160].

A meta-analysis confirmed that neurocognitive impairment is present during all phases of BD. That meta-analysis calculated the respected effect sizes for specific neurocognitive domains separately for each phase. During the acute manic/mixed states, these effect sizes showed a clear impairment in attention (0.79–0.90), verbal learning (1.43) and delayed free verbal recall (1.05), letter fluency (0.51) and semantic fluency (0.59), general executive function (0.72), and speeded set shifting (0.64). During acute bipolar depression, there were impairments in attention (0.80), verbal memory (1.20), phonemic fluency (0.93), and executive function in speeded set shifting (0.64). During the euthymic phase, these effect sizes showed a clear impairment in auditory (0.41) and sustained visual vigilance (0.69) and speeded visual scanning (0 .65), working memory (0.65), verbal learning (0.81) and long-delay verbal free recall (0.78), executive functions concerning problem-solving tasks (0.54), verbal interference (0.75) and set-switching tasks (0.73), immediate nonverbal memory (0.73), delayed nonverbal recall (0.80), visuospatial function (0.55), phonemic (0.51) and semantic (0.75) verbal fluency, and finally in psychomotor speed (0.66) [223]. Overall these results suggested that patients in a manic or depressed state had significantly greater effect size impairment in verbal learning than patients in a euthymic state.

The overall evidence suggests that the observed neurocognitive deficit in BD patients is not secondary and does not constitute a by-product of mood symptomatology or of exposure to medication. This is in spite of the observed

strong relationship between mood symptoms and neurocognitive impairment. The most probable explanation is that neurocognitive impairment reflects a deeper neurobiological dysfunction which probably includes the presence of premorbid developmental abnormalities [310].

27.4.4 The Effect of Age and Age at Onset and Personal Psychiatric History

It has been well established that the overall progression of the illness causes and worsens the neurocognitive deterioration. The progression is a concept that cannot be easily defined and operationalized; however, there are a number of factors and indices which can be used to conceptualize it. These factors include the age at onset, the duration of illness, and the number of previous episodes. A general belief is that the neurocognitive function is strongly associated with the severity of the disease [394].

27.4.4.1 Age

The age of the study sample seems to play an important role, since young patients with BD have better performance compared to young unipolar patients, but the reverse is true in the elderly [66]. Overall, age seems to play a complex role. It has been suggested by one meta-analysis that there is significant impairment of neurocognitive function with advancing age [20], while a second one reported that the difference between patients with BD and healthy controls attenuates with age, and probably this happens because the neurocognitive performance of healthy people deteriorates with age, at a rate which seems to be faster in comparison to what is observed in patients with BD [247], thus leading to a floor effect. A similar phenomenon has been observed in schizophrenia [128–130]. Another meta-analysis reported no effect for age [52].

27.4.4.2 Age at Onset

The severity of the neurocognitive is correlated with age at onset [47, 275], especially in psychomotor speed and in verbal memory [50]. The early onset of illness and particularly the onset during childhood or adolescence are associated with more severe impairment [189]. In pediatric BD patients, the observed neurocognitive deficit is similar to the deficit seen in adult BD patients [109]. Especially the impairment in attention is correlated with the age at onset [10].

27.4.4.3 Personal Illness History

During the course of the illness, the number of episodes [102], the number of prior hospitalizations [36, 97, 102, 353], and the longer duration [102] were associated with a worse neurocognitive function. Illness duration is related to the loss of inhibitory control [132] and to a general memory deficit [35] and verbal memory [252]. Also worse neurocognitive function might be associated with the number of episodes but not with the duration of the illness [97, 200]. The impairment in attention correlates with the age of first hospitalization and with the duration of the illness [10].

Some studies found that any history of mood disorder has an adverse effect on neurocognition and especially on memory [35, 36]. The number of past manic episodes, hospitalizations, and suicide attempts was correlated with a more severe neurocognitive deficit [252]. It has been suggested that manic episodes were correlated with impairment in verbal learning and memory [69] and in attention and executive function [237], while the number of past depressive episodes was reported to have adverse effect on verbal memory [126] and reaction times [242].

The reverse explanation has also been suggested, with the neurocognitive deterioration being the cause rather than the effect of worse course and outcome [256]. The possibility that neurocognitive differences between psychotic and nonpsychotic BD patients are in fact the result of an earlier onset of illness or current medication use rather than a result of psychosis per se cannot be excluded. In line with this, it has been reported that psychotic BD patients had more hospital admissions and a younger age at illness onset and a larger proportion of them were using antipsychotics. They also had less years of education [53], which is something that should be taken into consideration since it has been suggested that education plays a significant moderate role [223]. Finally, two meta-analyses

suggested no significant effect for age at onset and duration or severity of illness, as defined by the number of episodes [50, 52].

27.4.5 The Role of Other Clinical Factors

There are many other clinical factors which could influence neurocognitive performance of patients with BD. These include brain white matter lesions that are sometimes found in remitted BD patients and rarely in patients with schizophrenia but apparently do not underlie neurocognitive deficits per se [214], or lifetime comorbid alcohol use disorder which also does not seem to correlate with neurocognitive performance at least at earlier stages [371]. One study found that overweight and obese BD patients might have worse performance on verbal fluency [396].

Education could constitute an additional confounding variable, since patients with BD have lower educational level despite their IQ level which is comparable with that of controls. Thus controlling for it might attenuate the magnitude of the observed neurocognitive impairment [146]. It has been suggested that the neurocognitive impairment effect sizes seem to decrease as a function of education [20, 247] and shorter duration of education is related to a more pronounced deficit in different domains like letter fluency, WCST categories, and the Stroop test [52]. The explanation might include two arms. The first concerns the possibility that education is a marker related to the onset and severity of illness, since early and severe illness interferes with educational attainment, and the second concerns the possibility that education is a protective factor perse.

27.5 Research Concerning Cognitive Deficits in BD-II

In spite of the research efforts during the last few decades, there has not been found a specific neurocognitive profile for the different bipolar subtypes [332]. This is probably because research on BD-II patients is rare, and there are large methodological differences between studies. As a result, inconclusive data are in place. Research on the other subtypes of the bipolar spectrum is essentially lacking. There is only one study reporting data on "bipolar spectrum" patients. That paper suggested the presence of a broad neurocognitive impairment, particularly affecting verbal memory and the executive functions [329].

Some unsystematic reports in the literature suggest that BD-II patients have similar performance to controls [104, 312, 352], but others supported the notion that BD-II patients perform in-between healthy controls and BD-I patients [11, 104, 110, 181, 254, 328, 363, 393], but this maybe specific concerning verbal memory [252, 363] and executive functions [363]. On the contrary there are data suggesting that BD-II patients perform similar to BD-I [73, 110, 164, 254] or even perform worse than BD-I patients, at least in some specific neurocognitive domains, including reaction time and inhibition [168, 342].

The confusing literature cannot answer the question whether there is any qualitative difference between bipolar subtypes. Such a difference would suggest (although that would not be mandatory) that possibly there are different neurobiological mechanisms which underlie BD subtypes. However, both questions (concerning a quantitative and a qualitative difference) remain unanswered. For both, there are data in favor and against.

A global neurocognitive impairment might be present in BD-II patients, with only phonemic verbal fluency being preserved and with moderate to strong effect sizes ranging between 0.62 and 1.34 [11]. For premorbid IQ, there is only one study which has not found any differences between BD-I and BD-II patients, since both groups had worse performance in comparison to the performance of controls [110]. It has been also shown that the intellectual decline was less pronounced in patients with BD-II in comparison to those with BD-I [62, 342]. There are several studies suggesting an impairment in psychomotor speed and in attention [11, 110, 168, 179, 181, 363]; however, other studies did not support this [312, 328]. One study suggested that reaction time was similar to that of controls [41]. Moreover, another study showed that attention

was intact, but psychomotor speed was impaired [393]. As far as memory is concerned, there seems to be a deficit in verbal memory and verbal learning [11, 252, 342, 363, 393], but some studies disagree [110, 168, 181, 312, 328]. Other authors found that in BD-II patients, there is a presence of a less disorganized semantic system in comparison to patients with BD-I [73, 164]. Additionally delayed memory is rather intact [110]. There are controversial data concerning the deficit in visual memory, since some authors reported an impairment [11, 110, 342] while others disagreed [168, 179, 181, 312, 352]. Similarly, some authors supported the presence of impairment in executive functions and in working memory [11, 110, 168, 252, 328, 342, 363, 393], while others did not [312, 352]. Additionally, there is one study which showed that there is a deficit only in working memory but not in executive functions [181]. It has been observed that all the studies using the Stroop color-word test, which assesses interference, showed a deficit in inhibitory control in BD-II patients [11, 328, 342, 363]. Also, there might be a deficit in the emotional processing domain [179, 342]; however, the emotion recognition seems to be intact [104]. It has been also found that unmedicated depressed BD-II patients had intact decision-making performance [352]. On the other hand, one study suggested that medicated BD-II patients had worse performance in comparison to unmedicated BD-II patients in sustained attention [179].

One meta-analysis suggested that there is no difference between BD-II and healthy controls neither in the estimated current intelligence quotient (IQ) nor the premorbid IQ [332, 333]. Another meta-analyses found that neurocognitive impairment in BD-II patients is as severe as in BD-I patients except for the domains of semantic fluency and memory [54]. There are contradictory data concerning psychomotor speed and verbal and visual memory, and the impairment in these domains is probably small in magnitude. On the contrary there are robust data concerning the presence of a working memory deficit and a decrease of cognitive flexibility and impaired inhibitory control in BD-II. Patients with BD-II manifest a deficit also in recognizing emotions [333]. In quantitative terms, BD-II does not differ much from BD-I [21] although it seems that the opinion which prevails is that BD-II patients manifest better performance in comparison to BD-I but worse than healthy controls and are positioned in between these two groups.

27.6 Long-Term Development of the Neurocognitive Deficit in Bipolar Disorders

27.6.1 Methodological Issues

It is difficult to chart the long-term course of BD since there are no reliable indices to describe and chart the course in a global way. This happens because BD is a complex illness with different phases and clinical characters. Moreover there is no clear direction of causality. One possibility is that the accumulation of mood episodes impacts neurocognitive function negatively; however, the reverse is equally possible. A third possibility is that both the neurocognitive deficit and mood symptoms, independently from each other, reflect a specific pattern of clinical course and disease phenotype without any direct relationship to each other.

27.6.2 Premorbid Period

Patients with BD have a relatively intact neurocognitive functioning throughout childhood and adolescence, and the neurocognitive impairment emerges only after the overt symptom onset, and this is in contrast to what is known concerning patients with schizophrenia [234]. In accord with this, it has been found that children who later develop BD exhibit good academic functioning prior to illness onset [224, 284, 287, 368, 370].

However, some studies argue for the opposite. A prospective investigation of executive functioning in at-risk adolescents showed an impairment in executive function in those who later developed BD [265]. Also an increased prevalence of abnormal developmental history has been shown with delayed language acquisition

and motor and social development in a group of adolescents with BD [325]. A large Finnish cohort study which evaluated verbal, arithmetic, and visuospatial reasoning in healthy male conscripts (mean age 19.9 years) showed that premorbid visuospatial impairment was associated with later development of both BD and schizophrenia [360]. Also, another prospective study from Sweden suggested that 7% of 56 adolescents with developmental deficits at the age of 6 years went on to develop BD, compared with none from the control group [175].

27.6.3 Early Stages of BD

The neurocognitive maturation and the development of the child is probably adversely affected by the development of BD during childhood [280]. Generally, the overall neuropsychological deficit has been associated with earlier age at onset which however is unclear to which extent it represents simply a longer duration [275]. Immediately following illness onset, adolescents with BD exhibit poor performance in social and neurocognitive domains [284]. Similarly, in adults, the impairment is present already during the first mood episode [271]. At illness onset, there is a deficit in several domains including sustained attention [81], spatial/nonverbal reasoning, learning and recall, and several aspects of executive function [366] as well as memory, verbal fluency, and executive function [31].

27.6.4 Medium Stages of BD

Patients with two or more illness episodes manifest poorer performance than patients with just one episode [200], and within 1–3 years, a significant further deterioration in the executive function can be observed [364] which, along with processing speed, is considered to be the main long-term neurocognitive deficit in BD [270]. One study found that the rest of neurocognitive functions seem to be stable at 1 to 3-year follow-up [27, 364]. However, the complete picture of the results is controversial concerning the short-term deterioration [23, 121, 178, 353, 374, 379].

Latter in the course of the illness, repeated acute episodes negatively impact the neurocognitive functioning [234] which seems to correlate with both the number of affective episodes and the overall duration of illness [69, 81, 102, 116, 230, 402]. In turn, duration of illness and disease course are reported to correlate with verbal and visual memory and executive function [291, 305, 402]. The comparison of young, elderly, and chronic BD patients revealed that a greater number of chronic patients scored in the severely impaired range on a memory and executive battery than their counterparts with fewer past episodes [264]. Thus, it is possible that instead of reflecting long-term damage to the brain because of repeated acute episodes, poor neurocognitive performance in multi-episode patients may be the result of the presence of chronic residual mood symptoms. It is known that BD patients experience mood liability [220, 369] and residual symptoms, usually depressive, during periods of euthymia in spite of the fact that they were rated as euthymic by clinicians [316].

27.6.5 Later Stages of BD

Complex comorbidity is frequent in chronic patients, and it is often accompanied with incomplete remission. Thus, the length of BD could act as a confounding factor in patients with comorbidity, e.g., with alcohol dependence in comparison to a nonalcoholic group [374]. A weak point in the literature is that although the acute effects of alcohol or drug intoxication have been controlled for in some studies [23, 214, 373], the effect of past exposures has not been taken into account.

Continuous medication is also a confounding variable especially at later stages and when chronicity and complex comorbidity is in place. For example, although one study reported that there has not been observed further decline of neurocognitive function in patients under long-term lithium treatment [119], another study suggested

that the executive function deficit was negatively correlated with years of exposure to antipsychotic drugs [402]. This latter finding could reflect either a toxic effect of long-term antipsychotic medication, a toxic effect of chronic psychosis, or both.

In the long term, neurocognitive deficit seems to be associated with functional outcome. This relationship is particularly true for processing speed, attention, memory [38], as well as the visual/motor processing domain [349]. One study used cross-sectional data from a large case-register study of 14,000 people hospitalized for mood disorder, 81,380 patients with osteoarthritis, and 69,149 patients with diabetes. The results indicated that patients with BD have a 6% increase in the risk of dementia with every episode leading to admission, and overall this risk was higher in comparison to the two control groups [201, 202]. Although these data are in favor of a neurodegenerative process, it is also possible that the findings reflect different courses of the illness plus a different probability of long-term medication treatment and electroconvulsive therapy (ECT) with unknown long-term effects [277]. This effect has already been discussed since patients with more severe and frequent affective episodes perform more poorly on neurocognitive testing [102].

In accord with this, a course characterized by chronicity and residual symptoms with lack of remission between episodes is related to a progressive neurocognitive deficit, and in this frame, an important role is attributed to the specific clinical course of BD [102, 264]. As previously mentioned, there is a deleterious effect of psychotic symptoms [234], and antipsychotic medication is associated with poorer performance in IQ, memory, and working memory assessments [114].

The overall longitudinal course suggests that neurodevelopmental factors play a minor role in the emergence of neuropsychological dysfunction in BD [234, 310]. Psychopathological factors during the course of the disorder itself are probably related to the neurocognitive impairment, but the nature of this association remains unknown.

27.7 Awareness of the Neurocognitive Deficit in Bipolar Patients

Although many patients with BD frequently complain about neurocognitive deficits in attention, concentration, and memory, there are limited data on the relationship between subjective cognitive complaints with objective neuropsychological deficits.

The patients' subjective cognitive complaints do not seem to correlate or predict objective neuropsychological deficits [63, 71, 372]. One study has suggested that there might be a weak correlation between subjective complaints and deficits in attention, memory, and executive function [251]. Moreover, it has been shown that neither mood symptoms nor the severity of mania or depression correlates with patients' self-report [63]. One study reported that patients with BD might show more subjective complaints when there is a higher number of episodes and especially a higher number of mixed episodes, a longer duration of the illness, and when the onset of the illness occurred at an earlier age [251]. There seems to be some association between depressive symptoms and self-report neurocognitive complaints; however, the association between complaints and objective neurocognitive functioning is not moderated by mood symptoms [372].

27.8 Gender and the Neurocognitive Deficit

There is limited literature concerning the association of gender with neurocognitive deficits in bipolar disorder, and no conclusions can be derived. Males with BD manifest a more severe deficit in immediate memory [68] and executive function [30], while one study suggested that gender does not play any role on emotion recognition [104]. A better memory performance might be associated with female gender [35], while another study suggested that the verbal memory deficit might associate with female gender as an endophenotype [203]. Overall the data are conflicting with one out of three recent

meta-analyses being in favor of a gender effect [20], while the two others against it [52, 247].

27.9 The Neurocognitive Deficit as an Endophenotype for BD

Endophenotype constitutes a stable pattern of behavioral symptoms with a clear genetic connection. It can be used to bridge the gap between high-level symptom presentation and low-level genetic variability. Ideally, an endophenotype might be useful in the differential diagnosis between disorders when they present with similar symptoms. Additionally, an endophenotype is associated with a specific disorder in the population and should be heritable and state independent. It is expected that endophenotype and the specific diagnosis co-segregate within families, and the endophenotype is found in non-affected family members at a higher rate than in the general population.

The neurocognitive impairment in patients with BD is at least partially different in comparison to the deficit seen in patients with schizophrenia, and it is also at least partially state independent and definitely present during periods of complete recovery and euthymia, although the literature so far does not support the use of global measures of neurocognition as endophenotypes [65, 185, 216]. On the other hand, however, specific neurocognitive deficits might be relevant, although further research is needed. It has been reported that unaffected relatives of patients occupy an intermediate position between patients and healthy controls concerning specific neurocognitive functions [134].

27.9.1 Twin Studies

There is not much data concerning discordant monozygotic or dizygotic twins with respect to neurocognitive deficits or endophenotypes in bipolar disorder. Healthy co-twins' performance in aspects of concentration, verbal and visual memory, and verbal recognition and executive function is worse than controls [156], although a population-based study reported that the verbal memory deficit as an endophenotype might relate to female gender alone [203]. It has been also reported that neither BD patients nor their unaffected co-twins differed from control subjects in spatial working memory [283]. Another population-based study on healthy monozygotic and dizygotic twins showed that monozygotic high-risk twins manifested a significant deficit on selective and sustained attention, executive function, language processing, and working and declarative memory, while the dizygotic high-risk twins manifested lower scores only on language processing and episodic memory [77]. No deficit was found on response inhibition in discordant twins of BD patients (even with a psychotic or familial form). Lower performance in twins during testing could be explained by current depressive symptoms [215]. It is interesting that in most of these studies, the unaffected co-twins suffered from minor or subthreshold mental disorders and dysthymia. Overall, twin studies provide some but not strong evidence for the presence of an endophenotype on the basis of neurocognitive impairment.

27.9.2 Studies on First-Degree Relatives

There is a limited number of studies in children whose parents suffer from BD. They suggest that high-risk children differ from control children in terms of reaction time; however, the quality of data is rather low [389]. The same research group also reported that both high-risk and control children have similar performance in speech competence tests [173]. In two other studies, high-risk children showed impaired PQ but not VQ; however, half of them were suffering from depression [98, 218]. Another study found that high-risk children and controls had similar IQ; however, controls had better performance in some reading and arithmetic cognitive tasks, and there was an increased rate of VQ-PQ discrepancy [260]. A study on visual backward masking was also negative [242]. High-risk relatives had slower reaction time on a sustained auditory attention task

[282]. Moreover, it has been shown by a recent study that specific executive functions which are supposedly located in the ventral frontal cortex might be impaired in high-risk children in comparison to controls [133]. Also there might be a deficit in healthy high-risk children in spatial memory, in attention, and in executive functions, even after correction for multiple confounding factors [207]. Overall these studies provide evidence for the usefulness of executive function impairment as an endophenotype for BD.

27.9.3 Studies on Mixed Samples of Relatives

There are more data concerning families of BD patients. These studies utilized mixed samples of first- and second-degree patients.

One study which utilized only female relatives of patients (most of them psychotic) reported that their IQ was superior to the IQ of controls. Also it argued in favor of the usefulness of visual memory and executive function as endophenotypes [216]. Another study indicated that IQ was similar to controls [142], while on the contrary, another one reported the presence of a general and nonspecific deficit in IQ, with some pronounced effect on verbal fluency [263]. More recent data suggest a higher discrepancy between VQ and PQ in families of patients with BD in comparison to healthy controls; however, these results were inconsistent with previous findings of higher VQ in comparison to PQ [368].

A deficit in psychomotor speed is also reported [16–18, 399], and while studies suggested an impairment in concentration [120], others have found that the impairment concerns shifting, but not sustaining attention (concentration) [82, 83, 315]. One study found a deficit in delayed recall in well-educated siblings with high IQ [196]. The literature also suggests the presence of an impairment in concentration, in the visuospatial declarative memory [120]; in verbal working memory [48]; in verbal [16–18, 78], visual [134], and auditory verbal learning [222]; in working memory [143]; and in executive function [16–18, 48, 347, 399].

There is one report on increased emotional interference with a bias toward mood-related information [42]. At-risk youths make more errors when identifying facial emotions, and the magnitude of the deficit was similar to that observed in patients with BD [60]; however, another study reported no deficit either in BD patients or in their first-degree relatives [344].

It is interesting that in spite of an overall normal neurocognitive functioning, there might be some kind of subtle impairment present in first-degree relatives of BD patients. A study which utilized L-tryptophan challenge showed that under challenge, a deficit emerges in relatives of BD patients in comparison to controls in memory, focused and divided attention, and psychomotor performance. Additionally, the relatives of BD-I patients performed worse than the relatives of BD-II patients [330, 331].

27.9.4 Reviews and Meta-analyses Regarding Neurocognitive Endophenotypes in Bipolar Disorder

Overall, the literature supports the idea that the deficit in executive function could constitute an endophenotype for BD, especially concerning psychotic cases. However, there are some data which dispute the usefulness of specific executive functions, including cognitive control during episodic memory retrieval [78], response inhibition [215], and cognitive set shifting [315]. Also, while a widespread deficit in attention and memory in families of patients with BD is reported, there are some negative data concerning concentration [82], psychomotor performance [48, 347], working memory [315], spatial working memory [283], verbal learning [18], verbal memory [48], and executive functions [347]. It is almost certain that IQ cannot serve as an endophenotype of the illness.

Reviews and meta-analyses suggest that executive function and verbal memory deficit could serve as core endophenotypes for BD [20, 26, 291], but conclusions are premature. The effect sizes reported are 0.49 with the Stroop test and

0.37 with the TMT-B, while concerning immediate verbal memory the effect size is 0.42. Thus, in first-degree relatives, effect sizes were small ($d < 0.5$), but significantly different from healthy controls in particular concerning executive function and verbal memory [20].

In line with the above, another meta-analysis reported small effect sizes, that is, 0.20 for current IQ, 0.18–0.36 for attention and 0.17–0.22 for mental speed, 0.13–0.33 for various aspects of memory, 0.27 for verbal fluency, and 0.24–0.51 for executive functions with the effect for the Stroop test being the highest (0.51). This meta-analytic study demonstrated that impaired response inhibition might constitute the most prominent neurocognitive endophenotype of BD. Another executive measure, set shifting (TMT-B, 0.38; WCST perseveration, 0.36), and two other neurocognitive domains, verbal memory (0.27–0.33) and sustained attention (0.36), also met the criteria as potential endophenotypes of BD. These results suggest that the response inhibition deficit, a potential marker of ventral prefrontal dysfunction, seems to be the most prominent endophenotype of BD [50].

Conclusion

A wide range of studies suggest neurocognitive alterations and deficits in bipolar disorders and also to a lesser degree in unaffected relatives indicating neurobiological and also possibly genetic bases of these alterations and dysfunctions. Understanding of the affected domains and also their biological background would help to outline a complex picture of bipolar disorder and deepen our understanding concerning its neuroanatomical, neurochemical and also genetic underpinnings and would also help us identify endophenotypes which would play a key role not only in research but possibly diagnosis, differential diagnosis, and subtyping of bipolar disorder, thus informing us about course, prognosis, and treatment options. Neurocognitive deficits are often residual symptoms lingering between the acute phases and showing a progressive nature with the course of illness and the increasing number of episodes; therefore, understanding and treating these deficits would also be crucial for functional recovery and restoring quality of life.

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Combination Strategies in Treatment-Resistant Depression

28

Francisco López-Muñoz, Cecilio Álamo, and Pilar García-García

28.1 Introduction

Depression is a common psychiatric disorder, whose prevalence ranges, according to different studies, from the 5 % reported by the National Comorbidity Survey [10] to the 17 % reported by the DEPRES study (Depression Research in European Society) [73]. According to the World Health Organization (WHO), some 121 million people are currently suffering from depression, with an annual prevalence of 5.8 % for men and 9.5 % for women [152]. A study carried out in six European countries [33] found that prevalence over the life course was 12.8 % in the case of major depressive disorder (MDD) and 14 % for any depressive disorder (9.5 % in men and 18.2 % in women), whilst Kessler et al. [67] calculated that MDD has a lifetime prevalence of about 17–21 %. However, these figures may vary according to the population studied and the criteria or diagnostic instruments used.

But apart from these high rates of prevalence, depression is a serious illness that substantially affects sufferers' lives and those of their families and with high social, occupational and economic costs [52]. Moreover, it affects the patient's quality of life more intensely than other chronic pathologies [46] and accounts for three times more use of primary care services, on average, than other complaints [10]. Depression occupied fourth place in the ranking of incapacity; by the year 2020, it is expected to be in second place [147]. From the economic point of view, care for the depressed patient is very expensive, the health cost of mental illness in developed countries being estimated at 1 % of gross national product and 10 % of the total healthcare budget [134].

These high figures confirm the seriousness of the problem depression currently represents [74], not only in the healthcare context, but also at a social, employment and family level [129]. A large part of this problem is the consequence not only of its high prevalence, but also of its tendency for chronification and for the frequency of relapse. In this sense, without treatment, individuals with recurrent depression have a high risk of repeated depressive relapses or recurrences throughout their life with rates of relapse or recurrence typically in the range 50–80 % [70, 71]. Furthermore, it is estimated that approximately

F. López-Muñoz (✉)
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal
e-mail: francisco.lopez.munoz@gmail.com; flopez@ucjc.edu

C. Álamo • P. García-García
Department of Biomedical Sciences (Pharmacology Area), Faculty of Medicine and Health Sciences, University of Alcalá, Madrid, Spain

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half of depression patients fail to respond adequately to antidepressant therapy [3, 95, 130]. The present review deals precisely with some of the therapeutic strategies that have been implemented in efforts to solve this problem of nonresponse, though before going on we should define what is understood by treatment-resistant depression.

28.2 Treatment-Resistant Depression

The goal of the treatment of depressive disorders is to achieve full symptom remission. However, according to diverse studies, between 40 and 50 % of depressed outpatients do not respond satisfactorily to treatment with antidepressants in monotherapy, with the adequate dose, regime and duration [78]. Of these patients, between 12 and 15 % show partial response and between 19 and 34 % are considered nonresponders [63], with the result that the depression may acquire chronic proportions after several pharmacological interventions [107]. Indeed, more than 10 % of patients remain depressed despite multiple interventions [95]. In the specific case of selective serotonin uptake inhibitors (SSRI) – the antidepressants most widely used in clinical practice and generally prescribed as first-line treatment, especially in primary care [84], O'Reardon et al. [100] claim that 30 % of depressive patients fail to respond adequately to the initial treatment and that between 60 and 70 % fail to achieve total remission. Consistent with these findings, the report from the STAR*D programme (Sequenced Treatment Alternatives to Relieve Depression) revealed that only about one-third of depressed patients achieved remission with citalopram treatment [142].

Likewise, reviews of the scientific literature confirm that 34 % of patients with MDD involved in double-blind, placebo-controlled clinical trials fail to respond to antidepressant treatment or do so only partially, even at the highest recommended dosage of the therapeutic range, and about 10–15 % have no response (at least a 50 % improvement) [36]. Moreover, the figure for patients failing to achieve remission (absence or near absence of symptoms) is as high as 60–70 %, according to Rush et al. [119].

There are various reasons why a patient fails to respond or responds only partially to a prescribed antidepressant. These include pharmacogenetic variations, problems associated with the pharmacokinetics of the prescribed drug, poor adherence to prescribed treatment and possible interactions with other medication, as well as the patient's general state of health (e.g. thyroid dysfunction) and the presence of other psychiatric illness (anxiety disorder, particularly obsessive-compulsive and post-traumatic stress disorders) or substance abuse [78]. Other sociodemographic correlates include adverse life events, whilst Caucasian ethnicity, female gender and better education have been associated with better response to treatment [142, 150]. Also, we should not rule out misdiagnosis and inappropriate or inadequate treatment [50, 106], factors which constitute the basis of so-called pseudoresistant depression. Recently, Balestri et al. [5] have identified several of clinical predictors of treatment-resistant depression: longer duration and higher severity of the current episode, outpatient status, higher suicidal risk level, higher rate of the first-/second-degree psychiatric antecedents (MDD and others) and side effects during treatments.

For depression characterised by an unsatisfactory therapeutic response, there is general agreement on poor prognosis, since there is a tendency, to a greater extent than in cases of positive response, to chronicity, with more frequent and more severe episodes and with increased risk of suicide [124]. It is in such cases, when an unsatisfactory therapeutic response is obtained, that we speak of treatment-resistant depression (TRD). This type of depression, moreover, generates significantly higher medical costs versus non-TRD. These patients use a disproportionately larger share of healthcare resources, and cost employers more in lost productivity, than patients who do respond to treatment [51].

The term treatment-resistant depression emerged in the scientific literature in the 1960s [114], though terms such as "absence of response", "inadequate response" and "partial response" to antidepressants are also currently in use [35, 57,

116]. Over the past 30 years, numerous definitions of TRD have been proposed [141]. One of the most widely used definitions of TRD in the scientific literature is that based on a situation in which an episode of major depression has not improved after at least two adequate trials of different classes of antidepressants given in appropriate dosages for a period of 6–8 weeks. This definition corresponds to the so-called European Staging Method for TRD [131]. According to this method, after the first trial, patients are classified as nonresponders to the antidepressants. Furthermore, a condition called chronic resistant depression is proposed, defined as a resistant or refractory depressive episode lasting more than 1 year, despite adequate interventions.

Nierenberg and DeCecco [96] suggested that TRD in patients who received adequate treatment could be defined based on any of three criteria: failure to achieve a minimum response (i.e. <25 % decrease from HDRS score), failure to achieve a response (i.e. <50 % decrease from HDRS score) or failure to achieve remission (i.e. final HDRS-17 score <7). In the studies carried out by our group, we considered as TRD patients, following Nierenberg and DeCecco [96], Lam et al. [72] and Keller [63], nonresponders or partial responders, according to the criteria of Hirschfeld et al. [57], to treatment in monotherapy with an antidepressant over a period of 6–8 weeks, the period considered "adequate" for assessing the antidepressant response by the majority of authors [94]. In this context, the depressed patient's response to a pharmacological treatment is defined by a reduction of at least 50 % in the depressive symptoms assessed using a standard instrument, such as the HDRS (Hamilton Depression Rating Scale) or the MADRS (Montgomery-Äsberg Depression Rating Scale) [78]. Likewise, in our studies we defined remission as the absolute value of the HDRS-21 score ≤10 and improved patients as a score <4 points on the Clinical Global Impression scale (CGI-I) [116, 142].

Recently, Mrazek et al. [91] have assessed the burden of TRD in the USA, and they have estimated the annual costs for healthcare and lost productivity in 5,481$ and 4,048$ higher, respectively, for patients with TRD versus treatment-responsive depression. Moreover, TRD may present an annual added societal cost of 29–48$ billion, pushing up the total societal costs of MDD by as much as 106–118$ billion.

28.3 Strategies for Treatment-Resistant Depression

In patients with TRD, it is necessary to implement mechanisms for improving therapeutic results. Thus, in the last decades, a whole series of treatment approaches have been suggested. The scientific evidence currently available that might guide approaches to TRD is still limited. Indeed, no drug therapy has been specifically approved by the US Food and Drug Administration (FDA) for TRD.

The National Institute of Mental Health published the results of an extensive study it had sponsored (STAR*D; Sequenced Treatment Alternatives to Relieve Depression) and which was designed to give an appraisal of sequential treatment strategies after nonresponse (with an initial trial of citalopram) [118]. This study provided prospective data on response and remission rates after randomised treatment allocations in patients who were not in remission after one to four sequential steps of treatment (STAR*D levels I–IV) (Fig. 28.1). In STAR*D, response and remission rates were, respectively, 26.8 % and 21.3 % at level II [120], 15 % and 16.2 % at level III [38] and 17.4 % and 10.1 % at level IV [86]. These lower remission rates were attributed by authors to the inclusion of patients who were more chronically depressed, had lower socioeconomic status and suffered from more comorbid somatic and psychiatric disorders. Moreover, in one of the STAR*D studies, patients who achieved remission after any level were monitored for up to a year [120]. About 50 % of those who exited level 1 with citalopram, 70 % of those who exited level 2 and 80 % of those who exited level 3 relapsed within 12 months.

In dealing with partial- or nonresponders to antidepressant treatment, various alternatives have been proposed (Table 28.1): adjustment of

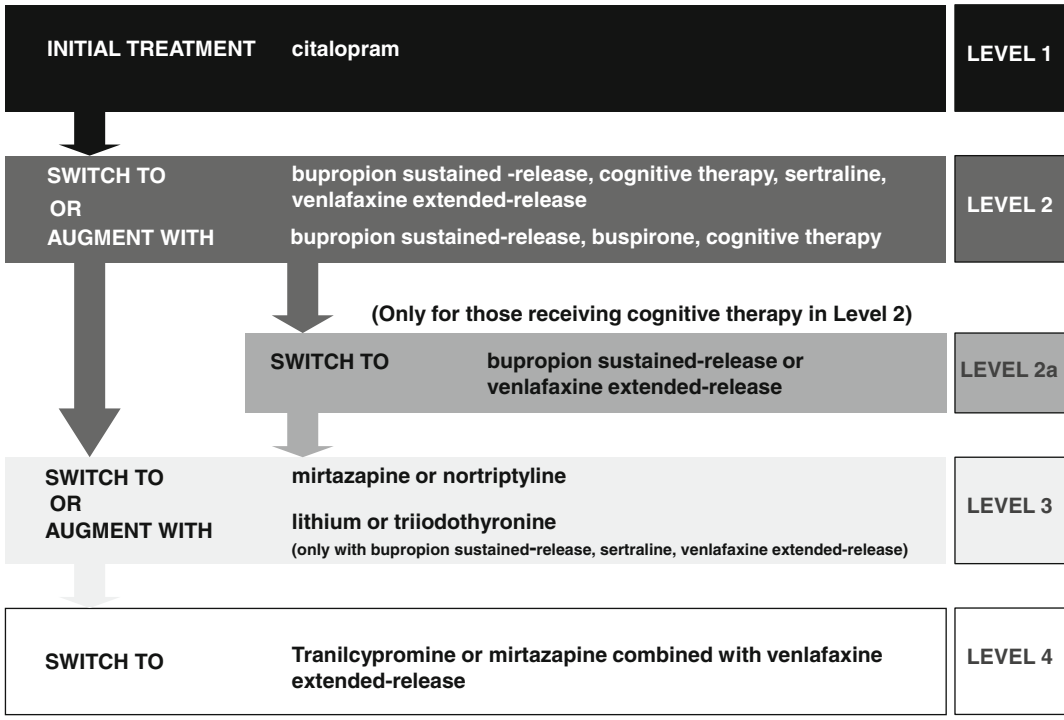

Fig. 28.1 Algorithm of STAR*D project (From Rush et al. [117])

Table 28.1 Therapeutic alternatives in cases of TRD

Re-evaluation of diagnosis
Comorbidity
Personality disorders
Nonpsychiatric cause
Drug interactions
Treatment optimisation
Increase of dose
Increase in treatment time
Monitoring of plasma levels
Pharmacological strategies
Substitution
Change of antidepressant family
Association
Augmentation: addition of a non-antidepressant agent
Combination: addition of another antidepressant agent

antidepressant dosage, which is generally increased (optimization strategy), substitution by another antidepressant agent (switch strategy), application of psychological therapies or the addition of a new drug [78]. With regard to the last of these strategies, there are two possibilities. On the one hand is the strategy called augmentation, consisting in the combination of the original antidepressant with another drug that is not an antidepressant per se, such as lithium salts, thyroid hormones, atypical antipsychotics, buspirone or pindolol [19]. On the other hand is a combination strategy, in which two antidepressant drugs are used, generally from different pharmacological families, with different pharmacodynamic profiles [78, 113]. Confirmation of diagnosis and optimising the dosage of the current medication is often the initial approach to treating patients who do not respond adequately. When this fails, clinicians must choose between other strategies (switch, augmentation or combination). The choice between these alternatives depends on previous experience with the drug and on the clinician's knowledge [78].

Switching antidepressants is a common strategy for TRD in clinical practice, generally through the substitution of an agent from a different fam-

ily of drugs [44]. However, few clinical trials have systematically examined the effectiveness of this procedure. Switching from a TCA to an SSRI is an option that has not been widely covered in the literature, nor has that of switching from one SSRI to another. Nemeroff [94] analysed the literature on switching studies, concluding that switching is effective in achieving a response in approximately 40–60 % of cases. Souery et al. [133] confirmed, in depressed nonresponders to a previous antidepressant treatment, switching to a different class of antidepressants was not associated with a better response or remission rate. Recently, with the end to review evidence supporting pharmacological treatments for TRD, Tundo et al. [146] reviewed original studies, clinical trials, systematic reviews and meta-analyses addressing pharmacological treatment for TRD in adult patients published from 1990 to 2013. The results of this review were switching from a TCA to another TCA provides only a modest advantage (response rate 9–27 %), whilst switching from a SSRI to another SSRI is more advantageous (response rate up to 75 %).

The theoretical rationale of augmentation is to obtain a different neurochemical effect by adding an agent affecting different neurotransmitter systems. Among the drugs used as tools for augmentation are buspirone; pindolol; lithium salts; psychostimulants; thyroid hormones; dexamethasone; yohimbine; atypical antipsychotics, such as olanzapine, risperidone, quetiapine, ziprasidone or aripiprazole; classic anticonvulsants, such as carbamazepine or valproate; new antiepileptic drugs, such as lamotrigine; and dopaminergic agonists, such as bromocriptine, pergolide, pramipexole or amantadine [78, 87]. It should be borne in mind, however, that various side effects, including metabolic syndrome and endocrine system disturbance, make the use of many of these drugs unadvisable in this context.

Classically, of all the drugs used in augmentation, the most consistent and well-documented results have been obtained with lithium salts and thyroid hormones (L-triiodothyronine; T_3). In a meta-analysis on lithium augmentation in TRD, Bauer et al. [7] identified 27 studies with a total of 803 patients. Considering solely placebo-controlled trials, response rates were significantly higher with lithium augmentation (45 %) than with placebo (18 %). However, data from the STAR*D programme revealed that, after 10 weeks of treatment, remission rates with T_3 augmentation ($n=73$; >25 μg/day) were better than with lithium augmentation ($n=69$; 900 mg/day) (25 % vs. 16 %) and that thyroid hormone was significantly better tolerated [98]. The results obtained with atypical antipsychotics are highly promising, and the new antiepileptic drugs also appear promising in this field. Recently, Zhou et al. [153] conducted a comparative analysis of the efficacy, acceptability and tolerability of 11 augmentation agents (aripiprazole, bupropion, buspirone, lamotrigine, lithium, methylphenidate, olanzapine, pindolol, quetiapine, risperidone and thyroid hormone) with each other and with placebo in adult TRD. Their conclusions were that quetiapine and aripiprazole appear to be the most robust evidence-based options for augmentation therapy in patients with TRD, but clinicians should be cautious of potential treatment-related side effects.

Finally, we should mention other types of therapy that have also shown their utility in patients with TRD, such as psychotherapy and physical therapies. With regard to the former, it has been found that the addition to pharmacotherapy of a structured psychotherapy, such as cognitive behaviour therapy, interpersonal therapy or long-term psychoanalytic psychotherapy, improves response rates in resistant or partially responsive depression [13, 42]. Additionally, data from the STAR*D study confirmed that cognitive therapy, either alone or in combination with a failed initial pharmacotherapy, was as effective a strategy as switching antidepressants, though associated with a slower rate of response [139]. With respect to physical or somatic therapies, electroconvulsive therapy (ECT), repetitive transcranial magnetic stimulation (rTMS), vagus nerve stimulation (VNS) and deep brain stimulation (DBS), light-based therapies, exercise, acupuncture and yoga have been studied in TRD patients with positive results [45, 60, 101, 103, 109, 110, 144]. Of these types of somatic therapies, ECT is the best studied. A fuller treatment

of this issue can be found in the review by Kennedy and Giacobbe [64]. The effectiveness and safety of Chinese medicine, like Guipi Decoction (GPD) as an adjunctive in the treatment of depression, have been evaluated by Sheng et al. [128] in a meta-analysis. They showed that GPD formulations were effective in the treatment of depression without causing any serious adverse effects. However, currently available evidence was of low quality and therefore inadequate to justify a strong recommendation of using GPD formulations in the management of depression [128].

Several treatments are currently being investigated as adjunctive treatments for depression. A new drug application (NDA) was recently filed for the use of brexpiprazole [102], a serotonin-dopamine activity modulator that is a partial agonist at 5-HT_{1A} and D_2 receptors and an antagonist at 5-HT_{2A} and alpha1_B/2_C adrenergic receptors [81]. Furthermore, cariprazine, a D_2, D_3 and 5-HT_{1A} receptor partial agonist [68], is currently undergoing clinical testing as adjunctive treatment for MDD [23].

28.4 Combination Strategies in Treatment-Resistant Depression

In general, combination therapy should be considered as an alternative to the switching strategy in partial responders, since it conserves the partial beneficial effects obtained, minimising the demoralising psychological effect of failure for the patient. Bares et al. [6] confirm that combined treatment is more efficacious than switch to monotherapy in the TRD. Moreover, it avoids the appearance of withdrawal symptoms, permitting the use of lower doses of the antidepressants employed; it improves certain symptoms not well dealt with by the first antidepressant and "ameliorates" some of its adverse effects; and it provides the possibility of obtaining a more rapid response than that obtained by the switching strategy [72, 78, 116, 130]. Nevertheless, we must also consider the possibility of drug interactions, as well as an increase in adverse side effects. These concerns are especially serious in the case of TCAs, whose administration should be accompanied by monitoring of plasma levels. Therefore, in nonresponders or partial responders suitable for treatment with two antidepressants in combination, a risk-benefit quotient should be calculated: higher incidence of adverse effects versus a reduction in morbidity and/or mortality due to suicide and the possibility of a "reduced therapeutic response" after repeated treatment failures [3].

Combining antidepressants for patients with TRD was first reported in 1963 by Sargant, with MAOIs and TCAs [123]. But the last years have seen a marked increase in the number of publications on combination treatments, even though the effectiveness of this strategy has not yet been sufficiently demonstrated in many controlled trials [78]. The combination strategies most well documented in the scientific literature up to the mid-2000s are SSRI with TCA, SSRI with mirtazapine and SSRI with bupropion [78].

The combination of SSRIs and TCAs was first reported in 1991 [149] with fluoxetine and desipramine. This TCA is the most widely employed as a combination tool in nonresponders to SSRIs, since it has an eminently noradrenergic pharmacodynamic profile. Indeed, it was recently shown that remission rates were significantly higher with desipramine plus fluoxetine than with either drug alone (54 %, 7 % and 0 %, respectively) [93]. Nevertheless, problems of safety with drug interactions considerably limit the use of this combination, since SSRIs are inhibitors of the same isoenzymes (CYP2D6) that metabolise TCAs, and this may lead to dangerous increases in the plasma concentrations of the tricyclic agents. For its part, the combination of SSRI and mirtazapine is also of great therapeutic interest, given its hypothetical synergic profiles, since mirtazapine, in addition to its α_2-autoreceptor antagonist properties, blocks the 5-HT_2 and 5-HT_3 receptors, enabling a reduction in the typical adverse effects of SSRIs on stimulating these receptors (sexual dysfunctions, nausea, anxiety, etc.) [99]. Moreover, placebo-controlled trials carried out with this combination have revealed significant differences in patients' improvement

(response rates of 45 % vs. 13 %) after 4 weeks [17]. As regards the SSRI-bupropion combination, very widely used in the USA, and excluding the STAR*D programme, all the data available come from open-label studies [154], such as that by DeBattista et al. [29], who obtained a response rate of 55 % 6 weeks after the addition of bupropion SR (150–300 mg/day) in a sample of 28 SSRI-resistant depressed patients.

More anecdotal data also support the use of combinations of venlafaxine with TCAs, with mirtazapine, with bupropion and with SSRIs, whilst the combination of two SSRIs is more controversial; even so, some pilot studies, with small numbers of patients, suggest the efficacy of combinations of citalopram with fluvoxamine and of citalopram with fluoxetine. Data have also been published on the combination of moclobemide with SSRIs or with TCAs, and even of MAOIs with TCAs or with SSRIs [78, 140], despite the potentially dangerous drug interactions that can occur – basically severe serotonergic syndrome [20] or agomelatine with bupropion [136].

Mirtazapine has indeed been used as an additive tool in the case of TRD to other antidepressants, though with very small numbers of patients. In a first open study, Carpenter et al. [16] added mirtazapine to 20 nonresponders for 4 weeks at high doses of SSRIs and other antidepressants. Four weeks later, 11 patients had responded to treatment, 6 did not respond and 3 had dropped out due to adverse effects (weight increase, sedation, fatigue). In a later study, placebo-controlled and randomised [17], patients in the mirtazapine group ($n=11$) showed response and remission rates higher than placebo ($n=15$), though in the case of remission, the differences were not statistically significant. Venlafaxine, an antidepressant with a similar action mechanism to dual-acting TCAs like imipramine, amitriptyline or clomipramine, but which lacks their typical anticholinergic and antiadrenergic effects, has also been used in TRD [28, 97, 122]. There are some published studies on the efficacy of venlafaxine addition in TRD patients, though sample sizes are generally very small. Thus, Gómez-Gómez and Teixido-Perramón [47], in a sample of 11 patients with resistance to TCA, obtained positive response and remission rates of 82 % and 64 %, respectively, after the addition of venlafaxine.

However, there are scarcely any consistent published data on the beneficial effect of adding other antidepressants to the regime of patients resistant to venlafaxine [116, 154]. Also, there are published reports of sporadic cases of positive response to the addition of mirtazapine ($n=3$) [16], bupropion ($n=8$) [65] and SSRIs ($n=4$) [48] to patients with partial response to venlafaxine. Malhi et al. [83] reported response and remission rates of 81.8 % and 27.3 %, respectively, with the combination venlafaxine plus mirtazapine, at 8 weeks, whilst Hannan et al. [53] report a percentage of clinical response at 8 weeks of 50 %.

In general, combination strategies, which constitute an alternative more and more frequently employed at the clinical level, tend to be effective in 50–60 % of cases, though this varies according to the drug employed [78]. The published data from the STAR*D (*Sequenced Treatment Alternatives to Relieve Depression*) programme provided an analysis of the true situation of remission rates with antidepressant treatments and the results obtained with different combination strategies [38, 86, 98, 120, 143]. One of the studies whose results were analysed in the STAR*D project involved a combination trial in a sample of 565 patients with TRD on citalopram, who were given, in addition, delayed-release bupropion [143]. The authors confirmed that bupropion could offer some advantages, such as greater reduction in the number and seriousness of symptoms, with few additional adverse effects. However, remission rates were modestly higher after the addition of sustained-release bupropion (39 %) compared to buspirone (33 %) [121].

Souery et al. [132], basing themselves on the results of the STAR*D studies, conclude that the optimum strategy for dealing with TRD has not yet been identified and stress the need for more clinical trials with a view to identifying the most effective treatment strategies. In this regard, since the mid-2000s, several studies, performed by our group, have been published which suggest the

suitability of the SSRI-reboxetine combination [1, 76, 77, 115, 116, 126], citing highly promising results, attributable to the fact that these antidepressants act on different and complementary monoaminergic action mechanisms.

28.5 The Role of Selective Noradrenaline Reuptake Inhibitor Reboxetine in Treatment-Resistant Depression as Adjunctive Therapy

Reboxetine is the prototype drug of the family of antidepressants that specifically inhibit the reuptake of noradrenaline (NARIs) and selectively increases noradrenergic neurotransmission at a central level (Fig. 28.2), without the potential problems of TCAs, such as desipramine or maprotiline [8, 22, 88, 90]. This selective pharmacodynamic profile explains its efficacy in relation not only to depressed mood but also to lack of energy, anhedonia and lack of interest in work and social relations [56]. This selective noradrenaline reuptake inhibitor has a very low affinity for muscarinic, H_1 histaminergic and α_1-adrenergic receptors [151], so that its use is not associated with the typical adverse side effects of TCA. Moreover, reboxetine does not inhibit or induce important CYP isoenzymes, which is why no clinically relevant drug interactions involving CYP2D6 are anticipated [69]. This is of crucial importance in relation to possible combination strategies in patients with TRD. It should also be borne in mind that desipramine, a well-established tricyclic drug, is thought to exert its primary antidepressant effects through increasing noradrenaline at the neuronal synapse. In addition, desipramine has shown itself to be effective in pharmacological strategies for TRD to SSRIs [37, 93], thanks to its synergic pharmacodynamic activity. Thus, experience with desipramine supports the notion that NARIs may have a role in the treatment of TRD.

In recent years, reboxetine has been one of the drugs most widely used as a combination tool in cases of TRD, especially with SSRIs, the agents commonly used as first-line antidepressant treatment. From the experimental perspective, Harkin et al. [54] showed, in animal models of depression, that the combination of sertraline and reboxetine permits a quicker pharmacological response than each antidepressant alone. On the basis of this, the use of reboxetine in clinical practice, as a combination strategy in cases of insufficient response to SSRIs, appears to be a highly plausible hypothesis.

Initially, Lucca et al. published their experience in the year 2000, with a small number of

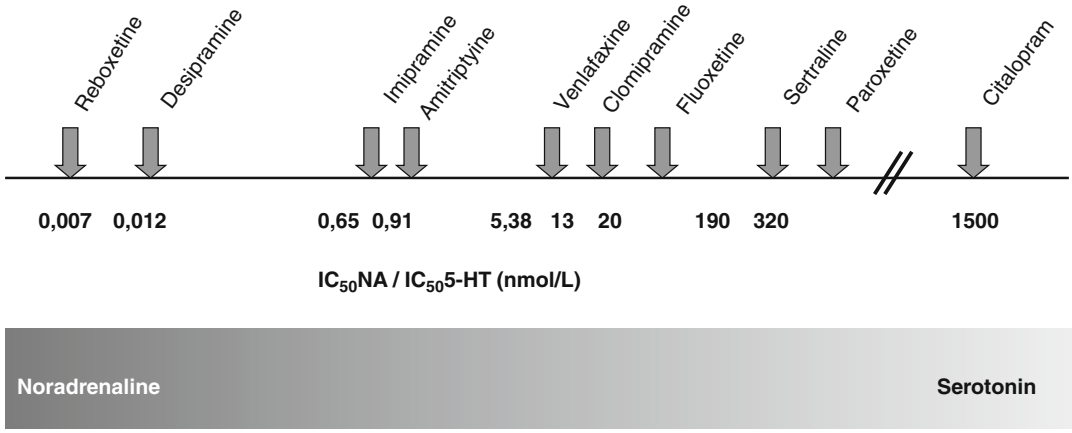

Fig. 28.2 Relative selectivity of different antidepressants in the inhibition of monoamine reuptake, represented by the ratio NA/5-HT. NA (noradrenaline); 5-HT (serotonin); IC_{50} (concentration necessary for inhibiting 50 % of reuptake) (Modified from Gorman and Sullivan [49])

patients: 14 patients diagnosed with MDD or bipolar disorder, nonresponders to conventional treatment with SSRIs, alone or in combination with pindolol. After 4 weeks of treatment, reboxetine was added at subtherapeutic doses (2–4 mg/day). Two weeks later, the results, assessed through the HDRS and CGI scales, were highly significant, except in the case of patients with psychotic traits. For their part, Devarajan and Dursun [30] provided data on four patients diagnosed with TRD (more than two SSRIs, one TCA, augmentation therapy with lithium, l-thyroxine and psychotherapy, venlafaxine and even, in three cases, ECT). These patients were treated with doses of 20–60 mg/day of citalopram and 4–6 mg/day of reboxetine for 16 weeks, obtaining, by the end of the treatment, reductions in HDRS score of between 73 and 88.8 %. The results of these preliminary studies suggest the potential utility of combination therapy with reboxetine in patients resistant to conventional treatment with SSRIs, as the action mechanisms of these agents affect different neurotransmission pathways [92].

Focusing on clinical studies carried out with reboxetine, Hawley et al. [55] reported data from a series of 24 patients with incomplete response to SSRIs, treated in combination with this NARI. These authors obtained a decrease in scores on the MADRS of 62.5 % at week 6 of the treatment and total remission (MADRS <10 points) in nine patients (37.5 % of the sample). Lucca's group [127] subsequently published the results obtained in a larger series of patients (27 patients diagnosed with MDD, with [$n=24$] and without [$n=3$] psychotic features), following the methodological procedure previously described. Of the total sample, 44.4 % of patients presented total remission, 29.6 % showed partial remission and 26 % did not improve; non-improvement was particularly common in the patients with psychotic traits, two of whom had to discontinue the medication due to therapeutic inefficacy. Elsewhere, Rapaport et al. [111] studied a total of 33 patients with MDD who responded only partially to treatment with fluoxetine (20 mg/day), adding reboxetine (4–8 mg/day) to the treatment for a period of 8 weeks. Of the 79 % of patients who completed the study, 39.4 % were classified as responders and 36.4 % as in remission. This association was well tolerated, the most common adverse effects reported being insomnia, dryness of the mouth, constipation and diaphoresis, though these were mild to moderate in intensity and transitory in nature.

In addition to these initial and generic studies, a series of articles on the assessment of this combination strategy have been published, including a open-label atomoxetine (another NARI approved only for the treatment of attention-deficit/hyperactivity disorder) study [18]. The studies by our group have shown the combination strategy with reboxetine to be a potentially useful tool in cases of TRD to SSRIs [77, 115, 116], mirtazapine [76, 116], venlafaxine [1, 116] and duloxetine [126]. Table 28.2 shows the work carried out to date with reboxetine as a combination tool.

In a first study of our group [115], we contributed data on a sample of 61 depression patients (45 patients with MDD, 9 patients with nonspecific depression disorder and 7 patients with dysthymia, according to the DSM-IV criteria) resistant or with partial response to SSRIs ($n=43$), venlafaxine ($n=12$) or mirtazapine ($n=6$), treated in combination with reboxetine and followed up for 6 weeks. Differences in reduction of HDRS score were already significant at week 2 after the addition of reboxetine. As regards type of diagnosis, the dysthymic patients showed a reduction in HDRS score significantly lower than that of the MDD patients ($p=0.031$). Mean reduction in HDRS-21 score was 48.9 %, and in CGI score, 38.9 %. At the end of the treatment, 62.3 % of the patients were classed as "improving" (CGI <4 points), 54.1 % as responding (HDRS ≤50 %) and 45.9 % as in remission (HDRS ≤10 points).

28.5.1 Reboxetine in Combination with SSRIs

With regard to SSRIs, as mentioned previously, O'Reardon et al. [100] point out that 30 % of depression patients fail to respond adequately to initial treatment with these drugs, and between

Table 28.2 Open studies on efficacy of the addition of reboxetine in TRD

Drug	*n*	Duration (weeks)	Dose (mg/day)	Assessment instruments	Efficacy				Reference
					Reduction scale score vs. baseline	Responders	Remission	Improving	
SSRI	34	6	2–8	HDRS	13.3 (49.4 %)	55.9	47.1	58.8	[115]
				CGI					
SSRI	43	6	2–8	HDRS	12.41 (48.9 %)	54.1	45.9	62.29	[116]
Venlafaxine	12			CGI					
Mirtazapine	6								
Mirtazapine	14	12	2–8	HDRS	11.92 (45.48 %)	35.7	28.6	64.3	[76]
				CGI					
Venlafaxine	40	6	2–8	HDRS	8.77 (34.9 %)	27.5	12.5	47.5	[1]
				CGI					
SSRI	141	12	2–8	HDRS	12.28 (46.79 %)	50.4	34.5	77	[77]
				CGI					
Duloxetine	79	12	2–8	HDRS	16.04 (65.5 %)	76	69.3	95,8	[126]
				CGI					

SSRI Selective serotonin reuptake inhibitors, *MADRS* Montgomery-Asberg Depression Rating Scale, *HDRS* Hamilton Depression Rating Scale, *CGI* Clinical Global Impression

60 and 70 % fail to achieve full remission. These patients may constitute a subgroup (i.e. melancholic depression) with poorer prognosis than nonresponders to antidepressants acting selectively on a single neurotransmission pathway, such as the SSRI [34, 82, 105]. Indeed, Malhi et al. [82] have reported an overrepresentation of melancholic (psychomotor changes) features in a "high treatment resistance" group ($n = 117$). It is in this broad range of patients that there is a need to implement the mechanisms necessary for improving therapeutic results, using combination strategies involving the addition of another antidepressant, an alternative that is becoming more and more popular. Especially interesting in this regard are combinations of antidepressants from different families of drugs, with complementary action mechanisms, such as NARIs, which may boost their respective antidepressant effects.

But moreover, from the neurobiological perspective, it has been confirmed, again as mentioned previously, that drugs which act selectively on the transporters of noradrenaline, such as reboxetine or desipramine, have an additional impact on serotoninergic transmission. Thus, extended administration of these drugs produces a desensitisation of presynaptic α_2-adrenergic heteroreceptors in serotoninergic neurons, which in turn augments serotoninergic neurotransmission, as suggested by Lucca et al. [80]. These presynaptic adrenoreceptors located at the serotoninergic terminals exercise an inhibitory influence on the release of serotonin, with the corresponding repercussions on 5-HT_{1B} autoreceptors. Furthermore, this response is reduced in the presence of an SSRI. This situation means that the administration of reboxetine, maintained over time, leads to a synaptic increase in the bioavailability of endogenous serotonin in the hippocampus, as demonstrated in the rat [11, 12, 89, 137].

From the clinical point of view, Kent [66] claims that the antagonism of the presynaptic α_2-adrenoreceptors could complement the action of the blocking of serotonin and noradrenaline reuptake, increasing the clinical response when a combination strategy with serotoninergic and noradrenergic agents is used. A similar phenomenon may occur with other drugs of a noradrenergic character, though not so selective, such as mianserin, as shown in a recent study of its combination with fluoxetine [39]. Fluoxetine would boost serotoninergic neurotransmission, on blocking the serotonin reuptake pump, and mianserin would boost noradrenergic transmission, on blocking the presynaptic α_2-adrenoreceptors. In this study, of double-blind design and 6 weeks' duration, in patients with MDD, the results revealed statistically significant differences in HDRS-score reduction in a fluoxetine plus mianserin group versus the fluoxetine group, suggesting the capacity of noradrenergic antidepressants (mianserin) for increasing the effectiveness of serotoninergic antidepressants (fluoxetine).

In the framework of our group, Rubio et al. [115] carried out a study of open and prospective design and 6 weeks' duration to assess the efficacy of reboxetine as a combination strategy, in 34 patients diagnosed with MDD who had not responded, or had done so only partially, to conventional treatment with SSRIs. Mean reduction in score on the HDRS-21 was 49.4 %, and on the CGI scale, 40.4 %. At the end of the treatment, 47.1 % of the patients were considered to be in remission, 55.9 % were assessed as responders and 58.8 % were judged to be improving. Dysthymic patients were those showing the poorest evolution, as was already observed to be the case in studies on monotherapy with reboxetine [62]. However, the small sample size of this subgroup ($n = 7$) precluded the drawing of definitive conclusions. No serious adverse effects were reported, the most frequent being nervousness and urine retention (5.9 %). Figure 28.3 shows the results of the subjective appraisal of this combination strategy by patients and doctors.

Subsequently, our group published a new study with a larger patient sample and a longer follow-up period [77]. This study, of open design, assessed the response to combination treatment with reboxetine in a sample of 141 patients with TRD to SSRIs (fluoxetine, $n = 29$; paroxetine, $n = 44$; sertraline, $n = 30$; citalopram, $n = 38$), over a follow-up period of 12 weeks. The initial mean dose of reboxetine was 2.76 ± 1.01 mg/day, and the mean maintenance dose was 5.08 ± 1.63 mg/day.

In general, the results obtained in this study were in line with the general data on combination strategies [61], with 77 % of "improved" patients, 50.4 % of responders to the combination treatment and 34.5 % of patients in remission, at 12 weeks of treatment (Figs. 28.4, 28.5 and 28.6). Percentage of patients in remission, as responders and improving were highest in the sertraline group (46.7 %, 63.3 % and 93.3 %, respectively). Differences in reduction of HDRS score were already significant by week 6 of the addition of reboxetine, both for the total group (−12.28 points) and for the four subgroups of patients analysed (sertraline −13.87; citalopram −11.97; paroxetine −11.86; fluoxetine −11.65). Compared to the rest of the studies published to date with reboxetine and bearing in mind their smaller samples and shorter follow-ups, our data confirmed a higher percentage of "improving" patients and a similar percentage of responders. There were 43 dropouts during the study (4 in the sertraline group, 8 in the citalopram group, 14 in the fluoxetine group and 17 in the paroxetine group); 15 patients were withdrawn from the study due to adverse effects and 28 for personal reasons unrelated to the study. The adverse effects reported during the 12 weeks of the study were judged as being of mild or moderate severity, even in the 15 patients who dropped out of the treatment due to adverse effects. Of these, eight cases were linked by the researchers to reboxetine (two cases of nervousness and restlessness, two cases of headaches, two cases of dry mouth, one case of insomnia and one case of anxiety). Nervousness was the adverse effect most frequently reported (5.21 %), followed by dryness of mouth (4.38 %) and insomnia (3.56 %). In no case was pharmacological treatment required for these adverse effects.

From the tolerability perspective, the low incidence of adverse effects observed in combination studies with reboxetine, including our own [77], may correlate with the pharmacokinetic profile of this drug [22, 115]. In relation to this aspect, it is worth pointing out, as we stressed earlier, that the in vitro (human microsomes) studies carried out

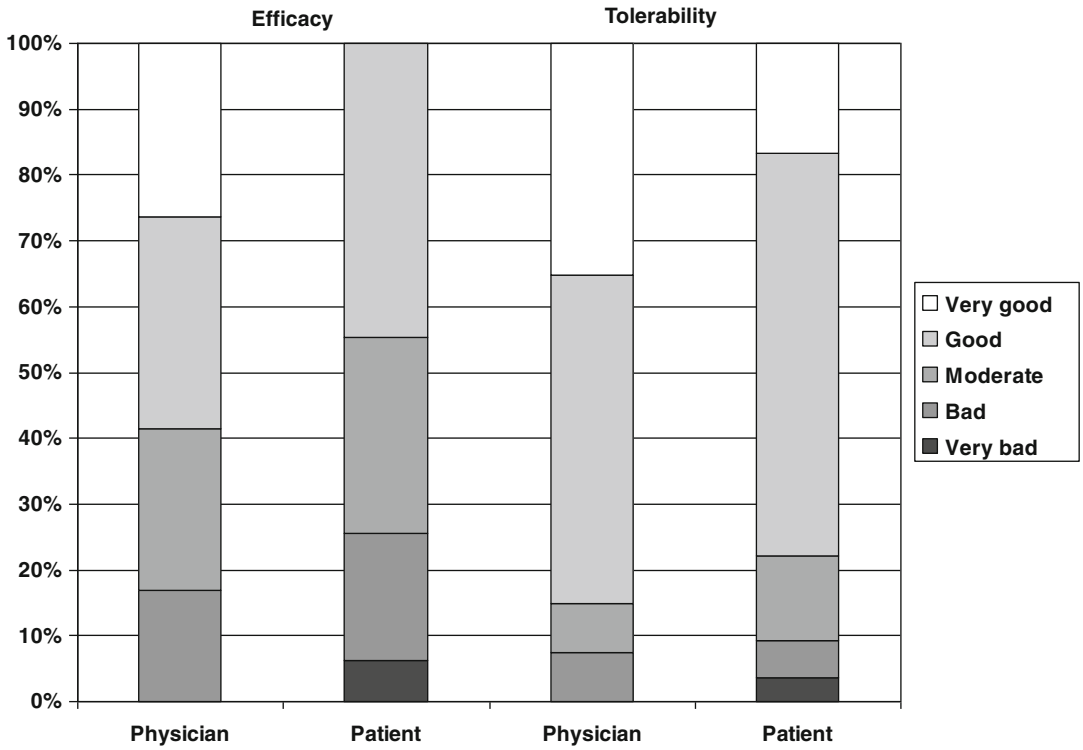

Fig. 28.3 Global subjective evaluation of the efficacy and tolerability of the combination SSRI plus reboxetine by patients and researchers ($n = 27$) (Modified from Rubio et al. [115])

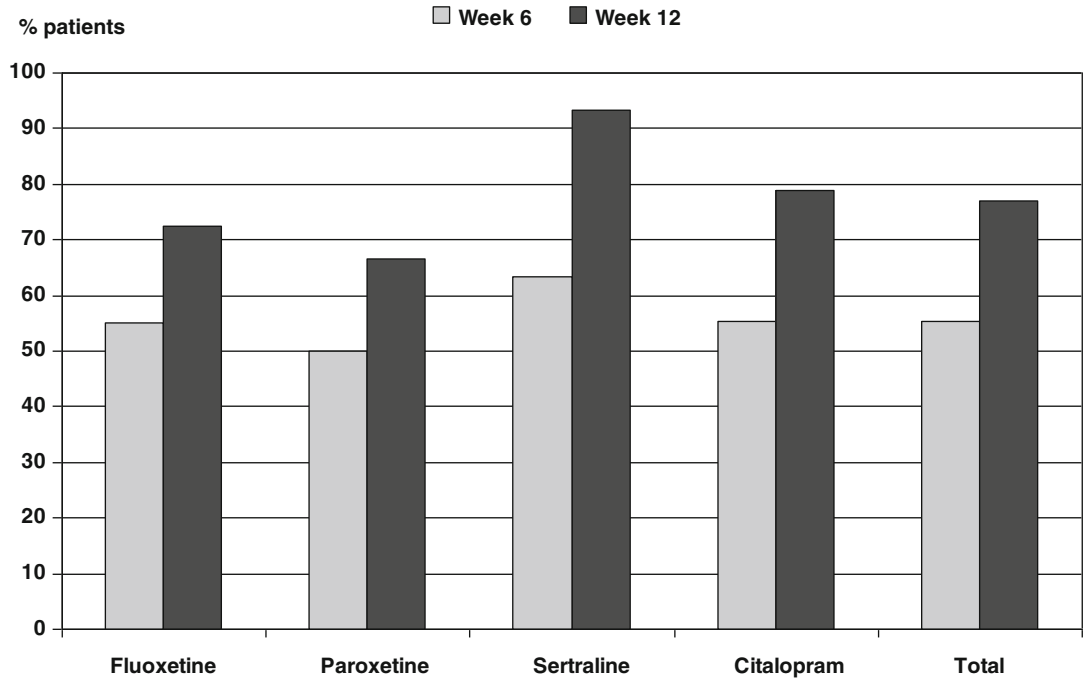

Fig. 28.4 Percentage of patients in improvement (score on the CGI <4 points) in patients treated with SSRI plus reboxetine. Analysis by intention to treat; *LOCF* last-observation-carried-forward (Modified from López-Muñoz et al. [77])

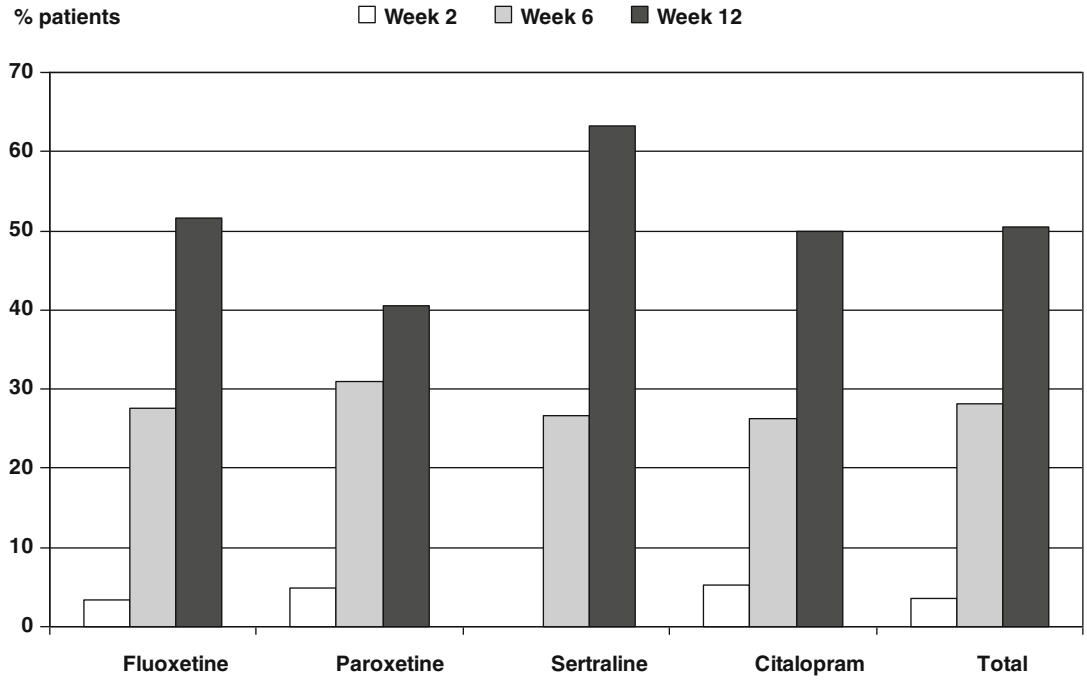

Fig. 28.5 Percentage of responders (reduction ≥50 % of total HDRS score) in patients treated with SSRI plus reboxetine. Analysis by intention to treat; *LOCF* last-observation-carried-forward (Modified from López-Muñoz et al. [77])

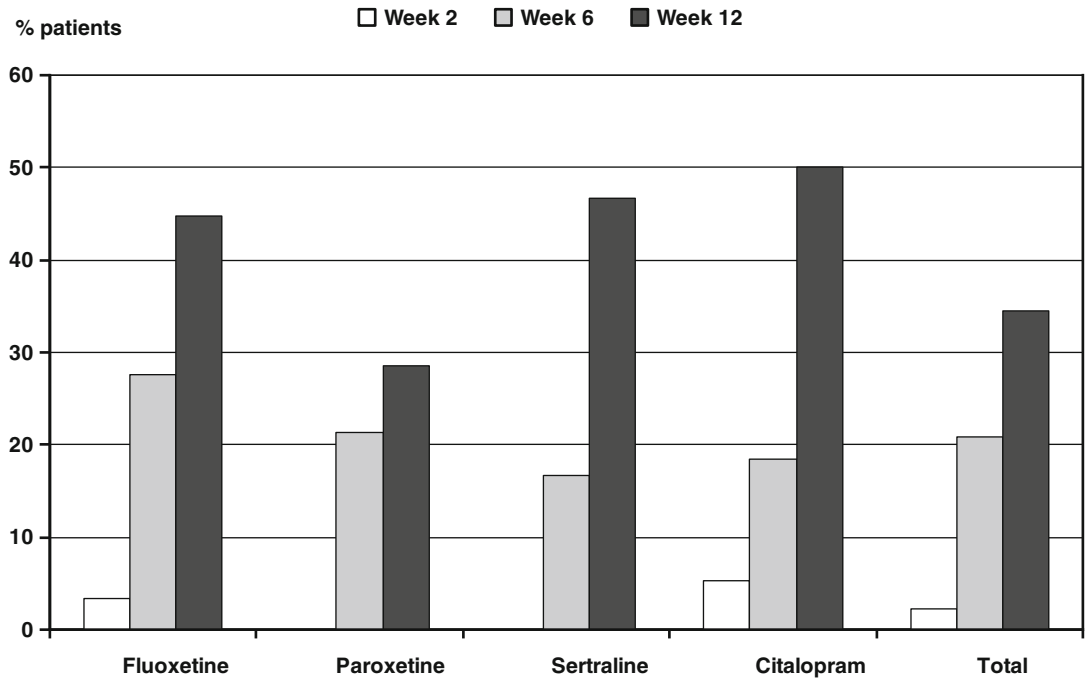

Fig. 28.6 Percentage of patients in remission (score on HDRS <10 points) in patients treated with SSRI plus reboxetine. Analysis by intention to treat; *LOCF* last-observation-carried-forward (Modified from López-Muñoz et al. [77])

to date show that reboxetine, at concentrations eight times higher than its C_{max}, does not inhibit any of the principal isoforms of CYP450, CYP2D6, CYP3A4, CYP1A2, CYP2C9 and CYP2C19 [14], nor does it have the capacity in vivo to induce the isoform CYP3A4 [108], which would greatly limit the likelihood of serious drug interactions with this antidepressant. In this regard, Avenoso et al. [4] confirmed that the administration of reboxetine to healthy volunteers (at therapeutic doses) did not modify the biotransformation of dextromethorphan to dextrorphan, the substrate widely used for assessing the inhibitory capacity of the isoenzyme CYP2D6. Likewise, the addition of 50 mg of quinidine, an inhibiting agent of the isoenzyme CYP2D6, to healthy volunteers treated with 1 mg of reboxetine, does not modify the pharmacokinetic parameters of the antidepressant [40]. All the data discussed here suggest that no serious drug interactions are envisaged with reboxetine, so that there are unlikely to be excessive numbers of adverse effects when this drug is used in combination with other antidepressants. In a randomised, double-blind study with 30 healthy volunteers, Fleishaker et al. [41] assessed the possibility of interactions between reboxetine and fluoxetine. Participants received, over a period of 8 days, 8 mg/day of reboxetine and 20 mg/day of fluoxetine, and the researchers found no statistically significant differences in the different pharmacokinetic parameters (C_{max}, T_{max}, $T_{½}$, ABC, V_d, binding to plasma proteins) in patients treated simultaneously with the two antidepressants versus each one administered individually. The authors concluded that concomitant administration of the two antidepressants was well tolerated, and no clinical impact was expected in depression patients treated with this combination strategy.

In conclusion, and in the light of the research carried out so far, it can be said that the combination strategy with reboxetine appears to be a potentially useful tool in cases of TRD to SSRIs, given that the action mechanisms of these drugs affect different neurotransmission pathways [27, 145], leading, in neurobiological terms, to an increase in the activity rates of the two

neurotransmitters, serotonin and noradrenaline [27]. Nevertheless, further controlled studies are necessary in order to reliably determine the efficacy of adding reboxetine in the case of depression patients resistant to treatment with SSRIs in monotherapy.

28.5.2 Reboxetine in Combination with Mirtazapine

Mirtazapine is an antidepressant also equipped with mixed noradrenergic-serotoninergic action, though its action mechanism is different from that of the mixed inhibitors (NSRIs), such as venlafaxine and duloxetine. The dual mechanism of mirtazapine, which translates into an increase in noradrenergic and serotoninergic activity, is due to the blocking of α_2-adrenergic auto- and heteroreceptors and to the selective antagonism it exercises on the $5HT_2$ and $5HT_3$ receptors [25]. Despite boosting both noradrenergic and serotoninergic pathways, there are a percentage of nonresponder patients to treatment with mirtazapine, estimated at as much as 31 % in certain specific studies [148]. In this regard, De la Gándara et al. [27] argue that the combination of reboxetine with mirtazapine will contribute dual action, noradrenaline reuptake inhibition and α_2-antagonism, so that the most refractory patients may benefit from a combination of serotonergic and noradrenergic mechanisms. However, up to now, there are no published data, other than those from our own group [76, 116], on combination strategies in nonresponders to mirtazapine.

The preliminary data reported by Rubio et al. [116] and referred to above, despite the small sample size ($n=6$), showed that the combination strategy with reboxetine could be useful in cases of TRD to mirtazapine, so that this same research group carried out a similar open study, with a larger sample ($n=14$) and with a longer period for monitoring the treatment (12 weeks) [76]. Maintenance dose of reboxetine in combination was 5.38 ± 1.89 mg/day, the mirtazapine dosage being kept constant throughout the study. Percentages of responders (HDRS $\leq$50 %) and those in remission (HDRS $\leq$10 points) at 12 weeks were 35.7 % and 28.6 %, respectively, whilst the figure for improvers (CGI-I <4 points) was 64.3 %. Figure 28.7 shows mean HDRS scores at the different assessment visits (0, 6 and 12 weeks), significant differences having been observed with respect to baseline at all the remaining visits.

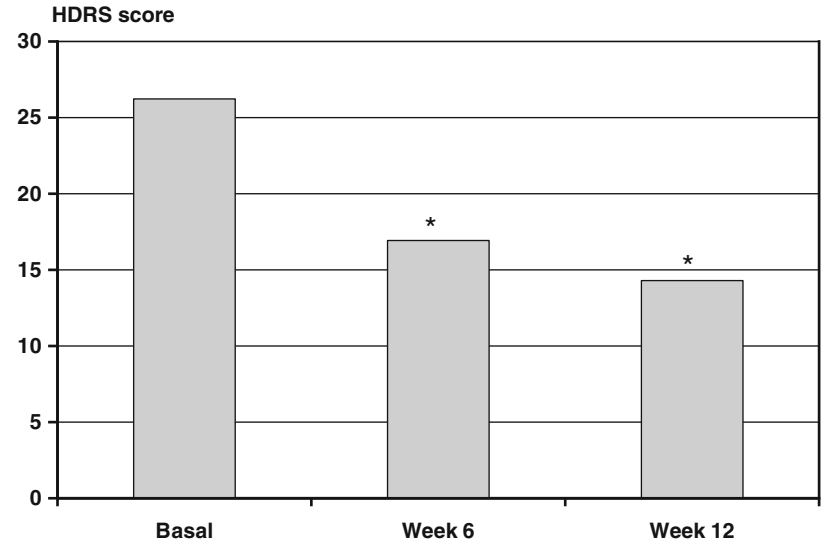

Fig. 28.7 Mean score on the HDRS in patients treated with mirtazapine plus reboxetine. *$p<0.05$ vs. baseline (Modified from López-Muñoz et al. [76])

From the perspective of tolerability, a previous experimental study revealed the scarcity of adverse reactions for the reboxetine-mirtazapine combination [125], a finding that was repeated in our own study at a clinical level; this could be correlated to the pharmacokinetic profile of reboxetine [22]. Likewise, the combination of mirtazapine with other antidepressants has also emerged as fairly safe [26].

By way of conclusion, the results of our study showed that this combination strategy could be useful and well tolerated in cases of resistance to mirtazapine, though we should not overlook the fact that this was an open study with a relatively small sample size.

28.5.3 Reboxetine in Combination with Venlafaxine

Venlafaxine is a dual-action antidepressant characterised by inhibiting the reuptake of serotonin and noradrenaline (NSRI), though at therapeutic doses, the capacity for blocking the reuptake of serotonin is much greater than that for noradrenaline. A meta-analysis confirms that only 44–46 % of patients with MDD enrolled in clinical trials with venlafaxine (in the subgroup of patients under age 54), even at the highest recommended doses of the therapeutic range, met the criteria of symptom remission at 8 weeks [32]. Given the pharmacodynamic properties of venlafaxine, which increases both serotonergic and noradrenergic neurotransmission, nonresponding patients to this antidepressant in monotherapy may constitute a subgroup with poorer prognosis than nonresponders to antidepressants acting selectively on a single neurotransmission pathway, such as the SSRI. In these partial responders, the strategy of increasing the dose of venlafaxine (basically using immediate-release formulations) may be problematic, since an increase in blood pressure has been reported with this antidepressant at doses above 200 mg/day [24].

Even so, it should be stressed that venlafaxine is approximately three- to fivefold more potent in inhibiting serotonin than noradrenaline reuptake in vitro [59]. And from the clinical point of view, it should be borne in mind that the dual mechanism of venlafaxine is dosage dependent, so that at low doses it tends to behave like an SSRI [26, 58]. Therefore, the combination of venlafaxine with serotonergic agents, especially fluoxetine, may involve increased risk of causing serotonergic syndrome [9]. Hence, in cases of combination with another antidepressant, reboxetine might be the most suitable alternative, seeking enhancement of noradrenergic neurotransmission.

Given the positive preliminary results obtained previously with reboxetine [116], our group has published the data obtained from a larger sample of patients with venlafaxine-resistant depression [1]. The study was open, prospective and multicentre and lasted 6 weeks, during which time we assessed response to the addition of reboxetine in 40 patients (19 women and 21 men), aged 18–65, who were diagnosed with MDD according to the DSM-IV criteria and were resistant to prior treatment with venlafaxine at maximum standard dosage. Mean maintenance dose of reboxetine in this study was 5.17 ± 1.81 mg/day and of venlafaxine 144.04 ± 60.3 mg/day. The authors reported a reduction of 34.9 % in the HDRS score (Fig. 28.8) and of 24.8 % in the CGI score between baseline and the end of the treatment ($p < 0.0001$). Percentages of responders and those in remission were 27.5 % and 12.5 %, respectively, whilst the percentage of improvers (CGI-I <4 points) was 47.5 %. The adverse effects most frequently reported in this study were constipation, nervousness, anxiety and insomnia (3.9 %). These data corroborate those reported by other researchers that have employed this antidepressant in routine clinical practice in patients with concomitant medication ($n = 1835$) [88]. Both researchers and patients filled out a subjective assessment scale on efficacy and tolerance for the combination treatment: 75.7 % of the researchers and 67.6 % of the patients considered the efficacy of the treatment as "good" or "very good", whilst tolerance was scored as "poor" by just 2.7 % of the patients. In either case the authors found significant differences in the degree of agreement between doctors and patients ($p < 0.0001$).

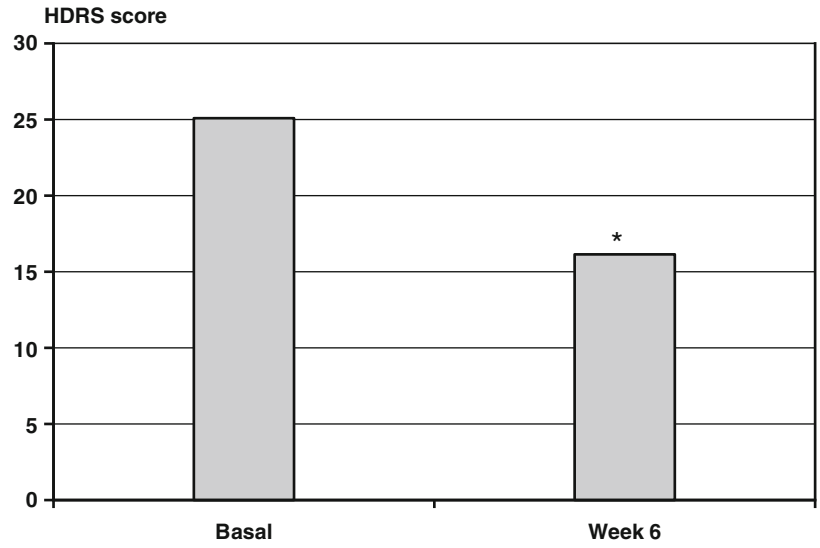

Fig. 28.8 Total reduction in HDRS score in patients treated with venlafaxine plus reboxetine * $p<0.0001$ vs. baseline (Modified from Alamo et al. [1])

Although these results seem promising, and as with the combination of reboxetine with SSRIs and mirtazapine, there is a need for controlled studies for definitively confirming the suitability of this combination strategy.

28.5.4 Reboxetine in Combination with Duloxetine

The results of studies with duloxetine confirm that only 40–58 % of patients with MDD, even at the highest recommended doses of the therapeutic range, met the criteria of symptom remission at 8 or 9 weeks [43]. However, there are no published data on the effectiveness of the combination strategy in patients who fail to respond to duloxetine, except reports of sporadic cases of positive response to the addition of bupropion ($n=3$) [104] and mirtazapine ($n=5$) [112]. Given the positive previous results obtained with the addition of reboxetine in patients with venlafaxine-resistant depression [1], we have raised the hypothesis of which an increase of noradrenergic activity could be beneficial for those patients who do not respond duloxetine suitably [126]. This antidepressant has a high affinity for noradrenaline and serotonin reuptake transporter, although their potency to inhibit human noradrenaline transporter is higher than for human serotonin transporter (inhibition constant in vitro of 7.5 and 0.8 nmol/L, respectively) [15].

We assessed, in a prospective 12-week open-label study, the effectiveness of the addition of reboxetine to 79 depressive outpatients (61.3 % women and 38.7 % men), aged 18–65 years (mean 50.51 ± 12.35 years), diagnosed with MDD, according to the DSM-IV criteria, that had previously not responded, or had done so only in a partial way, over 8 weeks of conventional treatment, in monotherapy, with duloxetine, to maximum standard recommended doses (60 mg/day) [126]. The mean maintenance dose of reboxetine was 5.85 ± 2.02 mg/day. Efficacy was assessed using the 21-item HDRS and the CGI-I. The mean score on HDRS at baseline was 24.48 ± 5.08, decreasing to 8.44 ± 5.90 at week 12 (65.5 % reduction; $p<0.0001$) (Fig. 28.9). Differences in decrease of HDRS score were already significant by week 2 of the addition of reboxetine (−5.13 points; $p<0.0001$). The percentages of responders (≥50 % reduction in HDRS) and patients considered as benefiting from complete remission (HDRS ≤10 points) at week 12 were 76 % and

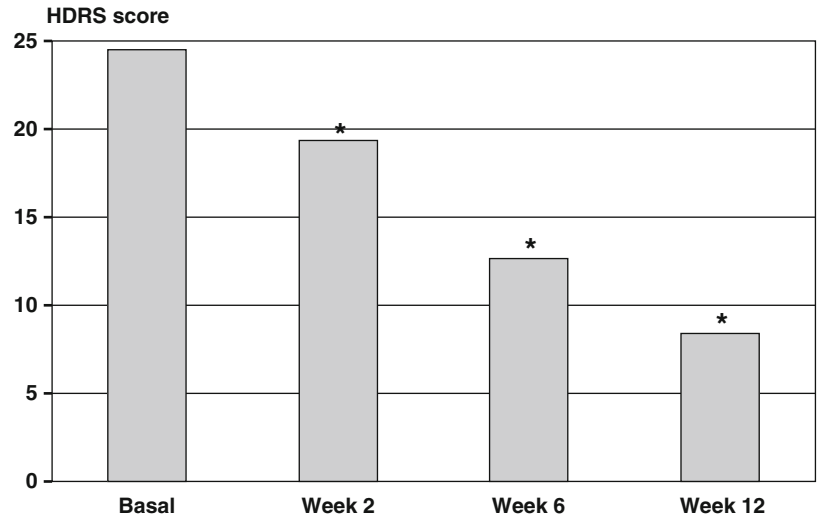

Fig. 28.9 Mean score on the HDRS in patients treated with duloxetine plus reboxetine. Analysis by intention to treat; LOCF (last-observation-carried-forward). * $p<0.0001$ vs. baseline (Modified from Seguí et al. [126])

69.3 %, respectively. By the end of the treatment, the score of CGI-I decreased 68.5 % ($p<0.0001$). Percentage of patient improving (CGI <4 points) was 95.8 %. The most common nonserious adverse events were dry mouth (21.24 %), increased sweating (11.95 %), constipation (9.73 %) and difficulty passing urine (2.65 %). All adverse events were judged as being of mild or moderate severity, including four patients dropped out of the treatment due to adverse effects related by the investigators to reboxetine (two cases of nervousness; one case of nervousness, insomnia and dry mouth; and one case of anxiety).

The results of this study suggest that the combination strategy with reboxetine may be an effective and well-tolerated tool in duloxetine-resistant patients. Nevertheless, double-blind, controlled studies with larger samples and longer follow-up periods are required to confirm these results.

Conclusions

Currently, drug therapy for psychiatric disorders is based primarily on the administration of a single drug capable of producing a positive response in the patient, or alternatively, of sequential monotherapies. Although the development of new antidepressants is desirable, other approaches should not be ignored. However, there is very little experience to date in approaching chronic psychiatric pathology, such as depression, by means of drug-combination strategies, compared to the case of other medical fields (e.g. hypertension, cancer, diabetes), which have seen the rigorous evaluation of combination treatments for enhancing response and improving outcomes. In fact, no combination or augmentation strategies have been approved by the FDA for the treatment of depression. In the specific case of TRD, the only in-depth study of drug combinations, with all their strengths and limitations, is the STAR*D trial.

Recent studies by our group [1, 76, 77, 115, 116, 126] have shown that the addition of reboxetine – the prototypical NARI agent – to patients with TRD to SSRI, mirtazapine, venlafaxine or duloxetine may be an effective and well-tolerated therapeutic strategy. However, to date there have been no controlled clinical trials for assessing the clinical efficacy of reboxetine as a combination tool in depression patients refractory to a previous antidepressant treatment. Obviously, the results of open

studies, without a control group, are methodologically questionable, though the use of placebo in this type of patient would also be questionable from an ethical point of view, given that the risk of autolithic behaviours is markedly higher [135]. On the other hand, observational studies provide a perspective from everyday clinical practice that can complement the data from controlled clinical trials.

The future lies in the development of new antidepressants with other mechanisms of action for improving depression. Monoaminergic theory by increasing monoamines by inhibiting serotonin and norepinephrine transporters has been a central theme in depression research since the 1960s [75, 79]. Recently, other theories forward to a complex mechanism have been introduced. For example, a central strategy has been to target glutamate receptors or modulation of cholinergic and gamma-aminobutyric acid (GABA)ergic transmission, neuronal plasticity, stress/hypothalamic pituitary adrenal axis, reward system and neuroinflammation [23]. In this sense, agomelatine, a melatonin MT_1 and MT_2 receptor agonist and a 5-HT_{2C} receptor antagonist [2]; tianeptine, a glutamatergic modulator [85]; vilazodone, a 5-HT_{1A} receptor partial agonist and SERT inhibitor; vortioxetine, a 5- HT_{1A} receptor agonist, 5-HT_{1B} receptor partial agonist and 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist and SERT inhibitor; and ketamine [31] are multimodal antidepressant examples [21, 23].

Despite these present shortcomings and to the development of new drugs, the study of antidepressant combinations should be encouraged and promoted, by both clinicians and the pharmaceuticals industry, and indeed by governments [138]. Moreover, such research should be subject to the same scientific rigour as that required for new monotherapies (large samples, adequate patient selection, controlled design, etc.), since there is no doubt that TRD currently constitutes a social-health problem of the first order.

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Antidepressant Drugs in Elderly

29

Cecilio Álamo, Francisco López-Muñoz,
and Pilar García-García

29.1 Introduction

In our society, scientific advances and the improvement of the living conditions are causing a "demographic transition" with a significant increase in the number of elderly people. Many of these elders, despite present comorbid diseases, have an active social life and want a good state of physical and mental health. The economic and social burden of aging and the decline in active social class are questioning the so-called welfare state which affects the practice of medicine [1].

Depression in the elderly is a major challenge for current psychiatry and a major problem of mental health. At this age, depression is more severe and complex which makes a correct diagnosis more difficult. Elderly with depression are more likely than younger patients to underreport depressive symptoms. They often present with nonspecific somatic complaints, such as insomnia, appetite disturbances, lack of energy, fatigue, chronic pain, constipation, and musculoskeletal disorders. In addition, they present a high risk of suicide. These facts condition a series of clinical aspects, prognoses, and therapeutic accompanied by high costs and health-care difficulties. The approach of depression in the elderly is not an easy task, but the difficulty does not justify a nihilistic attitude [2, 3].

From the quantitative point of view, the prevalence of depression in elderly is higher than in the young adult. According to the World Health Organization, depression is the most frequent mental disorder and the most disabling medical condition among geriatric patients, but it is often under-recognized and not adequately treated in 40–60 % of cases [2, 3]. While the general population prevalence of major depressive disorder (MDD) is approximately 5 %, an estimated 14–20 % of community dwelling older adults experience symptoms of depression, with even higher rates being observed in inpatients (12–43 %) and in those who reside in long-term care facilities, where the estimated prevalence may reach 40 % [4].

Depression in older adults is associated with a significant morbidity burden, impairing the ability to function both physically and cognitively and increasing the risk comorbid medical illnesses, delayed recovery, placement in long-term

C. Álamo • P. García-García
Department of Biomedical Sciences (Pharmacology Area), Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain

F. López-Muñoz (✉)
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal
e-mail: francisco.lopez.munoz@gmail.com; flopez@ucjc.edu

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care facilities, and mortality due to suicide and other causes [4]. Older patients are at increased risk of medical comorbidity, and this is a risk factor for inferior treatment response and poor antidepressant tolerability [5].

It is a common misperception that depression is a part of normal aging. While depression is common in old age, affective symptoms are not a normal part of aging. Nevertheless, depression in the older patient always merits early recognition and treatment. Pharmacotherapy is the cornerstone of management for depression, with guidelines recommending their use in mild, moderate, and severe depression. In elderly, due to its greater sensitivity, the therapeutic approach of depression needs medicine more efficient and with a better tolerability [2, 3]. However, the overall outcome of major depression in the elderly is poor, with a meta-analysis of 12 studies of elderly community patients showing that 21 % of patients had died and almost half of those remaining alive were still depressed after 2 years [6].

The treatment of depression in senile age must take into account the following general recommendations. It is necessary to always treat the symptoms of depression with an antidepressant and start with lower doses than those recommended for adult. The use of an antidepressants without anticholinergic and sedative effects is preferable, since they minimize the risk of cardiotoxicity and cognitive impairment. The cardiotoxicity of some antidepressants, especially those of the heterocyclic group, can be dramatic. Assess possible interactions with other drugs, since the elderly is usually a polymedicated patient with different somatic comorbidities. The treatment should follow the criteria established for the duration of the treatment [7], and if antidepressant response is obtained, the drug should be kept for long periods, even for lifetime. The use of antidepressants in the elderly should be performed only when necessary, but age should not deprive the patient of medication to improve their health and quality of life. The success of drug treatment will depend on a proper assessment of the risk-benefit relationship [3].

In the present review, we have used Embase, PubMed, ScienceDirect, and Google Scholar databases, which are considered the most exhaustive within the biomedical field. We also reviewed our personal collections of research. This review took into account all original articles, brief reports, reviews, editorials, letters to the editor, and so on.

29.2 Pharmacodynamic Classification of Antidepressant Drugs

Antidepressants are categorized into classes based upon their chemical structure or the neurotransmitters that they affect. The monoamine hypothesis has been the pharmacological target for the treatment of depression. At this moment, all antidepressants, including the presynaptic alpha-adrenergic receptor antagonist mirtazapine, use the same mechanism of action: the modulation of monoaminergic (noradrenergic and/or serotonergic, and dopamine to a lesser extent) neurotransmission at synaptic level. Attempts to find antidepressants that initiate their effects through non-monoaminergic mechanisms are intense but discouraging [8, 9].

The most important exceptions to this hypothesis are due to the research of the French laboratory Servier that developed an antidepressant called tianeptine, with a mechanism of action not well established but related to the modulation of glutamatergic mechanisms [10]. In addition, it developed the agomelatine, an antidepressant with agonistic action on MT_1 and MT_2 melatonin receptors. However, in both cases the monoaminergic contribution to the mechanism of action seems to be present. Thus, tianeptine releases dopamine in the nucleus accumbens and in prefrontal cortex [11], while agomelatine by blocking 5-HT_{2C} receptors increases norepinephrine and dopamine levels in prefrontal cortex [2, 3, 8].

According to its chemical and pharmacodynamic profile, especially its ability to inhibit the reuptake of monoamines and its affinity for different neurotransmitter receptors, antidepressants can be classified in some groups with different therapeutic options (Table 29.1).

Table 29.1 Pharmacological properties of more important antidepressant drugs

	Monoamine transporter inhibition			Receptor antagonism				
Pharmacologic classes	NE	5-HT	DA	α-1	α-2	Ach	H-1	5-HT_2
TCA								
Imipramine	+++	+++++	–	++		++	++++	+
Clomipramine	+++	++++++	0	+++		+++	+++	++
Amitriptyline	+++	++++	–	+++		+++	+++++	+++
Nortriptyline	+++	+	0	+		+	++++	+
Trimipramine	++	+	+	++		+++	+++++	+++
Doxepin	+++	+++	0	+++		++	++++++	
Dosulepin	+++	++						++
Second generation								
Mianserin				+	++++	+	+	+
Maprotiline	+++				++	++		++
Trazodone		++		+++	+	–	–	++
SSRIs								
Fluvoxamine	+	+++++	–	–		0	–	0
Fluoxetine	++	+++++	–	–		–	–	0
Paroxetine	++	++++++	+	–		++	0	0
Sertraline	+	++++++	+++	++		+	0	0
Citalopram	–	++++	0	+		–	+	0
Escitalopram	–	++++++	0					
NSRIs								
Reboxetine	++++	0	0	0				0
NDRIs								
Bupropion	0	–	+	–	++	0	–	
SNRIs								
Venlafaxine	+	++++	–	0		0	0	0
Desvenlafaxine	+	++++	–	0		0	0	0
Duloxetine	++++	++++++	+	–		–	–	0
Milnacipran	+++	+++	0	0	0	0	0	0
SARIs								
Mirtazapine	–	0	0	+	+++	+	++++++	0
Others								
Tianeptine[a]	0	0	0	0	0	0	0	0
Agomelatine[b]	0	0	0	0	0	0	0	++[a]

Potency of effects: 0 without effect, – weak, + ascending order of potency
Modified from Alamo et al. [12]
NE Norepinephrine, *5-HT* serotonin, *DA* dopamine, *α1; α2* adrenergic receptors, *Ach* cholinergic receptor, *H1* histaminergic receptor, *5-HT_2* serotonergic receptor, *TCA* tricyclic antidepressants, *SSRIs* selective serotonin reuptake inhibitors, *NSRIs* norepinephrine selective reuptake inhibitors, *NRDIs* norepinephrine-dopamine reuptake inhibitors, *SNRIs* serotonin-norepinephrine reuptake inhibitors, *SARIs* serotonergic-adrenergic receptors inhibitors
[a]Glutamatergic modulator and neuroprotection
[b]Melatonin receptors agonist

In general, we can say that the monoamine transporter inhibition by antidepressants is associated with its therapeutic effects, although it is also responsible for undesirable effects. On the other hand, the affinity of antidepressants by monoamine receptors relates to its adverse effects, although the alpha-2 receptor antagonism may be related to antidepressant efficacy of mirtazapine [13].

29.3 Criteria for Selection of Drug Therapy in Depressed Elderly

The selection of the most appropriate treatment in any pathology, especially in the elderly, should take into account the criteria of efficacy, tolerability, and safety. While clinical trials of antidepressants may exclude some individuals over the age of 65, it is not clear that evidence of safety and efficacy in such subgroup analyses is reflective of this age group more broadly. The generalizability of outcome data across populations can potentially be uncertain, and the comparative efficacy of different antidepressants in older adults could potentially differ than what is seen in younger populations.

On the one hand, the standard diagnostic criteria for depression are not always applicable to the elderly patient. The great variability of the chosen evaluation measures, both efficacy and tolerability, in the various clinical studies makes it difficult to compare between different groups. In addition, the elderly with depression suffer relapses frequently and require longer treatment courses. Furthermore, it should be underlined that the term "late life depression" usually includes extremely heterogeneous groups of individuals, aged between 60 and over 100 years [14].

As well, age-related changes that affect the pharmacokinetic and pharmacodynamic of drugs can affect the safety and potential harms with antidepressants in older adults. Furthermore, frequent comorbidities, which lead to polypharmacy, complicate the application of standardized treatments [15]. Older adults are at increased risk of anticholinergic side effects and orthostatic and sedative effects. These effects can exacerbate underlying conditions such as cardiovascular disease, cognitive impairment, and delirium and can increase the risk of falls and fractures [16].

All these circumstances make the main source of information, controlled clinical trials, more complex and therefore less comparable. Possibly because of these difficulties, the number of clinical trials conducted in depressed elderly is much lower than in the younger adult group.

Supporting as mentioned above, Canadian Agency for Drugs and Technology in Health can perform a study on *Antidepressants in Elderly Patients with Major and Minor Depression*: *A Review of Clinical Effectiveness and Guidelines*, in which a total of 760 citations were identified in the literature search. Following methodological screening, only 13 publications met the inclusion criteria and were included in this report. With these limitations, also seen in other meta-analysis and systematic review, extraction of results with applicability to the clinic is not an easy task.

29.4 Therapeutic Effectiveness of Antidepressants in Elderly

Initial reviews of controlled clinical trials, conducted in the last two decades of the twentieth century, agreed on two key findings: antidepressants are clearly superior to placebo and that older patients are just as likely as younger patients to go into remission given appropriate treatment [17]. Some reviews made in the initial years of the twentieth century come to similar conclusions: superiority of antidepressants with placebo and similar efficacy in the elderly than in the adult depressed. However, in acute treatment, systematic reviews of placebo-controlled antidepressant trials in the elderly suggest somewhat smaller effect sizes, particularly in trials restricted to participants aged 65 and over [18].

Furthermore, there are no differences in efficiency between the different groups of antidepressants in the elderly. Even dual-action agents such as tricyclic antidepressants (TCAs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) do not appear to confer any additional efficacy benefits over single action agents such as selective serotonin reuptake inhibitors (SSRIs) in the treatment of depression in the elderly [19].

In some studies the effectiveness of a similar efficacy of antidepressants in elderly than in young adults is questioned. A review of Tedeschini et al. [20] found that antidepressant treatment for major depression was effective, but that such treatment may have been less effective in later life than in a general adult population.

However, methodological limitations in the review mean that the reliability and generalization of the conclusions are uncertain.

Recently, Kok et al. [21] carried out a systematic review and meta-analysis, which included 92 double-blind randomized clinical trials, of depressed older patients of acute phase treatment and show that all classes of antidepressant were individually more effective than placebo in achieving response and remission, confirming previous reviews, although remission was less significant. Furthermore, studies directly comparing different classes of antidepressants do not indicate a difference in achieving remission or response. Authors suggest that antidepressants are well studied for the acute phase treatment in older patients with unipolar major depression. However, there is a paucity of double-blind clinical trials in treatment-resistant depression, psychotic depression, minor depression, and depressed nursing home patients.

Canadian Agency for Drugs and Technology in Health (2015) comes to similar conclusions in its recent review: a number of antidepressants appear to be effective in those aged 65 and older, relative to placebo, but there is limited evidence on comparative efficacy between antidepressants and quality issues with the available evidence.

It is very important to be aware that older people take longer to respond to antidepressant. NICE recommends that in older adults antidepressant treatment should be given for a minimum of 6 weeks before it is considered ineffective [22].

The goal of treatment in elderly is not only acute recovery but also the prevention of the recurrence. Most experts recommend 6–12 months of treatment after a first episode. However, this recommendation could stay short because monitoring during 2–3 years in depressed elderly, recurrence rates ranged between 50 and 90% [12]. Thus the long-term benefits of continuing antidepressant medication in the prevention of recurrence of depression in older people are not clear. Continuing antidepressant medication for 12 months appears to be helpful, but this is based on only three small studies with relatively few participants, using differing classes of antidepressants in clinically heterogeneous populations [23]. However, for some authors, older patients should be treated for at least a year from when clinical improvement is noted, and those with recurrent depression or severe symptoms should continue treatment indefinitely.

Instead, it has been described that the treatment-resistant depression affects up to one-third of the elderly patients with depression. The highest levels of acute or chronic stress factors, less social support, the earliest age of onset, melancholic like demonstrations, patients needing use of adjunctive medication in acute treatment, current age, and the highest level of current anxiety were also factors who predicted a worse long-term response [12, 24]

In general, systematic reviews of placebo-controlled antidepressant trials in the elderly suggest somewhat smaller effect sizes, particularly in trials restricted to participants aged 65 and over [18] and when they were compared with adult patients with MDD [20, 21].

It can be concluded that the clinical research in the field of antidepressants in the elderly has not been able to detect differences in efficiency between groups of antidepressants or any in particular. However, this does not exclude the need for the individualization of antidepressant therapy in the elderly. In this sense, it is essential to know the antidepressant pharmacodynamic profile. In fact, some pharmacodynamic characteristic of antidepressants that would be intolerable for a particular patient may be used therapeutically in others. Thus, mirtazapine, an antagonist of presynaptic α2-adrenergic receptors and a potent antihistaminic, can facilitate sleep and increase appetite and weight. These properties may be of particular interest in elderly patients with insomnia or weight loss. In contrast, bupropion or reboxetine, which inhibits reuptake of catecholamine, can be used to energize patients showing an excessive lassitude, lethargy, fatigue, or with excessive sedation during the day. In depressed patients with neuropathic pain, duloxetine may be useful, and nausea, agitation, insomnia, and hypertension appear more often at high doses [2, 3, 19]. The recent introduction of vortioxetine has created expectations of a particular profile of effectiveness in the elderly. This agent

proves efficacy in elderly patients and also appears to have cognitive enhancing properties which are largely independent of improved depressive symptoms [25].

There is evidence that in elderly, especially in frail elderly patients, it is advisable to "start low, go slow." In the acute phase, at least 6 weeks of treatment may be needed to achieve optimal therapeutic effect [18, 26]. Since the efficacy of all antidepressants versus placebo is similar in the elderly, selection is not conditioned by differences in efficiency, but by other factors as the profile of adverse effects, comorbidities, and concomitant use of other drugs that can produce drug interactions [12].

29.5 Tolerability of Antidepressants in Elderly

All antidepressants have a similar efficacy, even in elderly. Thus, we must select the right antidepressant according to their profile of adverse effects. Changes in the function and cerebral structure related to the aging increase the vulnerability to the undesirable effects of antidepressants. Loss of neurons in the cortex, locus coeruleus, and hippocampus increases the sedative and psychomotor effects of these drugs. Gradual loss of cholinergic neurotransmission in the central nervous system (CNS) increases the sensitivity to the anticholinergic effects of antidepressant. Reduction in the sensitivity of the carotid and hypothalamic centers facilitates the hypotensive effect of antidepressants. Loss of the number of neurons in the nigrostriatal pathway increases the extrapyramidal effects of some drugs [12, 27].

Pharmacokinetic and pharmacodynamic changes in elderly can also influence in antidepressant therapy. In general, SSRIs constitute the first-line treatment for depression in the elderly. Fluoxetine and paroxetine are effective, but due to the long half-life of the first and the potent anticholinergic effect of paroxetine, they would not be the first-line treatment of depression in the elderly patient [2, 3].

On the other hand, TCAs are effective in elderly patient but their used is limited by its adverse effects and cardiotoxicity in overdose. Thus, TCAs must have been reserved for patients with previous good TCA antidepressant response. Where possible, avoid TCAs in patients at high risk of cardiovascular disease, arrhythmias, and cardiac failure [18]. In patients with a recent history of myocardial infarction, TCAs are contraindicated. Furthermore, glaucoma, orthostatic hypotension, urinary retention, hypertrophy of prostate, and cognitive impairment are contraindications for TCAs [2, 28]. In patients with cardiac disease or those on hypotensive drugs, TCAs increase the risk of fall [18].

Monoamine oxidase inhibitors (MAOIs) have a narrow therapeutic margin and require restrictions with several drugs and nutrients that content tyramine. This group of antidepressants is not recommended in the elderly even if it is performed by personnel with experience in this type of antidepressants [29].

Although side effects can be idiosyncratic, most can be explained by the drug's mechanism of action at the central and peripheral synaptic levels. Antidepressants typically block the reuptake of certain neurotransmitters (norepinephrine, serotonin, and dopamine) and block some types of receptors. In many cases these adverse effects are predictable and are collected in schematic form in Table 29.2. Knowing these predictable effects, we can find therapeutic alternatives [12].

29.5.1 Cardiovascular Risks of Antidepressants in Elderly

The relationship between pharmacodynamic properties of antidepressants and cardiovascular risk involved is well known. Cardiovascular adverse effects of the antidepressants, especially with overdoses of TCAs, are probably the best studied. In fact, different clinical guides are not recommended in the use of TCAs in patients with cardiovascular risk, arrhythmias, heart failure, or coronary insufficiency. In these cases, the use of SSRIs, bupropion, or mirtazapine is recommended

Table 29.2 Pharmacodynamic aspects implicated in efficacy and tolerability of antidepressant drugs

Pharmacological effect	Drugs	Therapeutic effect	Adverse effects
5-HT uptake inhibition	TCAs	Antidepressant	Nausea
	SSRIs		Nervousness
	Venlafaxine		Insomnia
	Nefazodone		Sexual dysfunction
NA uptake inhibition	TCAs	Antidepressant	Tachycardia
	Venlafaxine		Hypertension
	Reboxetine		Tremor
			Sexual dysfunction
5-HT receptor agonism			
5-HT_{1A} postsynaptic	TCAs	Antidepressant	
	MAOIs	Anxiolytic	
	SSRIs		
	Mirtazapine		
	Nefazodone		
5-HT_{1D}	SSRIs		Headaches, migraines
5-HT_2	SSRIs	Antidepressant	Insomnia
		Antiobsessive	Anxiety
		Antibulimic	Sexual dysfunction
			Agitation, akathisia
5-HT_3	SSRIs		Loss appetite
			Increase gastrointestinal motility
			Nausea, diarrheas
Presynaptic 5-HT_{1A} receptors desensibility	TCAs	Antidepressant	
	SSRIs		
	Venlafaxine		
5-HT receptors antagonism			
5-HT_{1D} presynaptic	Pindolol	Antidepressant	
5-HT_2	TCAs	Antidepressant	Hypotension
	Mirtazapine	Improvement sexual function	
	Nefazodone		
	Agomelatine		
5-HT_3	Mirtazapine	Antiemetic	
Blockade central and peripheral muscarinic cholinergic receptors	TCAs		Blurred vision, constipation, dry mouth, urinary retention, tachycardia, Memory disorders
	Paroxetine (SSRIs)		
Histaminergic (H_1) receptors antagonism	TCAs		Sedation, drowsiness, dizziness, weight gain, hypotension
	Mirtazapine		
Adrenergic receptors antagonism			
Central an peripheral α_1 receptors	TCAs		Orthostatic hypotension, dizziness, tachycardia, sedation
α_2-receptors	TCAs	Antidepressant	Priapism
Down-regulation β-adrenergic receptors	TCAs	Antidepressant	
	Fluoxetine		
	Fluvoxamine		
	Paroxetine		
	Venlafaxine		
	Nefazodone		
Monoamine oxidase inhibition	MAOIs	Antidepressant	Hepatotoxicity, hypertensive crisis, headaches, irritability disorders, insomnia, sexual sphere disorders

as alternatives [30]. Peripheral antimuscarinic properties can induce a slight tachycardia, in which they take part in addition the norepinephrine reuptake inhibition. Its administration can lead to electrocardiographic alterations, like prolongation of spaces PR, QRS, and QT that usually are innocuous in the healthy young, but which they can cause a bundle branch block in elderly. TCAs and paroxetine, which has anticholinergic properties similar of the imipramine, are implicated in these effects [2, 3, 27, 31].

29.5.2 Antidepressants and Myocardial Infarction Risk in Elderly

Depression and myocardial infarction appear to have a bidirectional relationship. Depression is three times more common in patients after acute myocardial infarction (MI) than in the general population, and, notably, some evidence suggests that depression may increase the risk of coronary heart disease in healthy individuals and is associated with cardiac morbidity and mortality in individuals with established coronary heart disease. Mortality risk in patients after MI increases by as much as fourfold [32].

In the past decade, the role of antidepressant use in the development of MI has been widely debated. The literature contains no clear findings for risk of MI and antidepressant use in older people. Some studies have found an increased risk of MI associated with antidepressant use, especially with TCAs [18, 33] and with SSRIs [34]. By contrast, some have found a protective effect limited to SSRIs [30, 35]. The possible best cardiovascular tolerability of SSRIs, especially the lowest risk of relapse of MI, could be explained by the depletion and decrease of serotonin stored in platelets, reducing its aggregation. This effect is related to the inhibitory potency of reuptake of serotonin, being more patent with fluoxetine, sertraline, and paroxetine [36, 37].

Recently, Undela et al. [38] conducted a meta-analysis that included 14 observational studies with data from more than 800,000 participants among which were collected almost 86,000 MI. Their results support the hypothesis that the use of TCAs may increase the risk of MI in depression, but SSRIs showing no association. Other authors related that an increased risk of MI may be explained by confounding factors relating to depression itself rather than specific adverse drug effects [39].

The debate seems to be open, but taking into account the general comorbidity of the elderly, in patients at risk of developing cardiovascular diseases, such as diabetes, smoking, hypertension, and overweight, or in patients who have had a recent MI, the use of TCAs should be abolished [30, 40, 41].

29.5.3 Antidepressants and Stroke Risk in Elderly

There is a wide-ranging debate on the relationship between antidepressants and cerebral vascular accidents. In elderly, this is a problem of great clinical interest taking into account the relationship between depression and stroke. Furthermore, the use of antidepressant drug poststroke is common. A number of studies have assessed the association between depression and subsequent risks of stroke morbidity and mortality, suggesting that depression could be a modifiable risk factor for stroke [42]. However, the relation of depression with antidepressant use and stroke recurrence or rehabilitation remains unclear.

In a systematic review using Cochrane methods with 7 treatment trials, including 615 patients, and 9 prevention trials including 479 patients, there was no definitive evidence that antidepressants prevent depression or improve recovery after stroke [43]. This work was redesigned by Chen and Guo [44] by means of a meta-analysis which found that antidepressant therapy was effective in the management of depression. In two meta-analysis of randomized placebo-controlled trials in patients with poststroke depression, the use of antidepressants is associated with improvement in depressive symptoms [45] and with a significant reduction of newly developed poststroke depression [46].

However, in a case-control study, an increased risk of cerebrovascular events was noted in patients with current exposure to antidepressants. The increased risk was a 24 % for SSRIs, 34 % for TCAs, and 43 % for other antidepressants [47]. Epidemiological studies had shown antidepressant use was associated with an increased risk of developing stroke [48], and fatal stroke in patients with depression who received antidepressants was even stronger [49]. A large-scale study that followed more than 80,000 women aged 54–79 years found that women who suffer depression and use antidepressants had an increased risk of stroke. However, the results should be interpreted cautiously because medication use can be a marker of depression severity [50].

Trifiro et al. [51] in a longitudinal study, conducted in almost 1,000 European patients with more than 65 years, showed an increased risk of stroke in patients who received an SSRI in relation to those who did not receive it. Interestingly, this risk was not increased among patients who were treated with TCAs or other antidepressants.

However, the controversy about the type of antidepressant used and the risk of stroke appears in three large studies conducted among Taiwanese patients. In a nationwide population-based cohort study conducted in patients with depression or anxiety treated with SSRIs or TCAs, the use of SSRIs was associated with a reduced risk for stroke compared with TCAs [52]. A more recent cohort study among more than 60,000 patients with a first stroke demonstrated that the use of all types of antidepressants had a 40 % greater risk of recurrence, especially in ischemic stroke [53]. In another nested case-control study conducted in nearly 20,000 elderly Taiwanese patients who had survived a first stroke, the use of TCAs, but not SSRIs or other antidepressants, was associated with an increased risk of stroke recurrence [54].

Furthermore, increased risk in ischemic stroke, but not hemorrhagic, occurred in a database of American patients treated with SSRIs [1] and in a case-control study [47]. Similarly, in a study case-control which included 916 patients with subarachnoid or cerebral hemorrhage, no association was found with SSRIs [55]. Douglas et al. [56] conducted a case-control study in the UK with a cohort of 365,195 patients prescribed either an SSRI or TCAs that showed no evidence of an association between current SSRI or TCA use and hemorrhagic stroke. Recently, Aarts et al. [57] studied, in 4,945 participants treated with SSRIs, the association of these antidepressants with microbleeds and ischemic vascular lesions determined by brain MRI. In general population, the use of SSRI antidepressants is not related to the presence of cerebral microbleeds. This strengthens the idea that the platelet inhibitor effects of antidepressant drugs with affinity for serotonin are minimal.

In contrast, they are controversial data. A meta-analysis of 16 observational studies involving 506,411 participants reported that SSRIs are also associated with an increased risk of intracerebral hemorrhage [58].

On the other hand, taking into account that the stroke often produces marked physical and cognitive impairments leading to functional dependence, caregiver burden, and poor quality of life, the use of antidepressants is common. Patients treated with antidepressants, fluoxetine or nortriptyline, had better recovery from disability than patients who did not receive antidepressant therapy [59]. In the FLAME trial, fluoxetine 20 mg daily given to 118 patients with motor deficits, who were not depressed, improved motor recovery and reduced disability measured with the modified Rankin scale at 3 months [60]. In a Cochrane review of SSRIs for stroke recovery that identified 52 trials of SSRIs (28 trials with fluoxetine) in stroke recruiting 4,059 patients, patients treated with SSRIs were less likely to be dependent, disabled, neurologically impaired, depressed, or anxious. However, there was a substantial methodological heterogeneity, and there seemed to be an excess of adverse events in those allocated with SSRIs [61].

Several small trials have suggested that fluoxetine improves neurological recovery from stroke. However, there is currently insufficient evidence for its routine use. For this reason it has developed a plan of collaboration through

three clinical trials: the UK (FOCUS), Australia and New Zealand (AFFINITY), and Sweden (EFFECTS) that aim to determine whether routine administration of fluoxetine (20 mg daily) for 6 months after acute stroke improves patients' functional outcome [62]. We hope that the results of these clinical studies clear us doubts about the benefit ratio risk of SSRIs in the stroke.

29.5.4 Antidepressants and Blood Pressure Changes in Elderly

The potential cardiovascular effects of TCAs are well known. They can cause orthostatic hypotension, slowed cardiac conduction, and increased heart rate and are therefore best avoided in patients with preexisting cardiovascular disease [63]. The risk of orthostatic hypotension is greater in older people. Antidepressants prescribed with vasodilators attenuated baroreceptor responses and increase the risk of orthostatism [64]. Secondary (nortriptyline) amine tricyclics cause less orthostatic hypotension and sedation than do tertiary (amitriptyline) amine tricyclics. Thus, tertiary amine tricyclics should generally be avoided in elderly patients because of the high incidence of orthostatic hypotension, sedation, cognitive problems, and cardiac effects [65].

There is a strong correlation between hypertension and depression, probably due to a hyperactive sympathetic nervous system for both disease processes. The association between the TCAs and hypertension has been suggested by some studies [66], but evidence is variable. In addition, SNRIs, as venlafaxine or duloxetine [67], induces an increase blood pressure, as well as a sustained diastolic increase [68, 69]. Venlafaxine produces dose-dependent hypertension in 5 % of patients, and above 300 mg/day, the risk is 15 %. In hypertensive patients venlafaxine no increase blood pressure. Some patients (1/3) treated with venlafaxine experienced lower blood pressure.

However, SSRIs are usually considered neutral agents on blood pressure, but taking into account their broad employment [70], cases of hypertension and hypotension have been described [68]. Fluoxetine and sertraline increase autonomic tone and improve orthostasis [71].

29.5.5 Antidepressants and Risk of Arrhythmia in Elderly

In general, it is considered that absolute risk of drug-induced life-threatening arrhythmia may be relatively low. However, considering that millions of patients are treated with antidepressants, even small increments in risk of sudden cardiac death may have a major health impact. Furthermore, in elderly with comorbidity as previous cardiac diseases or treated with other negatively interacting drugs, the absolute risk of drug-induced arrhythmia may be considerable [72].

The QT/QTc is currently generally accepted as a risk marker for arrhythmia. Most TCAs seem to prolong the QT interval, but reports of "torsade de pointes" are few and especially related to amitriptyline and maprotiline [73]. In elderly, especially with somatic comorbidity, TCAs have been related with atrioventricular block [72].

SSRIs are widely used and considered safe, but 2011 Food and Drug Administration (FDA) and European Medicines Agency (EMA) have limited the recommended maximum doses of citalopram and escitalopram. For patients older than 65 years, the maximum daily dose was reduced to 10 mg for escitalopram and 20 mg for citalopram, since they can prolong QT interval [18]. A more recent pharmacovigilance study [74] shows that escitalopram, citalopram, and amitriptyline had a dose-dependent effect on QTc prolongation. In contrast, bupropion was associated with QTc shortening, while seven other antidepressants (fluoxetine, paroxetine, sertraline, nortriptyline, duloxetine, venlafaxine, and mirtazapine) had no significant effect. Another recent paper [75] reviewed cardiovascular effects of escitalopram (5–20 mg/day) *versus* placebo in over 3,000 patients. The mean difference from placebo in the QTc was considered clinically insignificant for all escitalopram doses, and rates of cardiac adverse events were also similar.

As a result, concerns about QTc alone should not prevent effective use of citalopram and escitalopram in patients for whom these drugs are indicated.

However, caution is necessary if other drugs that prolong the QT space are used concomitantly.

29.5.6 Antidepressants and Endocrine Metabolic Syndrome in Elderly

Endocrine metabolic syndrome (EMS) is a group of abnormalities (abdominal obesity, dyslipidemia, dysglycemia, and hypertension) that predispose a person to develop type 2 diabetes mellitus and cardiovascular disease. There is evidence indicating that EMS and depression overlap some pathophysiological elements. Studies done in patients with depression suggest that the prevalence of EMS is more frequent in patients of depression compared to healthy controls [2, 3, 76, 77], and individuals with diabetes have twofold higher odds of depression than those without diabetes [78].

The presence of diabetes in patients with depression is so common that some authors have questioned the role of antidepressants in this problem [79]. However, recent evidence from case reports, observational studies, and randomized trials suggests that long-term use of any type of antidepressants increases the risk of developing diabetes. Kivimaki et al. [80] observed that continuing use of antidepressant medication was associated with an increased relative risk of type 2 diabetes, although the elevation in absolute risk was modest.

However, the nature of the relationship between antidepressants and diabetes remains unclear [81], and evidence from trials of antidepressant-associated hyperglycemia in human is scarce. Derijks et al. [82] conducted a case-control study based on spontaneous reports of adverse drug reactions (ADRs) in the database of the international pharmacovigilance program of the World Health Organization (WHO ADR database). They observed that the use of antidepressants is associated with disturbances in glucose homeostasis and can produce both hyperglycemia and hypoglycemia. The association between antidepressant use and hyperglycemia was most pronounced for antidepressants with high affinity for the NE reuptake transporter, 5-HT_{2C} receptor, and H1 receptor. Thus, secondary amines of the TCAs, such as desipramine or nortriptyline, venlafaxine, desvenlafaxina [83], or duloxetine [84], which inhibit the reuptake of norepinephrine and mirtazapine [85] have been associated with an increase in plasma levels of glucose.

In contrast, the association with hypoglycemia was most pronounced for SSRIs, antidepressants with a high affinity for the serotonin reuptake transporter with the exception of paroxetine [86, 87]. In the same way, bupropion was related to a decrease in blood glucose due to the secretion of insulin in subjects with elevated glycemia and show a favorable effect decreasing the weight in obese subjects [68].

On the other hand, TCAs have been associated with a higher rate of dyslipidemia and abdominal adiposity and therefore with a more severe symptomatology of the EMS [88]. Imipramine shows an unfavorable profile since it increases the cholesterol total/HDL-cholesterol ratio [89]. The antidepressants mirtazapine [90], venlafaxine [91], and desvenlafaxine [83] have been associated with an increase in relation LDL/HDL-cholesterol. SSRIs show a favorable lipid profile with the exception of paroxetine, since it raises levels of LDL-cholesterol and triglycerides [92]. In contrast, fluoxetine presents a favorable lipid profile even in nondepressed diabetic patients [68].

The changes of body weight are a hallmark of depression, which can be shared with different somatic diseases and increased by various antidepressants. The TCAs increase the body weight even at low doses. Similarly, there are multiple references and controlled studies in relation to mirtazapine-induced weight gain [93]. Histaminergic blockade, which induces an increase in appetite for hydrocarbon, could explain weight increase, but this is not the only mechanism [94]. Acute administration of SSRIs has a neutral behavior on body weight [68]. However, Sussman et al. [95] showed that the prolonged administration of fluoxetine, paroxetine, or sertraline for more than 16 weeks led to an increase in weight, clinically significant in 13.8 % of the patients.

On the other hand, bupropion is related to a decrease in weight, mainly in subjects with a

high body mass index (BMI) [96]. Furthermore, agomelatine, in randomized controlled trials, found an increase of the weight by more than 7 % lower (5 % of patients) than with placebo (5.7 %), SSRIs (8.8 %), and venlafaxine (5.4 %) [97].

29.5.7 Antidepressants and Risk of Hyponatremia and Syndrome of Inappropriate ADH Secretion (SIADH)

Hyponatremia associated with antidepressant use is an adverse event that preferably and disproportionately affects older people [35]. The excess of ADH secretion induced by some antidepressants, mainly SSRIs, SNRIs, and to a lesser extent TCAs, causes a hyponatremia which translates clinically by malaise, nausea, headache, lethargy, muscle pain, confusion, loss of consciousness, and seizures [3, 98], named "syndrome of inappropriate secretion of ADH" (SIADH) [99]. A mild hyponatremia may cause some degree of lethargy, which can be misinterpreted as a worsening depression. This worsening can wrongly suggest an increase in the dose of antidepressant. This could make the hyponatremia worse [100].

Although it can be considered a rare adverse effect in young, the prevalence of hyponatremia in elderly treated with SSRIs ranges from 12 to 25 %, of which 9 % have clinical symptomatology [101]. The mortality rate among elderly patients with hyponatremia may reach 25 % [102]. Since the SIADH occurs in the first month of treatment with SSRIs and also with venlafaxine, it is recommended to control the levels of sodium in the elderly, to prevent its evolution toward more serious complications [2, 3, 98]. In elderly with kidney disease, SSRIs are best avoided [103].

29.5.8 Antidepressant Drugs and Bleeding Risk

Bleeding has been a concern since the introduction of SSRIs in most studies, including elderly patients [104]. Currently, there are about 15 epidemiological studies that show the existence of a risk of high digestive bleeding with SSRIs. This effect is mediated by the antiplatelet effect of SSRIs [105]. This risk is magnified with the association of nonsteroidal anti-inflammatory (NSAIDs) and the use of proton pump inhibitors can be protector [106].

In general, the epidemiological evidence supports a moderately increased risk of gastrointestinal bleeding, depending on patient susceptibility and the presence of other risk factors, as aging, previous history of upper-gastrointestinal bleeding or peptic ulcer, and in those who take NSAIDs, oral anticoagulants, antiplatelet drugs, or corticosteroids, associated to the use of SSRIs, extended probably to venlafaxine [106, 107]. Combined use of antidepressants and NSAIDs was associated with an increased risk of intracranial hemorrhage within 30 days of initial combination [108].

However, a case-control study conducted in Italy confirmed the gastrointestinal bleeding risk with SSRIs and venlafaxine, even in patients not receiving NSAIDs, corticosteroids, anticoagulants, or antithrombotic [109]. Furthermore, serotonergic antidepressants are associated with increased perioperative bleeding events, particularly abnormal bleeding [110].

Therefore, precaution in patients treated with SSRIs in individuals with risk of digestive bleeding or treated with NSAIDs or other anticoagulants is recommended. In these cases the selection of an antidepressant that does not affect serotonergic mechanisms is preferable.

29.5.9 Antidepressants and Osteoporotic Fracture Risk

Depression adversely affects bone density and increases fracture risk [111]. Evidence from longitudinal, cross-sectional, and prospective cohort studies suggests that the use of antidepressants at therapeutic doses is associated with decreased bone mineral density and increased fracture risk [112]. The principal antidepressants implicated are SSRIs that were associated with a statistically significant increase in the risk of osteoporotic fractures [113] especially in the early stages of

treatment, with a dramatic increase after initiation [114]. The risk with SSRIs, probably related with the degree of inhibition of reuptake of serotonin in osteoclasts, osteoblasts, and osteocytes [115, 116], can be higher than with TCAs or other antidepressants. Moreover, the risk of nonvertebral fractures in older women is increased both by treatment with SSRIs and TCAs [117], and the recent Canadian Multicentre Osteoporosis Study (CaMos) supports an association between SSRI and SNRI use and fragility fractures [118]. In fact, both the SNRIs and the TCAs also inhibit serotonin reuptake.

29.5.10 Sexual Dysfunction and Antidepressants in Elderly

Sexuality remains a prominent part of the lives of older persons, but many people believe that older adults do not or should not have sexual activity. Despite the fact that both depression and antidepressants can worsen the sexuality in the elderly, it is a condition that does not usually be investigated. Sexual dysfunction (SD) affects orgasm, sexual arousal and desire, ejaculation, erection, and dyspareunia. These effects may lead to no adherence, early termination of treatment, and worsening of quality of life [2, 3].

Patients treated with SSRIs, particularly escitalopram and paroxetine, and venlafaxine have significantly highest rates of overall SD. TCAs can also induced SD. In contrast, bupropion, mirtazapine, or agomelatine, and the recent agent introduced in therapeutic, vortioxetine, 10 mg daily, has been associated with an incidence rate of SD similar to placebo [18, 119]. Therefore, mirtazapine, bupropion, or agomelatine can be a valid alternative [120–122].

29.6 Safety of Antidepressants in Elderly

The main risk that can occur with antidepressants is a hypothetic increase in mortality. Prospective studies have consistently shown an association between depression and increased mortality in older adults. It is important to consider the mortality due to the depressive condition, associated with a poor adherence to medical treatment, bad self-care for diabetes and cardiovascular disease, smoking, lack of physical activity, cognitive impairment, and disability. On the other hand, mortality caused by voluntary or accidental overdose with suicidal intentions should be taken into account [2, 3].

Prospective studies have consistently shown an association between depression and increased for no suicide mortality in older adults [123] and persistent depressive symptom increased risk of dying in older primary care patients [124]. On the other hand, elderly with major depression in practices provided with additional resources to intensively manage depression had a mortality risk lower than that observed in usual care and similar to older adults without depression [125]. Furthermore, depression management mitigated the combined effect of multimorbidity and depression on mortality [126]. Therefore, the treatment of depression in the elderly appears to decrease the non-suicide mortality rate.

SSRIs are considered agents of first choice in the elderly for his tolerability and safety. For this reason is surprisingly a recent cohort study in elderly depressed patients that concluded that SSRIs and other antidepressants, as mirtazapine or venlafaxine, were associated with significantly higher rates of all causes of mortality and increased risk of several adverse effects compared with TCAs. However, as this is an observational study, it is susceptible to confounding by some bias as indications or characteristics between patients prescribed with different antidepressant drugs. In fact, doses tended to be lowest for TCAs (70 % of prescriptions were for ≤0.5 defined daily doses), compared with SSRIs (13.8 %). Furthermore, patients who are not treated are likely to have less severe depression and may have poorer physical health, such that they are considered too frail for antidepressant treatment [35].

Apparently, these data can be in contrast with other strong opinions. Thus, TCAs are drugs

classically included in Beers criteria of potentially inappropriate medication use in older adults [127], and the NICE and other clinical guidelines recommend that normally a SSRI should be chosen [40].

Another aspect to consider is the relationship between depression, suicide, and antidepressants. The risk of antidepressant overdose is increased in the elderly. The high risk of suicides in this population (especially from 75 to 80 years) and the physical and cognitive limitations increase errors in dosage. The higher risk is related to the cardiotoxicity, and the potentially most dangerous are TCAs and SSRIs are the safest. The TCA's toxicity is very fast; arrhythmias, seizures, hypotension, and coma appear in an hour [128]. Akathisia, tremor, agitation, hyperreflexia, delirium, fever, diarrhea, convulsions, clonus, hypertonia, tachycardia, and mydriasis predominate in the intoxication with SSRIs. The presentation is mixed among the TCAs and SSRIs with dual antidepressants. Venlafaxine produces seizures and QRS prolongation in 13 % and 36 % of the patients, respectively [2, 3, 129].

Suicide, at least in some groups of elders, has reached the proportions of a real epidemic. In the US population older than 85 years, suicide rate is five times of the general population [130]. Suicide rates have tended to decrease more in Europe and other countries where there has been a greater increase in the use of antidepressants [131, 132]. In fact, antidepressants reduce the risk of suicide among elderly patients [133], and the correct clinical use of antidepressants, especially in the elderly, is more of a benefit than a risk [134].

In general, SSRIs are relatively safe in overdose [128], but clinicians should warn patients of the possible risk of suicidal behavior and monitor patients closely, especially in the early stages of treatment. Also, caution should be exercised with high doses of citalopram or escitalopram for a possible relation to QT interval prolongation [74, 135]. In patients with high risk of overdose, it is important to avoid TCAs or venlafaxine by their cardiotoxicity. The dose of CNS affecting drugs in 2005–2012 was considered lethal in 8.6 % of the inquiries due to TCAs, but only in 1.6 % calls due to SSRIs [136].

Appropriate antidepressant use in elderly is more of a benefit than a risk; thus we must not penalize the entire population by denying effective care [2, 3, 130].

29.7 Potential Drug-Drug Interactions: An Important Criterium to Select an Antidepressant in Elderly

Drug interactions with antidepressants is of great interest since the vast therapeutic arsenal, the longer life expectancy of patients, and comorbidity have increased dramatically the number of described cases of drug interactions [137].

Comorbidity and polypharmacy in the elderly and its fragility practically guarantee the emergence of drug interactions. A prospective cohort study in hospitalized elders treated with five or more drugs had a prevalence of potential drug interactions of 80 % at the level of CYP450. Think of interaction help to avoid it [138]. In the older people, pharmacodynamic and pharmacokinetic processes responsible for these interactions are significantly altered, so the possibility of them is higher than in the young adult [27].

Regarding interactions, it is convenient to consider the epidemiological frequency of the combination, the frequency of interaction when drugs are coadministered, the therapeutic index of interacting substances (ratio between the therapeutic and toxic levels), or the possibility of establishing an individualized posology. For these reasons, it is very important to detect the clinically important interactions. So, antidepressants can cause pharmacokinetic and pharmacodynamic interactions with drugs with a low therapeutic range and used frequently in elderly as digoxin, theophylline, NSAIDs, oral antidiabetics, insulin or anticoagulants, alcohol, and other psychoactive drugs. A good knowledge of interactions not only stops the

prescription but helps it and provides alternatives [2, 3, 12, 137].

Conclusions

The heterogeneous group of elderly is necessary to perform an adequate therapeutic, to consider the possibility of physiological changes. These changes do not always flow in parallel with the chronology of the age.

The elderly have more medical comorbidity and more previous depressive episodes, both of which adversely affect outcome, and relapse rates appear higher than in younger subjects [5]. The somatic comorbidity requires established treatments to be compatible.

Similarly, in the elderly with an altered response of several organs, the concentrations of drugs in the tissue targets favor the emergence of adverse reactions. It's necessary to know the habits of the patient; the use of tobacco, alcohol, and caffeine; as well as the administration of other drugs, indicated for other pathologies or self-medicated, since they can modify the kinetic behavior of psychoactive drugs and cause interactions.

The elderly should begin treatment with doses lower than usual due to decreased tolerability to antidepressants and increase them slowly up to the therapeutic dose. Simple dosing regimen facilitates adherence.

The use of medications in the elderly should be performed only when necessary, but age should not deprive the patient's medication that improves their health and quality of life [2, 3, 18].

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Antidepressant Efficacy of Escitalopram in Major Depressive Disorder

30

Eiji Kirino

30.1 Introduction

Escitalopram is a selective serotonin reuptake inhibitor (SSRI) that selectively binds to the human serotonin transporter (SERT). This activity inhibits serotonin (5-HT) reuptake and increases the amount of serotonin in synaptic clefts, which results in antidepressant action.

Racemic citalopram (RS-citalopram), an SSRI widely used in patients with major depressive disorder (MDD), possesses both an active S-enantiomer and clinically inactive R-enantiomer [1, 2]. Escitalopram was produced by isolating the active S-enantiomer from RS-citalopram. In vitro and in vivo studies have shown that escitalopram inhibited the serotonin transporter protein more potently than citalopram [2–4]. For example, in vivo electrophysiological data indicated that escitalopram was four times more potent than citalopram in reducing the firing activity of presumed serotonergic neurons in the dorsal raphe nucleus of rat brain [5]. In November 2011, escitalopram was approved in 100 countries in Europe, North America, and other regions. Escitalopram is indicated for generalized anxiety disorder, social anxiety disorder, obsessive-compulsive disorder, panic disorder, premenstrual dysphoric disorder, and MDD [6].

E. Kirino, MD, PhD
Department of Psychiatry, Juntendo University Shizuoka Hospital, 1129 Nagaoka Izunokunishi, Shizuoka 4102211, Japan

Department of Psychiatry, Juntendo University School of Medicine, Tokyo, Japan
e-mail: ekirino@med.juntendo.ac.jp

30.2 Pharmacological Profile

30.2.1 Pharmacodynamic Profile

Escitalopram has a highly selective, dose-dependent, inhibitory effect on SERT. Its antidepressant action arises from its inhibition of serotonin reuptake into presynaptic nerve ending, which enhances serotonin activity in the central nervous system [1, 7]. Radioligand binding assays revealed that escitalopram showed particularly high selectivity for SERT compared to citalopram and several other SSRIs [7–9]. Escitalopram is "the most typical SSRI" of the SSRI agents, because it has virtually no binding affinity for other transporters [7, 9].

Escitalopram binds to two different sites of SERTs. It binds to the high-affinity binding site (primary site) of SERT, which controls serotonin reuptake in nerve endings, and it binds to the low-affinity binding site (allosteric site), which induces structural changes in SERT. The latter (allosteric action) is thought to stabilize and prolong binding of escitalopram to the primary site [3, 10–12].

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30.2.2 Pharmacokinetic Profile

The half-life of receptor occupancy for escitalopram was calculated to be approximately 130 h, much longer than the half-life of the plasma concentration, which was approximately 30 h [13]. An allosteric action may be involved in this prolonged occupancy. Escitalopram is metabolized in the liver, mainly by cytochrome P-450 (CYP) 2C19 and also by CYP3A4 and CYP2D6. Escitalopram inhibits liver metabolic enzymes, but primarily only CYP2D6 [14], with minimal inhibition of the other enzymes; the IC_{50} for CYP2D6 was higher than its effective blood concentration. In this regard, its interactions with other drugs would presumably be minimal.

30.3 Clinical Efficacy

30.3.1 Comparison with Placebo

In a placebo-controlled study [15], patients with MDD received escitalopram at a dose of 10 mg/day, and a control group was given placebo. After 8 weeks of therapy, the total Montgomery-Asberg Depression Rating Scale (MADRS) score changed by −16.3 in the escitalopram group and −13.6 in the placebo group. Thus, escitalopram had significantly greater efficacy than placebo. The total MADRS score of the escitalopram group began to show significant improvement compared to that of the placebo group by the second week of therapy. This demonstrated its fast-acting property. In addition, the remission rate (the percentage of patients with a total MADRS score of 12 or less) was significantly higher in the escitalopram group than in the placebo group. Thus, the initial therapeutic dose (10 mg/day) was demonstrated to be effective. Likewise, in other studies [15, 16], escitalopram 10 or 20 mg/day was more effective than placebo in the treatment of MDD. Reduction in MADRS scores, the primary endpoint, was greater with escitalopram than with placebo as week 1 [16] or 2 [15] and was maintained throughout treatment. Furthermore, Clinical Global Impression-Improvement (CGI-I) and Clinical Global Impression-Severity (CGI-S) scores were reported [15] and support the MADRS score findings: escitalopram produced significant lower CGI-I scores from week 1 and CGI-S scores from week 3 than placebo and this continued throughout treatment.

30.3.2 Comparison with SSRIs

Six randomized, double-blind, controlled studies [16–21] compared escitalopram and citalopram. Escitalopram was administered to patients with MDD for 4–8 weeks at 10–20 mg/day. All six studies [16–21] showed that the efficacy of escitalopram was equivalent to or greater than that of citalopram. Details of these studies are as follows. In the study of Burke et al. [16] ($N=491$; randomly assigned to placebo, escitalopram, 10 mg/day, 20 mg/day or citalopram, 40 mg/day), escitalopram (10 mg/day) was at least as effective as citalopram (40 mg/day) at endpoint. In the study of Lepola et al. [17], by week 8, significantly more patients had responded to treatment with escitalopram ($N=155$) than with citalopram ($N=160$). In the study of Lalit et al. [18], response rates at the end of 2 weeks were 58 % for escitalopram (10 mg/day) ($N=69$) and 49 % for citalopram ($N=74$) (20 mg/day). Response rates at the end of 4 weeks were 90 % for escitalopram (10–20 mg/day) and 86 % for citalopram (20–40 mg/day). The remission rates at the end of 4 weeks were 74 % for escitalopram and 65 % for citalopram. Additionally, there were lesser dropouts and lesser requirement for dose escalation in escitalopram than in citalopram. In the study of Moore et al. [19], MADRS score decreased more in the escitalopram ($N=138$) than in the citalopram arm ($N=142$). There were more treatment responders with escitalopram (76.1 %) than with citalopram (61.3 %), and adjusted remitter rates were 56.1 % and 43.6 %, respectively. In the study of Yevtushenko et al. [21] ($N=322$; randomly assigned to escitalopram, 10 mg/day or citalopram, 10–20 mg/day), at study end, the mean change from baseline in MADRS total score was significantly greater in the escitalopram arm than in the 10 and 20 mg/day citalopram

arms. Changes in the CGI-S and CGI-I scores and the rates of response and remission were significantly greater in the escitalopram group compared with those in the citalopram 10- and 20-mg/day groups. On the other hand, in the study of Ou et al. [20] ($N=240$, randomly assigned to escitalopram, 10–20 mg/day or citalopram, 20–40 mg/day), no significant differences were found in the change in the total Hamilton Depression Rating Scale (HAM-D17) score between the two groups.

The meta-analysis of Montgomery et al. [22] comparing escitalopram and citalopram supported these controlled studies: escitalopram was significantly more effective than citalopram in overall treatment effect, with an estimated mean treatment difference of 1.7 points at week 8 on the MADRS and in responder rate (8.3 percentage points) and remitter rate (17.6 percentage points) analyses, corresponding to number-needed-to-treat (NNT) values of 11.9 for response and 5.7 for remission. The overall odds ratios were 1.44 for response and 1.86 for remission, in favor of escitalopram. However, Trkulja [23] reported that MADRS reduction was greater with escitalopram, but 95 % confidence intervals (CI) around the mean difference were entirely or largely below 2 scale points (minimally important difference), and CI around the effect size (ES) was below 0.32 ("small") at all-time points. Risk of response was higher with escitalopram at week 8 (relative risk, 1.14; 95 % CI, 1.04–1.26), but number needed to treat was 14 (95 % CI, 7–111). All 95 % CIs around the mean difference and ES of CGI-S reduction at week 8 were below 0.32 points and the limit of "small," respectively. The report concluded that the claims about clinically relevant superiority of escitalopram over citalopram in short-to-medium-term treatment of MDD are not supported by evidence.

A long-term, double-blind, controlled study compared paroxetine to escitalopram given for 24 weeks to patients with severe disease [24]. In that study, escitalopram at 20 mg/day showed better efficacy than paroxetine at 40 mg/day. The total MADRS score changed by −25.2 in patients given escitalopram and by −23.1 in those given paroxetine. Thus, the outcome was significantly better for the escitalopram group, with an intergroup difference of 2.12. Furthermore, the HAM-D17 total score changed by −16.9 and −15.0 in the two groups, respectively; again this showed a significantly better outcomes for the escitalopram group than for the paroxetine group. In addition, the remission rate (percentage of patients with a total MADRS score of 12 or lower) was significantly higher (75.0 %) in the escitalopram group than in the paroxetine group (66.8 %). On the other hand, another study [25] that compared variable doses of escitalopram (10–20 mg/day) and paroxetine (20–40 mg/day) revealed equivalent efficacy in the two groups at week (end of acute treatment), although significantly more patients withdrew from the paroxetine group (34 %) than from the escitalopram group (21 %), and significantly more paroxetine patients withdrew due to lack of efficacy. In severely depressed patients (baseline MADRS total score ≥30), escitalopram was superior to paroxetine at week 27 (end of maintenance treatment).

In an 8-week double-blind randomized comparative study [26] with escitalopram (10 mg/day fixed-dose) or sertraline (50–200 mg/day flexible dose), no difference in efficacy was observed for either treatment. The mean changes from baseline to endpoint in MADRS scores were −19.1 and −18.4 for the escitalopram and sertraline groups, respectively, with response rates of 75 % and 70 % for escitalopram- and sertraline-treated patients, respectively. Both treatments were generally well tolerated. Consistent with these findings, a meta-analysis by Cipriani et al. [27] and comments by Patrick et al. in a related paper [28] advocated escitalopram and sertraline as the two "best" drugs in terms of efficacy and acceptability.

30.3.3 Comparison with SNRIs

In a double-blind, controlled study [29] of escitalopram (10–20 mg/day) vs. duloxetine (60 mg/day) for 8 weeks, the changes in the total MADRS scores were −18.0 ± 9.4 and −15.9 ± 10.3, respectively. This result showed that escitalopram was significantly superior to duloxetine. In another long-term, double-blind, controlled study [30] of

escitalopram (20 mg/day) vs. duloxetine (60 mg/day) for 24 weeks, the total MADRS score improved significantly to a greater extent in the escitalopram group than in the duloxetine group at week 8. This trend persisted until week 24.

Escitalopram has also shown equivalent or superior efficacy to that of venlafaxine extended release (XR) and better tolerated [31, 32]. In an 8-week double-blind randomized parallel-group trial [31], there were no significant differences in measures of efficacy between the two antidepressants, although tolerability measures favored escitalopram over venlafaxine XR. Another 8-week double-blind randomized parallel-group trial indicated that the efficacy of escitalopram was similar to venlafaxine XR; this was based on the mean change from baseline to week 8 in MADRS total score. However, escitalopram-treated patients achieved a sustained remission significantly faster than venlafaxine-treated patients. There were higher incidences of nausea, constipation, and increased sweating in the venlafaxine-treated patients, and significantly more of these patients had discontinuation symptoms when treatment was completed at week 8.

30.3.4 Relapse and Recurrence Prevention Study

An MDD relapse prevention study [33] was carried out in another group of patients aged 65 and older. Escitalopram was administered at a dose of 10 mg or 20 mg/day for 12 weeks. Patients that reached remissions (a total MADRS score of 12 or lower) were allocated to receive either escitalopram at 10 mg or 20 mg/day or placebo. The two groups were followed to determine the relapse rate. The cumulative non-relapse rate remained high in the escitalopram group but decreased over time in the placebo group. At the end of study, relapses were observed in only 9 % of the escitalopram group and 33 % of the placebo group; thus, the relapse rate was significantly lower in the escitalopram group.

An MDD recurrence prevention study [34] examined recurrences after 16 weeks of continuous therapy with escitalopram. Patients given escitalopram at a fixed dose of 10 mg or 20 mg/day were compared to controls given placebo for 52 weeks of maintenance therapy. Time to recurrence was significantly longer in patients who received maintenance treatment with escitalopram compared with patients switched to placebo, and MDD recurrence was 27 % in the escitalopram group significantly lower than the 65 % observed in the placebo group (Table 30.1).

30.4 Tolerability

Patients with MDD generally exhibited favorable tolerance to escitalopram, regardless of whether they received short-term or long-term therapy. Adverse events were typically mild and temporary [35]. The most frequent adverse events that occurred during escitalopram therapy included insomnia, nausea, excessive sweating, fatigue/somnolence, dysspermatism, and decreased libido [36].

30.4.1 Comparison with SSRIs or SNRIs

Escitalopram was compared to other SSRIs or SNRIs in a meta-analysis of patient data from 16 double-blind, controlled studies [37]. When attention was focused on adverse events that occurred at a frequency of 5 % or more, escitalopram showed significantly lower frequencies of diarrhea and dry mouth and the presence of more than one adverse event compared to the other SSRIs. Escitalopram was also associated with significantly lower frequencies of nausea, insomnia, dry mouth, vertigo, excessive sweating, constipation, and vomiting than the SNRIs.

30.4.2 Discontinuation Symptoms

Discontinuation symptoms typically occur at the end of treatment with antidepressant drugs. A detailed study [38] compared discontinuation symptoms in patients with MDD during the post-therapy observation period after 27 weeks of

Table 30.1 Summary of clinical and safety outcomes of escitalopram treatment of major depressive disorder as reported by randomized controlled trials

Study	Study design	Subjects (N)	Dose (mg/day)	Clinical outcomes	Safety outcomes
Wade et al. [15]	8-week double-blind placebo-controlled trial	MDD (380)	Escitalopram 10 (fixed doses)	MADRS score changed by −16.3 in the escitalopram group and −13.6 in the placebo group	Well tolerated in all patients. Nausea episodes significantly increased in the escitalopram group than in the placebo group
Burke et al. [16]	8-week randomized double-blind placebo-controlled fixed-dose multicenter trial	MDD (49)	Escitalopram 10, 20; citalopram 40 (fixed doses)	Escitalopram (10, 20 mg/day) produced significant improvement at study endpoint relative to placebo Escitalopram (10 mg/day) was as effective as citalopram (40 mg/day) at study endpoint	Discontinuation due to adverse events for the escitalopram (10 mg/day) group was not different from those for the placebo group, and no difference between the escitalopram (20 mg/day) and citalopram (40 mg/day) groups was noted
Lepola et al. [17]	8-week randomized double-blind placebo-controlled trial	Moderate to severely depressed patients ($n=469$)	Escitalopram 10–20; citalopram 20–40 (flexible doses)	Significantly more patients responded to escitalopram than to citalopram or the placebo	Escitalopram was as well tolerated as citalopram. Both escitalopram and citalopram had placebo-level adverse event withdrawal rates
Lalit et al. [18]	4-week randomized double-blind parallel-group controlled multicentric trial	MDD (214)	Citalopram 20–40; escitalopram 10–20; sertraline 50–150 (flexible doses)	Response rates after 2 weeks were 58 % for escitalopram (10 mg/day) and 49 % for citalopram (20 mg/day) Response rates after 4 weeks were 90 % for escitalopram (10–20 mg/day) and 86 % for citalopram (20–40 mg/day). Remission rates after 4 weeks were 74 % for escitalopram and 65 % for citalopram	There were fewer dropouts in the escitalopram group than in the citalopram group
Moore et al. [19]	8-week double-blind randomized trial	MDD (280)	Escitalopram 20; citalopram 40 (fixed doses)	MADRS score decreased more in the escitalopram group than in the citalopram group There were more treatment responders with escitalopram (76.1 %) than with citalopram (61.3 %). Adjusted remission rates were 56.1 % and 43.6 %, respectively	Tolerability was similar in both groups

(continued)

Table 30.1 (continued)

Study	Study design	Subjects (N)	Dose (mg/day)	Clinical outcomes	Safety outcomes
Yevtushenko et al. [21]	6-week prospective randomized double-blind active control trial	MDD (322)	Escitalopram 10; citalopram 10, 20 (fixed doses)	Mean change in MADRS was significantly greater in the escitalopram group than in the 10 and 20 mg/day citalopram groups	Prevalence of adverse events was significantly lower with escitalopram than with citalopram. Nausea and headache were the most frequently reported adverse events
				Changes in CGI-S and CGI-I and response and remission rates were significantly greater in the escitalopram group than in the citalopram 10 and 20 mg/day groups	
Ou et al. [20]	6-week double-blind trial	MDD (240)	Escitalopram 10–20; citalopram 20–40 (flexible doses)	No significant differences were found between the two groups for changes in the HAM-D17 total score	No significant differences were found between the two groups for adverse events
Boulenger et al. [24]	24-week long-term double-blind controlled trial	MDD (459)	Escitalopram 20; paroxetine 40 (flexible doses)	Escitalopram (20 mg/day) showed better efficacy than paroxetine (40 mg/day). The total MADRS score changed by −25.2 in escitalopram group and by −23.1 in paroxetine group	Withdrawal rate due to adverse events was significantly lower for escitalopram (8 %) than for paroxetine (16 %). There were no significant differences in the incidence of adverse events during treatment. HAMD-17 total score change indicated significantly better outcomes for escitalopram (−16.9) than for paroxetine (−15.0). The remission rate was significantly higher (75.0 %) with escitalopram than in paroxetine (66.8 %)
Baldwin et al. [25]	27-week double-blind randomized parallel-group trial	MDD (323)	Escitalopram 10–20; paroxetine 20–40 (8-week flexible-dose acute period followed by 19-week fixed-dose maintenance period)	Equivalent efficacy was found in both groups at week 8	Paroxetine demonstrated significantly more discontinuation symptoms relative to escitalopram based on DESS scores
				Significantly more paroxetine-treated (34 %) than escitalopram-treated patients (21 %) withdrew from the study. Significantly more paroxetine-treated patients withdrew due to lack of efficacy at week 27	
				In severely depressed patients, escitalopram was superior to paroxetine at week 27	

Ventura et al. [26]	8-week randomized double-blind multicenter parallel-group trial	MDD (212)	Escitalopram 10 (fixed doses); sertraline; 50–200 (flexible doses)	No differences in efficacy were observed for escitalopram and sertraline treatments	Both treatments were generally well tolerated
Khan et al. [29]	8-week randomized double-blind multicenter parallel-group trial	MDD (278)	Escitalopram 10–20 (fixed at 10 for the first 4 weeks followed by flexible doses), duloxetine; 60 (fixed doses)	A significantly greater proportion of escitalopram-treated patients than that of duloxetine-treated patients completed the 8-week study (87 % vs. 69 %). At week 8, escitalopram treatment resulted in significantly greater improvement than duloxetine treatment	Significantly fewer escitalopram-treated patients discontinued because of adverse events than duloxetine-treated patients (2 % vs. 13 %)
Wade et al. [30]	24-week double-blind randomized parallel-group trial	MDD (294)	Escitalopram 20; duloxetine 60 (fixed doses)	MADRS score improvement was significantly better in the escitalopram group than in the duloxetine group at week 8. This trend persisted until week 24	Withdrawal rate due to adverse events was lower for escitalopram (9 %) than for duloxetine (17 %), and significantly more patients treated with duloxetine reported insomnia (12.6 % vs. 4.9 %, respectively) and constipation (8.6 % vs. 2.8 %, respectively)
Gorwood et al. [33]	12-week open-label trial followed by a 24-week randomized double-blind placebo-controlled trial	MDD (305)	Escitalopram 10, 20 (fixed doses).	Significantly fewer escitalopram-treated patients (9 %) than placebo-treated patients (33 %) relapsed	Escitalopram was well tolerated with 53 patients (13 %) withdrawn as a result of adverse events during the open-label phase, and 3 (2 %) escitalopram-treated patients and 6 (4 %) placebo-treated patients were withdrawn as a result of adverse events during the double-blind treatment phase (not significant)
Kornstein et al. [34]	16-week open-label trial followed by 52-week randomized, double-blind placebo-controlled trial	MDD (139)	Escitalopram 10, 20 (fixed doses)	Time to recurrence was significantly longer in patients who received maintenance treatment with escitalopram than in patients switched to placebo	Long-term escitalopram treatment was well tolerated
				MDD recurrence was 27 % in the escitalopram group, which was significantly lower than the 65 % observed in the placebo group	

therapy with escitalopram (20 mg/day) or paroxetine (40 mg/day). Discontinuation symptoms were evaluated in terms of the Discontinuation Emergent Signs and Symptoms (DESS) score. During the observation period, the drug doses were gradually decreased over 1–3 weeks, followed by 1 week of alternate-day dosing and, subsequently, 1–3 weeks of placebo. The escitalopram group exhibited smaller changes in the total DESS score and significantly less frequent discontinuation symptoms compared to the paroxetine group, both at the end of alternate-day dosing and after 1 week of placebo administration. On the other hand, it has been reported that an antidepressant withdrawal syndrome may induce manic states in patients treated for major depression, even in the absence of a history of bipolar disorder [39–42], and there has been a case report of a young woman with unipolar depression who developed a manic state after abrupt discontinuation of low-dose escitalopram [43]. This manic state remitted when escitalopram was reintroduced within a week after the interruption of treatment [43]. Therefore, careful observation should be performed when discontinuing escitalopram, although escitalopram induces less discontinuation symptoms compared with other SSRIs.

30.4.3 Suicidality

Suicidality was studied in a detailed meta-analysis [44] conducted on data from 34 placebo-controlled studies on SSRIs. That analysis included >40,000 patients, and approximately 2,600 had been treated with escitalopram. They found one instance of suicide, which occurred 6 days after treatment cessation. Another analysis of placebo-controlled studies [46] specifically included patients with MDD or anxiety disorders that used escitalopram. They reported no suicides during the first 2 weeks of treatment or during the entire period of escitalopram (<24 weeks), but one suicide occurred in the placebo group. Furthermore, there was no indication of increased risk of nonfatal self-harm or suicidal thoughts among patients that received escitalopram compared those that received placebo [45]. Rather, escitalopram reduced the MADRS item 10 ("suicidal thought") or HAM-D item 3 ("suicidal thought") scores to a significantly greater extent than placebo [16, 45, 46]. For an estimated >12 million patients with MDD and/or anxiety disorders treated with escitalopram, pharmacovigilance information revealed a suicide rate of 1.8 per 1 million patients; this rate was similar to that in patients treated with citalopram (2 per 1 million) and considerably lower than that in patients treated with tricyclic antidepressants (12 per 1 million) or monoamine oxidase inhibitors (MAOIs) (14 per 1 million) [45].

30.4.4 Sexual Dysfunction

A small, retrospective study [47] ($N=47$) indicated that two-thirds of patients with SSRI/SNRI-induced sexual dysfunction reported mild or marked improvements after switching to a regimen with escitalopram. However, several reports have suggested that escitalopram may be associated with increased sexual dysfunction in both men and women compared to bupropion or sertraline [48, 49].

30.4.5 QT Prolongation

The cardiovascular safety of antidepressants has been the subject of recent debate. Additionally, the prescribing information and recommended dosing for citalopram have been modified to address concerns about the risk of QTc prolongation [50, 51]. Cardiovascular effects of escitalopram were assessed in participants in double-blind randomized placebo-controlled studies [52]. Escitalopram-placebo differences in mean changes in ECG values were not clinically meaningful. The difference compared to placebo in systolic or diastolic blood pressure (BP) was not clinically or statistically significant. The mean differences when compared to placebo in the corrected QT [Fridericia's (QTcF)] interval were 3.5 ms (all escitalopram doses), 1.3 ms (escitalopram 10 mg), and 1.7 ms (escitalopram 20 mg,

$p = 0.2836$ for 10 vs. 20 mg). One out of 2,407 escitalopram patients had a QTcF interval >500 ms and a change from baseline of >60 ms. The incidence and types of cardiac-associated adverse events were similar among patients treated for 8–12 weeks with placebo (2.2 %) or escitalopram (1.9 %) as well as patients treated for 24 weeks with placebo (2.7 %) or escitalopram (2.3 %). These data demonstrate that escitalopram, like other SSRIs, has a statistically significant effect on heart rate and no clinically meaningful effect on ECG values or BP compared with the observed placebo-level incidence of cardiac-associated adverse events. However, caution is required in administering escitalopram to aged individuals, patients with liver dysfunction, patients with defective CYP2C19 activity, or patients that have received other drugs that confer a risk of QT prolongation [53, 54].

30.4.6 Overdosage

In a retrospective analysis [55] of 28 patients that underwent a supratherapeutic ingestion of escitalopram (5–300 mg), only one patient reported adverse events. That patient was admitted to a hospital for persistent lethargy, but the outcome was good. However, when escitalopram is taken at high doses or in polysubstance ingestions, CNS depression may occur. Patients ($N = 13$) that had taken escitalopram (mean dosage 126 mg) as a co-ingestant in polysubstance ingestions exhibited CNS depression (54 %), cardiovascular effects (54 %), and ECG changes (23 %) [56]. In a case report [57], after an overdose of escitalopram (100–200 mg), a 38-year-old man exhibited severe, prolonged serotonin syndrome and elevated serum escitalopram concentration.

30.4.7 Hyperglycemia

The exact mechanism responsible for the impairment of glucose control in patients taking SSRIs such as escitalopram is still unclear; however, there has been a case report on escitalopram-induced hyperglycemia in an 83-year-old female patient with diabetes [58], suggesting that escitalopram may cause the loss of glycemic control.

30.5 Patient Acceptability

A meta-analysis by Cipriani et al. [27] reported on the efficacy and patient acceptability of 12 new antidepressant drugs. In that meta-analysis, patient acceptability was defined as the persistence observed in taking a drug during an 8-week therapy. Escitalopram and sertraline showed the best profile of acceptability, leading to significantly fewer discontinuations than did duloxetine, fluvoxamine, paroxetine, reboxetine, and venlafaxine. Especially, among those 12 drugs, escitalopram was associated with the highest rate for both efficacy and acceptability.

The rates of discontinuing therapy were analyzed among pooled data from double-blind, controlled studies of escitalopram vs. paroxetine [59] or duloxetine [60]. The pooled data for paroxetine was derived from two studies that treated patients for 24 [24] and 27 weeks [25]. The discontinuation rate at the end of the study period was significantly lower for patients on escitalopram (16.8 %) than for those on paroxetine (27.9 %). When the reason for discontinuing therapy was restricted to adverse events, the discontinuation rates remained significantly lower for escitalopram (6.6 %) than for paroxetine (11.7 %).

The pooled data for duloxetine were derived from two studies that treated patients for 8 [29] and 24 weeks [30]. The discontinuation rate at the end of the study period was significantly lower for escitalopram (12.9 %) than for duloxetine (24.6 %). When the reason for discontinuing therapy was restricted to adverse events, the discontinuation rates remained significantly lower for escitalopram (4.6 %) than for duloxetine (12.7 %). Thus, escitalopram was associated with high therapy continuity.

MDD has a relatively high likelihood of recurrence. Thus, high therapy continuity with escitalopram represents an advantage for patients with this disease. There may be several reasons for the high therapy continuity of escitalopram. First, it

has high efficacy and good tolerability, as shown in the clinical studies mentioned in Sects. 2 and 3 above. Thus, dropouts from escitalopram therapy due to insufficient efficacy or adverse events appeared to be limited. Furthermore, the demonstrated efficacy of escitalopram at an initial dose of 10 mg [15] could be detected in early therapeutic phase by patients [15, 30]. It was speculated that early signs of improvement most likely led to increased adherence, which, in turn, led to prevention of relapse [33] and recurrence [34].

The fact that escitalopram demonstrated preventive effects on relapse [33] and recurrence [34] represented major benefit to patients that desire to be reintegrated into society. For instance, for a company employee that wants to return to work, escitalopram may facilitate the return-to-work program, and thus, the patient would expect to return to work smoothly.

Conclusion

This review provided an overview of escitalopram focusing on its efficacy, tolerability, and patient acceptability in the management of MDD. In terms of efficacy, escitalopram was superior to placebo and equal to or better than paroxetine or other SSRIs and SNRIs. In addition, escitalopram exerted a stable antidepressive action. Escitalopram had high tolerability, because adverse events related to escitalopram therapy were generally mild and temporary. Moreover, discontinuous symptoms were apparently milder than those related to paroxetine therapy.

The meta- and pooled analyses [27] showed high patient acceptability of escitalopram, which indicated that patients found it easy to continue this antidepressant therapy. Therefore, escitalopram can be regarded as an antidepressant drug associated with high therapy continuity, and the high efficacy of escitalopram is in part based on improved adherence due to high tolerability. In addition, the high therapy continuity of escitalopram can be expected to prevent relapses and recurrences. A comparison with placebo demonstrated that escitalopram had preventive effects on both relapse and recurrence of MDD.

A review of Murdock et al. [7] discussed the positioning of escitalopram in the management of MDD. Preliminary studies have suggested that escitalopram was as effective as other SSRIs and venlafaxine XR (venlafaxine hydrochloride extended release); furthermore, escitalopram may provide the advantage of cost-effectiveness and cost utility. However, additional longer-term, comparative studies that evaluate specific efficacy, tolerability, health-related quality of life, and economic indices would be needed to determine definitively the position of escitalopram relative to other SSRIs and venlafaxine in the treatment of MDD. Nevertheless, available clinical and pharmacoeconomical data indicate that escitalopram is an effective first-line option in the management of patients with MDD.

Because MDD recurs readily, it is important to select antidepressant drugs that allow high therapy continuity for pharmacological treatments. The effects of escitalopram highlighted in this review indicated that it is an antidepressant drug appropriate for first-line therapy.

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31 Antidepressant Medications and Suicide Risk: What Was the Impact of FDA Warning?

Gianluca Serafini, Paola Solano, and Mario Amore

31.1 Introduction

Suicide is one of the most relevant public health issue worldwide as shown by the fact that the World Health Organization (WHO) launched a warning reporting that there will be a 20 % increase of deaths due to suicidal behavior by 2020 [1]. Moreover, the WHO suggested that every 40 s an individual dies by suicide, and many more attempt suicide worldwide. Most cases are related to the presence of a psychiatric condition and in particular more than 50 % of deaths occur in comorbidity with major affective disorders [2]. Overall, 1–2 % of the entire population completed suicide during the life span [3], but suicide risk may be considered generally modest during a specific brief period. Given that numbers of suicide events as well as the size of investigated cohorts of suicidal patients are generally quite small, the main findings of most studies should be interpreted with caution.

As mentioned, some of the most relevant risk factors for suicide include the presence of psychiatric conditions such as major depressive disorder (MDD), in particular if associated with hopelessness or in comorbidity with substance abuse [4–7]. Suicidal behavior and self-harm have been reported to be generally increased in subjects with major depression [8, 9], and antidepressant (AD) medications are commonly used as a conventional treatment option in these patients although they may be also associated with dysphoric-mixed-agitated/psychotic states potentially increasing suicidal risk [10–12].

Recently, the effects of psychoactive treatments on suicidal behavior raise a growing interest among clinicians since contrasting evidence has been reported concerning the effect of these medications on suicide risk. Although AD compounds significantly reduce the severity of depressive symptoms [13, 14], existing evidence also showed that suicide rates and self-harm may increase with the use of these medications especially in younger individuals [15–17].

A large body of literature addresses the need of identifying those AD compounds associated with the higher suicide risk, but robust evidence including reliable randomized controlled trials (RCT) able to inform about the best AD treatment strategy is still lacking to date. Notably, the relationship between AD compounds and suicide events is difficult to investigate, mainly due to the relative low frequency of suicides. According to a recent meta-analysis of data submitted to the US Food and Drug Administration (FDA) including over 350 AD trials and approximately

G. Serafini, MD, PhD (✉) • P. Solano • M. Amore
Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), Section of Psychiatry, University of Genova, IRCCS San Martino, Largo Rosanna Benzi 10, Genova 16100, Italy
e-mail: gianluca.serafini@unige.it

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100,000 patients, only eight suicide deaths had been documented [15].

This chapter aimed to address the relationship between AD compounds and suicidal behavior, first by providing a historical background about the main topic and then by carefully reviewing the most important findings regarding this theme in the current literature. For this purpose, the FDA boxed warning concerning the increased suicide risk related to the use of AD medications will be addressed and discussed both in respect to the whole population and different age groups.

31.2 Historical Background

The initial studies suggesting a possible association between AD treatment and increase of suicidality were essentially case studies dated back to 1991 and reporting that high doses of fluoxetine may be associated with suicidal behavior in adolescents [18]. However, subsequent studies did not universally confirm these results [19]. In May 2003, GlaxoSmithKline advised the FDA in the USA that paroxetine use during the course of a clinical trial has been associated with suicide-related adverse events in pediatric patients [20]. At the end of 2003, the Medicines and Healthcare Products Regulatory Agency, the UK's counterpart to the FDA, sent a letter to all clinicians working in the UK to advice against using fluoxetine in children and adolescents. In October 2004, the FDA reached a split decision (specifically, 15 yes, 8 no) according to which pharmaceutical companies have been dictated to add a "black box warning" (Fig. 31.1) concerning the heightened risk of suicidal thoughts and behaviors in subjects who were using AD medications. In particular, AD medications have been associated with an increased risk of suicidal thinking, feelings, and behavior in young and adolescent subjects. As reported, the warning is about AD medications that may cause de novo "suicidality" in some individuals [21]. The introduction of a "black box" warning represented the most serious warning which was placed in the labeling of a prescription medication. Advertisements that serve to remind health-care professionals of a product's availability (so-called reminder ads) are not allowed for products with "black box" warnings. Moreover, a black box warning does not prohibit the use of a certain compound, e.g., AD drugs in children and adolescents. Rather, it introduces a warning about the risk of suicidality and encourages prescribers to carefully balance this risk associated with AD's use with clinical needs.

However, this FDA warning was rapidly associated with a burning debate since it was argued that it would have discouraged depressed

The results of pediatric depression studies to date raise very important problems. First, the poor effectiveness results, except for Prozac, make it very difficult for practitioners to know what to do to treat a very serious, life-threatening illness.While we believe that these drugs may be effective in children, studies have not shown this to be true. Second, and of equal importance, the analyses we initiated in 2002 appear to show that the drugs in the pediatric controlled depression trials can lead to suicidal behaviors or thinking. While no suicides occurred in the trials, suicides certainly have been reported in treated patients, and the devastating results of these suicides were a critical part of the February 2, 2004, Advisory Committee meeting.

FDA generally supports the recommendations that were recently made to the Agency by the Psychopharmacologic Drugs Pediatric Advisory Committees regarding reports of an increased risk of suicidality associated with the use of certain anti-depressants in pediatric patients. FDA has begun working expeditiously to adopt new labeling to enhance the warnings associated with the use of anti-depressants and to bolster the information provided to patients when these drugs are dispensed.

Fig. 31.1 Extract from FDA black box warning "Antidepressant Drug Use in Pediatric Population," issued 15 October 2004 (http://www.fda.gov/NewsEvents/Testimony/ucm113265.htm)

patients from seeking help and clinicians from prescribing AD medications even if they were clinically indicated [22]. Moreover, a large meta-analysis of placebo-controlled randomized trials including 100,000 patients found that AD medications may increase suicidal behavior till the age of 40 years [23]. Although the exact mechanism involved in the supposed increased suicidality was poorly understood, the assumptions underlying the introduction of the "black box" warning supported the paradoxical idea that AD drugs can have two separate (apparently opposite) effects, one promoting suicidal behavior and the other preventing suicidality, respectively. The hypothesis that does not recognize the preventative effect and assumes only the existence of the promoting effect was not able to explain the protective effect which was observed in older subjects. The relative susceptibility to these two effects seemed to vary according with age. In older subjects the preventative effect tended to predominate, whereas in younger subjects it appeared the opposite. Exactly, the risk may be considered doubled in youths [23] although many suicides and suicide attempts in this population have been missed by the FDA analysis [24].

This controversial topic was further stressed by the study of Gibbon and colleagues [25] who analyzed data on prescription rates for selective serotonin reuptake inhibitors (SSRIs) from 2003 to 2005 in children and adolescents using available data (through 2004 in the USA and 2005 in the Netherlands). SSRI prescriptions in these populations were significantly reduced by approximately 22 % in the USA and the Netherlands after the FDA warnings [25]. According to these data, youth suicide rate increased by 49 % between 2003 and 2005 in the Netherlands with a significant inverse association with SSRI prescriptions, whereas a 14 % increase of youth suicide rates in the USA (2004–2005) has been reported. However, the prescription decline has been observed in 2005 whereas the suicide increase occurred in 2004 [26].

Over the last decade, other researches brought up further claims regarding negative consequences related to the FDA black boxed warning. However, it is difficult to distinguish the effects of the black boxed warning from those of public awareness regarding suicidality. As Stone [26] correctly suggested, although adverse outcomes such as suicides increased in 2005 concomitantly with a dramatic reduction in AD use, the two phenomena may be unrelated. Similarly, although AD administration has been associated with a significant reduction of suicide risk, not necessarily an increase in the prevalence of AD use results in significantly reduced rates of suicide. Therefore, a causal link between the introduction of the black box warning, changes in AD use, diagnosis of depression, and treatment cannot be definitely drawn [27].

Lu and colleagues [28] analyzed a very large cohort of health-care claim data of patients (from 2000 to 2010) including 1.1 million adolescents and 1.4 million young adults and reported a significant reductions in AD use during the course of 2 years after the FDA advisory among adolescents, young adults, and adults. They suggested that, after the FDA warning was issued, the rates of AD use remained below the formerly expected levels according to prewarning patterns in all age groups. This reduction in AD prescriptions was paralleled by a significant post-warning decrease in the rate of new diagnoses of major depression in all age groups (children, young adults, and all adults) by primary care providers. Although the evident reduction of AD prescriptions after the FDA warning, no compensatory use of alternative depression treatments occurred [29]. Unfortunately, the FDA introduction of the black box warning that occurred in 2007 does not seem to be sufficient to attenuate the detrimental effect on depression treatment.

Considering this historical background and the effects related to the introduction of the black box warning regarding suicidality in subjects who were taking AD drugs, the present chapter critically reviews the current literature about the potential associations between AD use and completed/attempted suicide and is mainly aimed to inform clinicians about the relationship between use of modern AD medications and suicidality after the FDA warning.

31.3 The Resonance of the FDA Black Boxed Warning: Antidepressants and Suicide Risk

Different studies investigated the resonance of FDA black boxed warning on suicidality using both experimental and ecological study designs. Here, as follows are summarized studies concerning AD prescribing rates after FDA boxed warning and studies about the increased/reduced suicidality after the black boxed warning, respectively.

31.3.1 Antidepressant Prescribing Rates After FDA Boxed Warning

The issue of AD prescription volumes after the FDA boxed warning has been investigated in different studies, though no concluding evidence about a potential decline in national AD prescribing patterns after the FDA boxed warning was reported according to the majority of studies (Table 31.1). For instance, Mittal and colleagues [30] reported that after a 2-year period, AD prescriptions for depressed children and adolescents in community-based and outpatient clinic settings declined compared to the period before the FDA boxed warning. However, this decline seems to be not persistent during the 5 years following the implementation of the FDA boxed warning. Specifically, the authors reported that during 2002–2003, ADs were prescribed in 4.1 million visits, 3.2 million visits in 2004–2005, and 2.8 million visits in 2006–2007, respectively. Based on these findings, AD prescribing volumes reversed during 2008–2009 with an increase to 3.6 million visits. Importantly, a significant decline in visits related to AD prescribing rates in the post-FDA boxed warning period (2006–2007) has been found compared to 2002–2003. Similarly, Clarke and colleagues [31] retrospectively examined 57,782 youths aged 10–17 and found that both new (incident) and refill AD dispensing continued to decline through 2009 with no sign of leveling off. However, among youths who started AD treatment, the cumulative supply of AD medication remained consistent across the pre- and post-period. This suggested that cumulative treatment episode duration did not decrease, possibly as a function of greater days' supply with each new refill in the post-period. Moreover, the authors reported that prescribers dramatically curtailed preauthorized refills in the post-warning period. Reeves and Ladner [32] suggested that AD-induced suicidality was an uncommon occurrence but even a legitimate phenomenon; therefore, a close monitoring was needed and an adequate follow-up care should be provided for patients after the beginning of an AD treatment. Katz and colleagues [33] investigated the effects of the FDA warning on AD prescribing patterns/outcomes in order to ascertain whether it may be associated with any unintended health consequences. The authors reviewed health-care databases including more than 265,000 Canadian children, adolescents, and young adults to investigate annually changes in the rates of AD prescription and outcomes in these populations in the 9 years before and 2 years after the warning, respectively. This population has been confronted with young adults used as a comparison group since this population was not targeted by the FDA warning. Results demonstrated that the rate of AD prescriptions is reduced among children and adolescents (relative risk (RR) 0.86, 95 % confidence interval [CI] 0.81–0.91) and young adults (RR 0.90, 95 % CI 0.86–0.93). In particular, there has been a reduction of 14 % in the rate of AD prescription among children and adolescents after the warning, whereas rates of newer AD use, except for fluoxetine, declined by 32–40 %. Fluoxetine use increased by 10 % in the period following the warning. Importantly, the rate of completed suicides among children and adolescents raised significantly after the warning (RR 1.25, 95 % CI 1.08–1.44; annual rate per 1000 = 0.04 before and 0.15 after the warning) coupled with no equivalent change in the rate of completed suicides among young adults (RR 1.01, 95 % CI 0.93–1.10; annual rate per 1000 = 0.15 before and 0.22 after the warning). In another study, Wijlaars and colleagues [34] evaluated a cohort of 1,502,753 children and adolescents in

Table 31.1 Evidence about the most relevant studies about AD use and suicide rates before and after the FDA black boxed warning

Study	Type of study	Sample	Main results
Lu et al. [28]	Association between AD use before and after the FDA warning and suicide attempts in patients admitted to hospital or emergency departments	1.1 million adolescents 1.4 million young adults 5 million adults	Safety warnings and widespread media coverage reduced AD use; similarly, suicide attempts among young people increased
Leon et al. [44]	Longitudinal 27-year observational study using prospective assessments of AD-associated suicidal behavior	757 subjects older than 17 years	The risk of suicide attempts or completed suicides was reduced by 20 % among participants taking antidepressant medications
Isacsson and Rich [21]	Longitudinal study on association between AD treatment and suicide rates in adolescents in Sweden during the period between 1992 and 2003 (baseline), and 2004–2010 (after the FDA warning)	845 suicides in the 10- to 19-year age group	The increase occurred among individuals not treated with antidepressant drugs
Barber et al. [38]	Survey carried out by the Centers for Disease Control and Prevention's youth risk behavior	100/100,000 suicides subjects aged 10–17	A reduction from 2000 to 2009 as well as a more recent increase in high school students' self-reported suicidal thoughts, plans, and attempts have been observed
Mosholder and Willy [48]	Meta-analysis based on data from 22 randomized, short-term, placebo-controlled, pediatric trials	2298 pediatric subjects who had been treated with nine different AD medications that were compared to 1952 individuals who received placebo	78 (54 with active drug and 24 with placebo) suicidal adverse events occurred in these trials, but no completed suicides have been reported. Active drug treatment was associated with an almost double rate of serious suicidal events compared with placebo
Hammad et al. [49]	Meta-analysis based on data from 20 trials	4582 pediatric depressed subjects	AD use in pediatric patients is associated with a modestly increased risk of suicidality
Bridge et al. [50]	27 studies published and unpublished randomized, placebo-controlled, parallel-group trials about second-generation antidepressants in subjects younger than 19 years	3241 subjects aged 6–18 with MDD 718 subjects aged 6–18 with OCD 1162 subjects aged 5–18 with other anxiety disorders	There was an increased risk difference of suicidal ideation/suicide attempt across all trials and indications for drug *vs.* placebo (0.7 %; 95 % CI, 0.1–1.3 %), benefits of AD greater compared to the risks
Katz et al. [33]	Reviewed national databases for AD prescription volumes and suicide rates for 9 years before and 2 years after the FDA warning	265,000 Canadian children, adolescents, and young adults	Overall, a 4 % decrease in AD prescription has been observed; suicides among children and adolescents raised after the FDA warning (RR 1.25, 95 % CI 1.08–1.44; annual rate per 1000 = 0.04 before and 0.15 after the warning). No change in suicides among young adults was reported (RR 1.01, 95 % CI 0.93–1.10; annual rate per 1000 = 0.15 before and 0.22 after the warning)

(continued)

Table 31.1 (continued)

Study	Type of study	Sample	Main results
Bailly [43]	Meta-analysis of RCT in pediatric population	1665 subjects aged 5–18	A modest suicidal behavior increase has been reported with SSRI compared to placebo, though no increase was found in completed suicides in pediatric population. Fluoxetine being the most safe
Wohlfarth et al. [51]	A meta-analysis of 22 RCTs on children and adolescents including studies on MDD, OCD, GAD, social anxiety, SSRIs, and SNRIs	3832 children and adolescents	No risk difference in terms of suicide events in anxiety disorders and OCD patients has been found. Conversely, a 1.4 % risk difference for those in the MDD studies (NNH 71.4) has been observed
Kaizar et al. [53]	Meta-analysis of 24 RCTs on children and adolescents with MDD, OCD, anxiety, and ADHD who were treated with citalopram, fluvoxamine, paroxetine, fluoxetine, sertraline, venlafaxine, mirtazapine, nefazodone, and bupropion	1592 subjects aged 6–17 with MDD 319 subjects aged 6–17 with OCD 75 subjects aged 5–17 with ADHD 322 subjects aged 5–17 with anxiety	An increased risk of suicidal thoughts and behaviors has been reported for those with MDD (OR 2.3), but even if the AD was an SSRI (OR 2.2)
March et al. [52]	Meta-analysis of four RCTs on sertraline in MDD and OCD	987 subjects aged 10–17 with MDD; 286 aged 7–15 with OCD	According to two MDD studies, the risk difference in terms of suicidal thoughts and behaviors was increased for sertraline to 1.56 % (NNH 64.2). No difference was reported in OCD studies with sertraline
Hammad and Mosholder [54]	Survey carried out by the Centers for Disease Control and Prevention's youth risk behavior	9/100,000 suicides in the USA in adolescents aged 15–19 2/100,000 suicides in the USA in adolescents aged 10–14	The FDA warning may have discouraged the appropriate use of ADs in the treatment of child and adolescent depression
Tiihonen et al. [63]	Ecological cohort study on Finnish adolescents with a follow-up of 3.4 years	15,390 Finnish patients aged 10–19 hospitalized for a suicide attempt	RR for suicide attempts was 1.84. Overall, 44 suicide deaths were reported with no differences in those who were taking antidepressants or not, except for paroxetine (RR 5.44)
Leon et al. [61]	A naturalistic study on three decades of prospective assessment propensity quintile-stratified safety analyses	All ages Unexposed to AD 3433 Exposed to AD 3283	The risk of suicide attempts or completed suicides was significantly reduced when participants received antidepressants
Gibbons et al. [57]	Cross-sectional study aimed to evaluate the association between AD prescription rate and suicide rate in children prior to the FDA findings	933 suicides of children aged 5–14	Higher SSRI prescription rates were associated with lower suicide rates in children and adolescents

Olfson et al. [35]	Cross-sectional study aimed to assess suicide rates for subjects aged 10–19 together with national AD prescriptions	10,604 adolescents receiving AD treatments	Overall, a 1 % increase in the use of ADs in adolescents was associated with a reduction in suicides by 0.23/100,000 adolescents per year
Hall and Lucke [58]	Australian prescription data in general practice for subjects aged 15 or older matched to suicide rates for the same age group	3250 subjects aged 15 or older	Suicide rates were inversely related to AD prescriptions in this age group
Sondergard et al. [59]	Register linkage study on subjects aged 10–17 aimed to evaluate AD prescriptions and suicide	2569 adolescents	SSRI treatment and suicidal behavior were not linked. It has been estimated that none of the suicides ($n=42$) had been treated with an SSRI 2 weeks prior to their suicide
Kamat et al. [62]	Meta-analysis on children and adolescents' AD prescriptions and suicide rates	12,865 children and adolescents	A significant positive correlation between suicide rates and AD rates ($p=0.031$) was reported
Simon and Savarino [60]	A naturalistic study on children and adolescents linking new episodes of depression, AD prescription, and treatment type with suicide attempts	131,788 children and adolescents	The highest risk of suicide attempt was observed in the month before the beginning of AD treatment, but the risk declined in the months after starting the treatment

AD Antidepressant, *FDA* Food and Drug Administration, *GAD* Generalized Anxiety Disorder, *MDD* Major Depressive Disorder, *NNH* Number Needed to Harm, *OCD* Obsessive Compulsive Disorder, *RR* Relative Risk, *SNRIs* Serotonin and Norepinephrine Reuptake Inhibitors, *SSRIs* Selective Serotonin Reuptake Inhibitors

The Health Improvement Network, a UK primary care database. Trends in the incidence of depression, symptoms, and AD prescribing were examined during the period 1995–2009, accounting for deprivation, age, and gender. The authors used segmented regression analyses to evaluate changes in prescription rates. Overall, 45,723 (3 %) children had at least one depression-related entry in their clinical records. Specifically, 16,925 (1 %) children have been treated with SSRIs. A decrease of SSRI prescription rates from 3.2 (95 %, CI: 3.0, 3.3) per 1000 person-years at risk (PYAR) in 2002 to 1.7 (95 %, CI: 1.7, 1.8) per 1000 PYAR in 2005 and a subsequent increase to 2.7 (95 %, CI: 2.6, 2.8) per 1000 PYAR in 2009 have been reported. Prescription rates of some contraindicated SSRIs such as citalopram, sertraline, and especially paroxetine dropped dramatically after 2002, whereas AD rates of fluoxetine and amitriptyline remained quite stable. However, rates for all ADs, except paroxetine and imipramine, increased again after 2005. These results recommend caution for general practitioners (GPs) in detecting depression and prescribing ADs following the FDA warning. Consistently with Olfson and colleagues' [35] evidence, a plausible explanation is that the FDA warnings slowed previous growth in the rate of AD treatment.

31.3.2 Increased Suicidality After the Black Boxed Warning

Lu and colleagues [28] conducted a large study including approximately 1.1 million of adolescents, 1.4 million of young adults, and 5 million adults. Specifically, the authors investigated associations between AD use before and after the warning and suicide attempts in patients admitted to hospital or emergency departments. They found that safety warnings concerning ADs and widespread media coverage reduced AD use, but simultaneously suicide attempts among young subjects increased. This study has been criticized by several authors [24, 27, 36–39] who questioned the sensitivity of the proxy measure (e.g., the proportion of suicide attempts by psychotropic drug poisoning including both intentional and unintentional overdoses) that later has been considered by the same authors as an imperfect proxy for suicide attempts [40]. In addition, the authors subsequently suggested that more direct analyses of suicide attempts and deaths seem to indicate no clear increase of suicide attempts after the FDA warnings [40]. In fact, only a small decrease of adolescent suicides and no change in the fraction of young adults who receive ADs were found. Furthermore, based on the Centers for Disease Control and Prevention data, most self-harm injuries in youths do not involve poisoning, and just half of emergency visits for psychotropic poisonings are intentional in nature [28, 41]. Another criticism that has been raised concerns the fact that not all intentional self-harm in youths reflects suicidal intent in clinical practice [42]. Bailly [43] reported mixed evidence and suggested that relative to placebo, SSRIs are efficacious for pediatric affective disorders although they may be associated with a modest increase in the occurrence of both suicidal ideation and behavior. However, the study showed that SSRI utilization was overall associated with a significant decrease in the suicide rates in children and adolescents, presumably due to their efficacy, relevant treatment adherence, and low toxicity in overdose.

Leon and colleagues [44] reported in a longitudinal, observational study with prospective assessments for up to 27 years which was conducted in five US academic medical centers that AD medications were associated with a significant reduction in the risk of suicidal behavior. Quintile-stratified, propensity-adjusted safety analyses using mixed-effect grouped-time survival models show that the risk of suicide attempts or suicides was reduced by 20 % among participants taking ADs (hazard ratio, 0.80; 95 % CI, 0.68–0.95; $z = -2.54$; $p = 0.011$).

Other authors [45] hypothesized that the warning, contrary to its intention, may have increased young suicides by leaving a number of suicidal young individuals without treatment with ADs. In particular, they analyzed individual data of all 845 suicides in the 10- to 19-year age group in

Sweden at the time frame 1992–2003 (baseline) and 2004–2010 (after the warning). Importantly, after the warning suicide in this age group increased for five consecutive years (60.5 %). The increase occurred among individuals which were not treated with ADs. Finally, there is also evidence suggesting that the decision to introduce the "black box" warnings was based on biased data and invalid assumptions [21].

Isacsson and Rich [21] suggested that the FDA decision was unsupported by the observational data regarding suicide in young people existing in 2003. The authors recommended that drug authorities may reevaluate the imposed warnings on AD drugs by analyzing the current public health consequences after the introduction of the warnings. They also reported that clinicians should be encouraged to treat depression in young individuals using ADs if indicated. According to the authors' suggestions, clinicians have to be aware about the increased suicide risk in depressed young subjects, closely monitoring this population of patients.

31.3.3 Decreased Suicidality After the Black Boxed Warning

There are studies that do not show an increase in suicide attempts or deaths in young people after the FDA warnings [38]. For example, based on a survey which was carried out by the Centers for Disease Control and Prevention's youth risk behavior, a reduction from 2000 to 2009 and, conversely, a more recent increase in high school students' self-reported suicidal thoughts, plans, and attempts have been observed [46]. Two national samples of hospital visits reported no increasing trend in young self-harm after the 2003–2004 FDA warnings [46]. Consistently with the aforementioned results, no increase in youth self-harm has been registered by the California's EPIC website [47].

Different studies confirmed these results, for instance, three meta-analyses of clinical trial data demonstrated that AD treatment increases, rather than decreases, the risk of suicidal behaviors in youths and adolescents [48–50].

In particular, Mosholder and colleagues [48] conducted a meta-analysis based on data from 22 randomized, short-term, placebo-controlled, pediatric trials involving 2298 pediatric subjects who had been treated with nine different AD medications that were compared with 1952 individuals who received placebo. The authors reported that overall, 78 (specifically, 54 with active drug and 24 with placebo) suicidal adverse events occurred in these trials, but no completed suicides have been reported (combined incidence rate ratio across all trials for serious suicidal adverse events = 1.89). Therefore, active drug treatment was associated with an almost double rate of serious suicidal events compared with placebo [48]. The second meta-analysis, Hammad et al. [49] included only 20 trials in the risk ratio analysis of suicidality among all placebo-controlled trials submitted to the FDA. No completed suicides have been reported in any of these trials, and the multicenter trial was the only study in which a significant risk ratio (4.62; 95 % confidence interval [CI], 1.02–20.92) was observed. As reported, 1.66 (95 % CI, 1.02–2.68) was the overall risk ratio for SSRIs in depression and 1.95 (95 % CI, 1.28–2.98) for all medications across all indications. In conclusion, the authors suggested that AD drug use in pediatric patients is associated with a modestly increased risk of suicidality.

Interestingly, Wohlfarth and colleagues [51] conducted a meta-analysis on 22 RCTs in child and adolescent populations ($n = 3832$) including samples of patients with MDD, obsessive compulsive disorder (OCD), generalized anxiety disorder (GAD), and social anxiety who were treated with SSRIs and SNRIs. The authors concluded that there was no risk difference in terms of suicide events in the sample of patients who were treated for anxiety disorders and OCD, whereas there was a risk difference for those in the MDD studies of 1.4 % (number needed to harm (NNH) 71.4). Similar results have been reported by March and colleagues [52] who analyzed four RCTs conducted using sertraline in MDD and OCD and found that in the two MDD studies, the risk difference for both suicidal thoughts and behaviors was increased of 1.56 %

for sertraline (NNH 64.2). Moreover, suicidal thoughts and behaviors associated with sertraline were more frequently observed in depressed children than depressed adolescents. However, there was no increase in suicidal thoughts and behaviors associated with sertraline when used for the treatment of OCD. These results suggested that the increase of suicidality in children and adolescents who were treated with SSRI compounds could be presumably a factor related to psychopathology rather than a specific drug-related effect. Notwithstanding with these results, Kaizar and colleagues [53] conducted a meta-analysis of 24 RCTs with a sample of children and adolescents with MDD, OCD, anxiety, and attention deficit hyperactivity disorder (ADHD). Moreover, citalopram, fluvoxamine, paroxetine, fluoxetine, sertraline, venlafaxine, mirtazapine, nefazodone, and bupropion demonstrated an increased risk of suicidal thoughts and behaviors for those with MDD (OR 2.3), in particular whether the AD was an SSRI (OR 2.2).

Furthermore, Bridge et al. [50] included 27 studies published and unpublished randomized, placebo-controlled, parallel-group trials about second-generation AD medications in subjects younger than 19 years. Based on pooled risk differences in rates of primary study-defined measures of responder status, this study demonstrated that while there was an increased risk difference of suicidal ideation/suicide attempt across all trials and indications for drug vs. placebo (0.7 %, 95 % CI, 0.1–1.3 %) (number needed to harm, 143 [95 % CI, 77–1000]), the pooled risk differences within each indication were not statistically significant. In this study, however, similarly to the previously mentioned reports, there were no completed suicides. According to the main results, the authors reported that benefits of AD medications seem to be greater compared to the risks from suicidal ideation/suicide attempt across indications. Findings from these meta-analyses are generally in line with data on self-harm derived by WISQARS nonfatal emergency department visits which were conducted on subjects aged 10–17 that showed increased rates of self-harm in years 2004 and 2005, concomitantly with the timing of the FDA warnings.

Overall, no concluding evidence seems to emerge, and the use of AD in young and adolescent populations still remains a tricky matter of concern according to the current knowledge about this topic.

31.4 Antidepressant Drugs and Suicide Risk: The Contribution of Ecological and Postmortem Studies

Ecological studies, despite a geographic variability related to AD use, demonstrated an inverse association between suicide rates and AD prescription volumes. Unfortunately, ecological population-based approaches are usually limited, and cautions are necessary when interpreting data.

Hammad and Mosholder [54] warned against the danger of a discouraging effect that could lead to a decrease in the appropriate use of AD in the treatment of child and adolescent depression. Also other authors highlighted the risk on the potential for discouraging appropriate use of AD drugs based on the sparked debate upon the FDA warning [15, 49, 55]. This could be mainly associated to the unintended consequence of eventually exposing more patients than occurring with AD drugs to the suicide risk of untreated depression [25]. These concerns were enhanced by the upturn in the 2004 US rate of adolescent suicide [25]. Moreover, significant falls were also reported in AD drug prescriptions for pediatric patients after the regulatory agency actions [25, 29, 55]. However, 6 years later data from the Centers for Disease Control and Prevention on the overall trend in suicide rates among adolescents showed that the rate of suicide decreased after the unexplained increase of 2004 [56] whereas in 2007, the most recent year with available data, the rates were the lowest reported in the last 25 years.

Some studies have been also carried out prior to the FDA warning. Gibbons and colleagues [57] examined the association between AD prescription rate and suicide rate in children aged 5–14 years by analyzing associations at the

county level across the USA. After adjustment for sex, race, income, access to mental health care, and county-to-county variability in suicide rates, higher SSRI prescription rates have been associated with lower suicide rates in children and adolescents. The aggregate nature of these observational data precludes a causal interpretation of the main findings, but the authors suggested that more SSRI prescriptions are associated with lower suicide rates in children and may reflect AD efficacy, treatment compliance, and better-quality mental health care. Furthermore, Olfson and colleagues [35] examined regional suicide rates for subjects aged 10–19 years with national AD prescriptions (1999–2000) and reported an inverse relationship between AD and suicide in this age group. Specifically, a 1 % increase in the use of AD in adolescents was associated with a reduction of suicides by 0.23/100,000 adolescents per year. Hall and Lucke [58] confronted Australian prescription data in general practice for subjects aged 15 or older which were matched to suicide rates for the same age group (1991–2001) suggesting that suicide rates were inversely related to AD prescriptions. The Danish pharmacoepidemiological register linkage study ($n=2569$) concerning prescriptions and suicide, 1995–1999, demonstrated no link between SSRI treatment and suicide in subjects aged 10–17 years. As suggested by Søndergård and colleagues [59], none of the 42 suicides had been treated with an SSRI 2 weeks prior to their suicide. In addition, Simon and Savarino [60] conducted a naturalistic study in a sample of children and adolescents linking new episodes of depression, AD prescription, and treatment type with suicide attempts ($n=131,788$), 1996–2005. Subjects have been followed 90 days prior to starting treatment and 180 days following the commencement of treatment. The risk of suicide attempts was highest in the month before the initiation of an AD treatment and dropped in the months after starting treatment regardless of whether they are receiving ADs from primary care physician or psychiatrist or psychotherapy.

Notwithstanding with the FDA's black boxed warning, an observational study on mood disorders that considered three decades of prospective assessment propensity quintile-stratified safety analyses found that the risk of suicide attempts or suicides was significantly reduced when participants received AD medications [61]. However, Kamat et al. [62] reported data from the Organisation for Economic Co-operation and Development (OECD) health dataset (1995–2008) showing a significant positive correlation between suicide rates, AD rates ($p=0.031$), and unemployment ($p=0.028$). These results also showed a significant negative correlation between suicide rates and inpatient psychiatric beds ($p=0.039$). However, the actual coefficients are less than ±0.16 indicating weak relationships and, after adjusting for other variables, the only variable that resulted statistically significantly associated with suicide rates is AD prescribing ($p=0.005$, $r^2=0.09$).

Tiihonen and colleagues [63] conducted an ecological cohort study including 15,390 Finnish patients aged 10–19 years which were hospitalized for a suicide attempt with a follow-up of 3.4 years (from 1997 to 2003) in order to investigate the relationship between AD treatment and suicides. The authors reported that the adjusted RR for suicide attempts in those using ADs was significant (1.84). Moreover, they reported 44 deaths in those aged 10–19 years with no differences in those who were treated or not treated with AD drugs, except for paroxetine for which an RR of 5.44 was reported.

However, ecological data could not establish cause-effect associations nor determine whether the changes in prescribing were due to untreated depression with drugs or prescription of fewer AD drugs for other conditions. Although being careful for the unintended consequences of regulatory agency actions is important, ecological data may be not the best guide to ascertain whether drugs are being used appropriately in a specific population of patients. Recently, Gusmao and colleagues [64] conducted a large naturalistic study that aimed to describe trends in the use of AD and rates of suicide in Europe, adjusted for gross domestic product, alcohol consumption, unemployment, and divorce. Moreover, the study explored whether any observed reduction in the

rate of suicide in different European countries preceded the trend for increased use of AD medications. Data were obtained for 29 European countries between 1980 and 2009 and showed an inverse correlation in all countries between recorded standardized death rate (SDR) for suicide and AD defined daily dosage (DDD), with the exception of Portugal. Every unit increase in DDD of an AD per 1000 people per day, adjusted for these confounding factors, reduces the SDR by 0.088. The correlation between DDD and suicide-related SDR was negative in both time periods considered, albeit more pronounced between 1980 and 1994. Thus, the authors concluded that suicide rates have tended to decrease more in European countries where there has been a greater increase in the use of AD drugs. Gusmao and colleagues' [64] findings underline the importance of the appropriate use of AD medications as part of routine care for people diagnosed with depression, therefore reducing the risk of suicide.

There are also postmortem studies about AD drugs and suicidality. In a large meta-analysis concerning AD treatments and children and adolescent suicidality, Gordon and Melvin [19] examined 11 meta-analyses of placebo-controlled trials, 17 pharmacoepidemiological studies, one meta-analysis of observational studies, and six postmortem studies. Based on the six postmortem studies, the authors suggested that if AD drugs are related to adolescent suicide events, AD medications should be presumably present in toxicology assays of adolescent suicide victims. Therefore, they assessed six toxicology studies for the presence of AD drugs in adolescents who died by suicide. Their findings suggested that it was reasonably uncommon for adolescents who have died by suicide to have been taking newer AD drugs, such as SSRI at therapeutic doses. The infrequent presence or absence of AD medications at autopsy suggests either the adolescent was not prescribed the AD or was not taking it in the days prior to their suicide [45, 65–68].

Several limitations should be considered when analyzing the main results of postmortem studies. First, not all toxicology assays were available for those who died by suicide. Unfortunately, these studies were not able to exclude subjects who had an injury to death period of less than 3 days [68]. Finally, it was not possible to know how many subjects may have withdrawn their AD treatment (AD withdrawal has been reported as a mechanism increasing suicidal behaviors) [45].

31.5 Antidepressant Use and Suicide Risk in Different Age Groups

Investigating the effect of specific medications in specific populations of patients is of paramount importance due to the differences of life span suicidal behaviors, psychopathology, and brain functioning [69].

31.5.1 Adults and Elderly

Recently, Gibbons and colleagues [70] concluded that AD drugs lowered suicidality relative to placebo among adult patients while demonstrating no difference in suicidality among youths. They suggested that AD medications possessed a robust efficacy in reducing suicidal behavior when compared with placebo. Further evidence has been provided by Erlangsen and Conwell [71], who identified an age-dependent decline in suicide rate for AD recipients. One possible explanation could be that older adults respond better to AD drugs than younger age groups. In addition, based on the increasing gap with age between estimated prevalence of depression and AD prescription rate in individuals dying by suicide, there is the urgent need to assess major depression in the elderly. Logistic regression analyses showed a 2–3 % decline in suicide rates with each additional year of age for men and women in treatment with AD compounds, respectively. Conversely, an opposite trend was found for untreated subjects. Moreover, fewer persons aged 80+ dying by suicide had received AD prescriptions during the last months of their life when compared with younger subjects [71]. Aiming to evaluate the AD use volume in different age group in respect to suicide risk, Phillips

and Nugent [72] reported an overall increase in AD use in all age groups coupled with a decrease in suicide rates for the young individuals and elderly. The elderly showed the largest increase in AD usage coupled with the biggest decrease in suicide rates, whereas only a moderate increase of suicide rates for the middle-aged group was found. Moreover, immediately after the FDA issued the black box warning, the Committee on Safety on Medicines (CSM) in the United Kingdom (UK) reported that in adult depression SSRIs remain beneficial. In addition, Gunnell and colleagues [73] carried out a meta-analysis of data from the Medicines and Healthcare Products Regulatory Agency of published and unpublished RCTs of SSRIs which were compared with placebo in adults and elderly. Although they found no evidence that SSRIs increased the risk of suicide or suicidal thoughts when compared with placebo, they reported weak evidence for an increased risk of self-harm (OR¼1.57, 95 % CI 0.99–2.55). In the same year, Fergusson and colleagues [74] found, in another meta-analysis including published RCTs of SSRIs which were used in any disorder, an increase in suicide attempts for adult patients receiving SSRIs when compared with placebo (OR = 2.28, 95 % CI 1.14–4.55) or therapeutic interventions other than tricyclic AD drugs (OR = 1.94, 95 % CI 1.06–3.57).

There are also studies reporting an inverse correlation between elderly suicide rates and AD prescription rates. For example, Shah and colleagues [75] reported that there was no significant correlation between elderly suicide rates and AD prescription rates in the large British National Formulary categories, individual psychotropic drug groups, and individual psychotropic drugs.

31.5.2 Children and Adolescents

The FDA black boxed warning concerning the possible association between children and adolescent suicide rate and AD treatments stimulated debates, and several studies about this topic appeared in literature (*see above*), though no concluding evidence has been reported [76].

A large meta-analysis of 35 randomized controlled trials of AD treatments compared with placebo in pediatric and adolescent patients with a total sample of 6039 individuals has been recently conducted. Suicidal behavior, suicidal ideation, and suicidal behavior or ideation were examined as suicide-related outcomes. There were trends indicating that active treatments increased the risk of these events in absolute terms. Regarding efficacy, the results showed that AD treatments did have a statistically significant effects compared to placebo, but the effect was less for the trials which were conducted on major depression. Overall, there was evidence of an increased risk in suicide-related outcomes on AD treatments, while AD treatments were also shown to be efficacious [77]. Phillips and Nugent [72] reported a decrease in overall suicide rates for the young and elderly coupled with an increase in AD use in all age groups. Hetrick and colleagues [78] meta-analyzed 16 RCTs including SSRIs on 2240 children and showed an increased risk of suicidal ideation and behavior in individuals receiving an SSRI compared with those receiving a placebo (RR 1.80; 95 % CI 1.19, 2.72). The Treatment for Adolescents with Depression Study (TADS) [52] is a large RCT on adolescents with major depression that included treatment with fluoxetine alone, cognitive behavioral therapy alone, combined treatment, and placebo. Since this study was the first to prospectively define suicide-related events in youths, thus its findings on suicidality are also historically important to consider when addressing the risk and benefits of ADs in youths. In particular, this study demonstrated that subjects receiving fluoxetine had lower suicidal ideation at follow-up than after the beginning of treatment as well as when compared with those in the placebo group.

Henry and colleagues [79] carefully reviewed a series of published and unpublished efficacy and safety data regarding AD use in children and adolescents. Based on epidemiological data, the authors failed to confirm the relationship between newer AD prescription and completed suicide in large populations of youths. In addition, Hetrick et al. [80] conducted a large Cochrane review on newer-generation AD compared to placebo in

children and adolescent depression considering 19 trials including 3335 participants. They found that there was evidence of an increased risk (58 %) of suicide-related outcome for those on AD medications compared with placebo (17 trials; $N = 3229$; RR 1.58; 95 % CI 1.02–2.45). This equated to an increased risk in a group with a median baseline risk from 25 in 1000 to 40 in 1000. The authors suggested caution is needed in interpreting the main results of this meta-analytic study considering the methodological shortcomings related to the internal and external validity. They also stated that both the size and clinical significance of the present findings need to be replicated. In summary, fluoxetine could be the medication of first choice based on the guideline recommendations, but, importantly, clinicians have carefully considered that an increased risk of suicide-related outcomes has been reported in those treated with AD drugs.

31.6 Association Between Type of Antidepressant Medications and Suicide Risk

Some reports suggested an increase of both suicide ideations and behaviors in patients of all age groups treated with specific AD medications.

31.6.1 Adults and Elderly

A study in a large cohort of patients aged 20–64 and diagnosed with major depression showed significant associations between different types of administered AD drugs and rates of completed and attempted suicides or self-harm. The group of AD medications classified as "other AD medications" (mainly including venlafaxine and mirtazapine) has been associated with the highest rates of both completed and attempted suicides or self-harm. Conversely, the use of mirtazapine, venlafaxine, and trazodone was associated with an increased risk of attempted suicides or self-harm compared with the most commonly prescribed AD, citalopram [81]. Multiple evidences have suggested that both venlafaxine and mirtazapine are associated with a greater risk of suicide and self-harm at a population level [82, 83]. Clinicians should be careful when prescribing these drugs in patients at high risk for suicide given the existence of published studies supporting the assumptions that they are the most lethal non-TCA AD drugs when taken in overdose [84]. Nevertheless, clinical trials—subject to their own sources of bias—indicated that venlafaxine and mirtazapine are at least as effective as SSRIs in alleviating depressive symptoms [85, 86].

Furthermore, Coupland and colleagues [82] reported that SSRIs were associated with the highest adjusted hazard ratios (HRs) for attempted suicide/self-harm (5.16, 95 % CI 3.90–6.83). There was no evidence that SSRIs or drugs in the group of other AD medications were associated with a reduced risk of any of the adverse outcomes compared with TCAs; however, they may be associated with an increased risk of specific outcomes. Moreover, Garlow and colleagues [87] found that compared to placebo, fluoxetine was not associated with a clinically significant increase in suicide ideation among adults with minor depressive disorder throughout a 12-week treatment study.

Conversely, Grunebaum and colleagues [88] carried out a post hoc analysis of data from a randomized, double-blind, 8-week clinical trial of the SSRI paroxetine controlled release ($n = 36$) vs. the norepinephrine-dopamine reuptake inhibitor bupropion extended release ($n = 38$) in patients aged 18–75 with DSM-IV major depressive disorder and past suicide attempts or current suicidal thoughts. The authors suggested a pathway by which SSRI treatment may exert a stronger effect compared with norepinephrine-dopamine reuptake inhibitor treatment on the possible reduction of suicidal thoughts during the initial weeks of pharmacotherapy in these patients with higher suicide risk. There was a strong effect on HDRS psychic depression (depressed mood, guilt, retardation, helpless, hopeless, worthless) (estimate = −2.2; 95 % CI, −3.2 to −1.1; t67.16 = −4.01; $P < 0.001$), that is one of the clusters most strongly correlated to suicidal ideation. The net drug effect demonstrated that mean psychic depression score was 2.2 points lower after 1 week of paroxetine

treatment relative to bupropion treatment. The significance level of this effect was <0.001 at weeks 1 and 2, 0.012 at week 3, and 0.051 at week 4, respectively, whereas results for other depression scale factors were not significant ($p > 0.05$). Makris and colleagues [89] used Swedish Registers and identified 12,448 suicides with forensic data for AD medications and information on inpatient-treated mental disorder during the period 1992–2003. Higher suicide seasonality was found for individuals treated with SSRIs compared to those with other AD treatments or without any AD treatment. The finding is more evident for men, violent suicide methods, and those without history of inpatient treatment.

Furthermore, Valenstein et al. [90] analyzed a sample of veterans with major depression for new AD starts (1999–2004) and found that most AD drugs did not differ in terms of suicide death. However, across several analytic approaches, though not instrumental variable analyses, fluoxetine and sertraline have been associated with a lower risk of suicide death than paroxetine. These findings are in line with the FDA meta-analysis including randomized controlled trials and reporting a lower risk for "suicidality" for sertraline together with a trend toward lower risks with fluoxetine compared with other AD medications.

Zisook and colleagues [91] analyzed 665 patients in the single-blind, 7-month randomized trial Combining Medications to Enhance Depression Outcomes study. Specifically, participants received escitalopram plus placebo, bupropion sustained release (SR) plus escitalopram, or venlafaxine extended release (XR) plus mirtazapine. Baseline ideation did not affect depressive symptom outcome. Bupropion-SR plus escitalopram most effectively reduced suicidal ideation. According to the main findings, venlafaxine-XR plus mirtazapine may pose a higher risk of suicide attempts.

A further caveat concerning the increased suicide risk during a second treatment with a different AD medication needs to be mentioned. Examining data collected between July 2001 and September 2006 from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D), Perlis and colleagues [92] found that of 1240 subjects entering level 2 with a score less than 3 in the suicide item, 102 (8.2 %) experienced emergence or worsening of suicidal thoughts/behaviors. Emergence or worsening at level 1 was strongly associated with reemergence or worsening at level 2 (crude OR = 4.00 [95 % CI, 2.45–6.51], adjusted OR = 2.95 [95 % CI, 1.76–4.96]). Relevantly, overall magnitude of risk was similar among next-step pharmacological augmentation vs. switching.

These results suggest that individuals who experience emergence or worsening of suicidal thoughts or behaviors with one AD treatment may warrant closer follow-up during the next-step treatment, as these symptoms may recur regardless of which modality is selected.

31.6.2 Children and Adolescents

There are studies suggesting an increase of suicide ideations/behaviors in children and adolescent patients treated with specific AD medications. Cooper and colleagues [93] conducted a retrospective cohort study including 36,842 children aged 6–18 years recruited between 1995 and 2006 who resulted new users of at least one of the specified AD medications. The authors reported that there was no evidence that risk of suicide attempts significantly differed for commonly prescribed SSRI/SNRI AD drugs. This study is bolstered by the presence of 419 cohort subjects who had a medically treated suicide attempt and four completed suicides in the whole sample. Furthermore, the adjusted rate of suicide attempts did not significantly differ among current users of SSRI and SNRI AD drugs compared with users of fluoxetine. Notably, users of multiple AD medications concomitantly had increased risk for suicide attempt. Moreover, based on the original venlafaxine publication, only 6 % of patients receiving venlafaxine experienced suicide-related events compared with 0.6 % receiving placebo [94]. After these data were carefully recorded by a second group of researchers, the numbers changed to 4.4 % of patients receiving venlafaxine compared with 0 % receiving placebo, respectively [50]. Either

way, venlafaxine has been associated with increased suicidality in adolescents, although this finding did not get adequate attention by a clinical point of view. Schneeweiss and colleagues [83] conducted a large 9-year observational study in Canada on 20,906 adolescents aged 10–18, who initiated a script for an AD with recorded depression matched with hospitalization for self-harm and suicide. Of those adolescents with a new diagnosis of depression, 266 attempted suicide, and three died of suicide in the first year of use. In addition, there were no differential effects of any of the individual SSRIs and TCAs. Whittington and colleagues [95] in a meta-analysis including five RCTs on suicide-related events and AD prescriptions in children and adolescents found that relative to fluoxetine, paroxetine, and sertraline, venlafaxine was most likely to be associated with suicide-related events. Finally, fluoxetine was likely to be associated with suicidal behavior or attempted suicide. The results were qualified due to the wide confidence intervals.

Furthermore, Miller and colleagues [96] conducted a study comparing two age groups for suicidality and new AD treatment. They used a population-based health-care utilization data with a total of 102,647 subjects aged between 10 and 24 years and 338,021 individuals aged between 25 and 64 years. Among the 10–24-year-old group, prior to propensity score matching, 75,675 initiated SSRI therapy and 5344 a treatment with SNRIs. There were 5344 SNRI users and 10,688 SSRI users after matching. Among the older cohort, 36,037 SNRI users were matched to 72,028 SSRI users (from an unmatched cohort of 225,952 SSRI initiators). Regardless of age group, patients initiating SSRIs and those initiating an SNRI had similar rates of deliberate self-harm. These results were not changed even after restricting to patients with no AD use in the past 3 years. However, Hetrick et al. [80] in their large Cochrane review focused on newer generation of AD compared to placebo in children and adolescent depression and reported no evidence that the magnitude of intervention effects compared with placebo was modified by individual drug class.

Finally, Hysinger and colleagues [97] reported that suicidal behavior among early and late adolescents who were taking AD medications differed in terms of methods used, previous psychiatric history, and proximal symptoms. Of the 250 cases which were reviewed, 65.6 % were female, 26.4 % were aged 10–14 years, and approximately one-half of the sample had a positive history of suicide attempts. It has been found that medication ingestion was the most frequent method of suicidal behavior for both early and late adolescents. However, early adolescents were significantly more likely to use hanging as a suicide method, to have a history of sexual abuse, and significantly less likely to have a history of substance abuse when compared to late adolescents. In addition, early adolescents were also more likely to have a history of a psychotic disorder and report hallucinations before the suicide attempt when compared to late adolescents.

31.6.3 All Age Groups

There are also studies addressing the association between AD use and method of suicide. Phillips and Nugent [72] reported a decrease in overall suicide rates for the young and elderly but underline an increase of suicide rates for the middle aged despite an increase in AD use in all age groups. Firearm suicides in men and women declined, but suicide by drug poisoning rose, particularly in women. In young males and elderly both males and females, better treatment of severe depression may have contributed to declining suicide rates. However, rising rates of prescription drug use are associated with higher levels of suicide by drug poisoning.

31.7 Studies that Did Not Find a Significant Association Between Antidepressant Use and Suicide Risk

There are studies in the current literature that fail to report any association between AD use and suicide rates. For instance, using a

population-based cohort study within the Dutch Integrated Primary Care Information (IPCI) database, Cheung and colleagues [98] did not show any association between the different AD drug classes and suicide attempts overall nor during the first weeks of treatment. Furthermore, no increased risk in the initial treatment period nor after restricting analyses to specific indication or age and gender groups was reported. Although the number of reported cases of suicide attempts was low as reported by the same authors, the study did not demonstrate an increase in terms of suicide risk after starting treatment with any type of AD medication.

In addition, Coupland and colleagues [82] conducted a cohort study using a large UK primary care database in order to quantify associations between different AD medications and suicide as well as deliberate self-harm (including suicide attempts) during the first 5 years of follow-up for adults diagnosed with major depression. Using citalopram as a reference, the authors found no differences in the suicide risk related to individual SSRIs or between SSRIs and TCAs. However, they also reported that the hazard ratio for suicide increased significantly during treatment with the venlafaxine and mirtazapine relative to SSRIs (2.6, 95% confidence interval 1.7–4.0). Interestingly, similar findings were reported for self-harm. Odds of self-harm increased with trazodone and decreased with amitriptyline. Across all AD drugs, hazard ratios for self-harm and suicidal behavior were increased during the first 28 days of treatment and during the 28 days after treatment discontinuation for completed suicides, respectively. However, when interpreting these results, it's important to pay attention to drug dosages. In Coupland and colleagues' study [82], a defined daily dose (DDD) of one corresponds to the generally accepted minimum effective AD dosages (e.g., 20 mg for citalopram and fluoxetine, 75 mg for amitriptyline). Most of the exposure to SSRIs in this study occurred at doses >0.5 DDD or >10 mg of citalopram as for the "other" AD medications. However, 64% of person-years exposure to TCAs occurred at doses ≤0.5 DDD, corresponding to ≤37.5 mg of amitriptyline. Some of these differences may be explained by the fact that TCAs often require a slower titration than other AD medications. Clinicians may also be conservative in their dosing as reflected, for example, in the National Institute for Health and Care Excellence (NICE) guidelines, which suggest that low dosages of TCAs may be maintained if clinical response is achieved (www.nice.org.uk/guidance/cg90). However, lower TCAs dosages can be used in a variety of other indications such as insomnia or headaches, so hazard ratios for the TCAs in Coupland and colleagues' study may be underestimated based on an indication bias. As emphasized by the same authors, the study results may be vulnerable to indication bias and, although conducted on large cohorts, may lack of sufficient power. Unfortunately, this study fails to show how patients may individually respond to specific AD drugs as well as how starting AD drugs or withdrawing from them may differentially confer suicide risk. It is likely that the association between higher suicidality and changes in AD treatment could be due to the fact that treatment changes often occur in periods of higher suicide risk.

Dubicka and colleagues [99] conducted a large meta-analysis to determine the pooled risk of self-harm and suicidal behavior in youths from randomized trials of newer ADs in order to calculate odds ratios for the combined data. The authors found that self-harm or suicide-related events occurred in 71 of 1487 (4.8%) depressed youths who were treated with ADs vs. 38 of 1254 (3.0%) of those who took placebo (fixed effects odds ratio 1.70, 95% CI 1.13–2.54, $P=0.01$). There was a slight trend for individual suicidal thoughts, attempts, and self-harm to occur more often in youths taking ADs than in those given placebo; however, none of these differences were statistically significant.

Finally, Olmer and colleagues [100] in a case-control study investigated the relationship between ADs and suicide attempts. Overall, 103 medical records of patients admitted after a suicide attempt (case group) were compared with 103 medical records of matched depressed patients admitted without suicide attempts (control group 1) and with 25 patients with and

without suicide attempts on separate hospitalizations (control group 2). As a result, no difference between cases and controls was reported.

31.8 Antidepressant Medications and Suicide Risk: Management and Treatment

Clinicians should be confident about depressive symptom improvement when an AD is initiated but simultaneously use cautions in the presence of suicide risk. In summary, they should:

1. Share with patients the assumption that the initial phase of AD treatment is a powerful indicator of the increased overall risk.
2. Carefully monitor patients during this starting time, in particular in the case of adolescents.
3. Warn patients and family members about the eventual worsening of depressive symptoms, as well as the emergence of suicidal ideation and self-harm.
4. Note any escalation—especially concerning suicidal ideation and self-harm—as a signal that the patient needs a rapid and systematic evaluation and treatment.
5. Advise patients that the interruption of AD drugs may also trigger a period of higher suicide risk justifying an intensified surveillance for a period of at least 4 weeks.

As previously anticipated and consistently with the aforementioned management indications, Isacsson and Rich [21] identified three major issues that could significantly guide to better treatment outcomes: (1) a careful reevaluation of the FDA warning in the light of the most recent findings; (2) a careful education of all health-care providers about the importance of aggressively treating depression, especially in youths and adolescents; and (3) an awareness about the higher risk of suicide of specific psychiatric conditions that should be closely monitored. These recommendations are necessary regardless of the type of AD treatment which has been provided.

However, it's also important to note that all medical treatments are associated with some risks, and AD drugs may be not excluded with this regard. As suggested, major depression is one of the major drivers of self-harm and suicidal behavior (it has been found in 87–92 % of suicide cases as reported by Angst and colleagues [101] and Hawton et al. [84]).

Therefore, in summary, clinicians should use vigilance for those patients who have been indicated as having a higher suicide risk, but in parallel they should also be careful to not a priori deny potentially effective drugs to some patients on the exclusive basis of unclear and controversial evidence.

31.9 Pharmacogenomics: A Future Prospective

Pharmacogenomics could lead to significant improvements of our current knowledge about AD treatment since it permits to create personalized treatments together with a better prediction of possible drug resistances.

For example, CYP2D6 is one of the most studied liver enzymes that may be related to psychiatric conditions and suicide risk [102]. Subjects with an increased number of CYP2D6 active genes, who are assumed to have a high enzyme activity in drug metabolism/elimination, seem to be more likely to die by suicide and to have a lifetime history of suicide attempts than those with a reduced number of CYP2D6 active genes [103–105]. CYP2D6 is involved in the metabolism of many psychoactive medications, such as AD medications. Thus, the effect of CYP2D6 on suicidal behavior can be partially due to drug therapeutic failure during AD treatment with CYP2D6 substrates and/or to differences in the central nervous system (CNS) regulation. Several studies found a poor response to AD drugs or early dropout from monotherapy treatment with CYP2D6 AD substrates in ultrarapid metabolizers [105–107]. CYP2C19 has also been found to be involved in the metabolism of SSRIs and TCAs; importantly, ultrarapid metabolizers have been identified as a high-risk group for poor treatment response and suicide risk [108]. Recently, for the first time a study

investigated the association between the CYP2D6- and CYP2C19-combined metabolic groups and the severity of the suicidal intent. The authors showed that a high CYP2D6-CYP2C19 metabolic capacity was related to increased severity of suicidal behavior. Consistently, most psychoactive drugs which were used for preventing or treating depressed and suicidal subjects are metabolized by CYP2D6 and CYP2C19 [102]. Unfortunately, studies investigating the individual adverse response to specific AD treatments and potentially providing genetic information for the best AD compounds in suicidal patients are yet to be carried out.

31.10 Conclusions: Major Limitations and Future Perspectives

Despite the large number of studies, no concluding evidence has been found, and the exact nature of the association between suicide rates and AD treatment remains controversial. However, a more careful and responsible AD use is absolutely required in clinical practice together with the need of adapting treatment strategies and closely monitoring the different investigated populations. At the same time, it is vital that primary care providers, who see and treat a substantial proportion of depressed patients, know that the risk posed by untreated depression—in terms of morbidity and mortality—has always been far greater than the very small risk associated with AD treatment [22]. The decision regarding the AD treatment should be balanced carefully assessing the suicide risk of patients as well as evaluating the whole clinical situation.

Some of the most relevant shortcomings of the abovementioned studies should be considered. For instance, observational studies may suffer from methodological limitations such as the achievement of inappropriate data and measures that may lead to questionable conclusions [109]. For example, projections of trends in rates of AD use or depression diagnoses that are based on historical trends are subject to error. Furthermore, methodological limitations may not allow this issue to be addressed adequately. The extremely low basal rate of suicide attempts and completed suicides needs to be interpreted as a limiting factor in cross-sectional studies analyzing the association between AD agent administration and suicidal risk. Meta-analytic approaches on larger samples seem to be only partially able to cover this gap [110].

Reliable tools helping clinicians in predicting how individual patients may differentially respond to particular AD medications are absolutely required [111]. Further additional studies together with deeper pharmacogenomic knowledge are also required, but to date cautions are needed when prescribing all AD drugs carefully weighing up all the potential risks and benefits related to the single cases.

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Neurobiology and Pharmacological Prevention of Suicide in Mood Disorders

32

Xenia Gonda, Zoltan Rihmer, and Peter Dome

32.1 The Neurobiology of Suicidal Behaviour

Suicide is a complex and multicausal phenomenon, and in spite of our broadening knowledge concerning its biological background, we are still very far away both from completing our picture and understanding of it and from obtaining effective methods of preventing it via pharmacological methods.

Suicide is complex not only concerning its etiopathology but also in its phenomenology. Suicidal behaviour presents in a variety of actions along a spectrum from suicidal ideation to attempted suicide to completed suicide. However, the varied phenotypical manifestation points to a varied neurobiological background, as increasing evidence indicates that suicidal attempts and completed suicides emerge on different neurochemical and genetic backgrounds. In about half of the completed suicides there is a previous attempt present, and suicide attempts should be differentiated according to lethality, violent or non-violent nature and intent to die, since suicide attempts are a more heterogeneous group also biologically [62, 72, 171]. There is also a great overlap between psychiatric disorders and suicidal behaviour, as almost 90 % of suicidal acts are carried out by psychiatric patients, mostly affective disorder patients, and to a lesser extent schizophrenic patients. Therefore, it is crucial to understand the neurobiology of suicide independently of that of psychiatric disorders as well as its relationship to these illnesses and to understand how suicide emerges both within and outside the framework of these illnesses.

Recently, understanding of the neurobiology of suicide has seen great advances from expanding methodologies in neurochemical studies encompassing the role of several neurotransmitter systems including the serotonergic, cholinergic,

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X. Gonda (✉)
Department of Psychiatry and Psychotherapy, Semmelweis University, Budapest, Hungary

Laboratory of Suicide Research and Prevention, National Institute for Psychiatry and Addictology, Budapest, Hungary
e-mail: gonda.xenia@med.semmelweis-univ.hu

Z. Rihmer • P. Dome
Department of Psychiatry and Psychotherapy, Semmelweis University, Budapest, Hungary

Laboratory of Suicide Research and Prevention, National Institute for Psychiatry and Addictology, Budapest, Hungary

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glutamatergic, GABAergic, dopaminergic, adrenergic and opioid systems, from the expansion of genetic studies including twin-, adoption- and family studies, as well as association, GWAS and epigenetic studies, and also from studies investigating the impact of stress on glia function and signalling. Furthermore, our current knowledge is already paving the way for further studies understanding the association of brain circuitry, protein modifications and microRNA changes in suicidal behaviour [48]. However, in order to understand suicide, we should incorporate all aspects including evolutionary and social contexts as well.

32.1.1 Animal Models of Suicidal Behaviour

Although suicide is a phenomenon occurring almost exclusively in humans, animal models are needed for experimental studies. Suicidal behaviour is difficult to incorporate into one model, because of its complexity, and its poorly understood contributing mechanisms, as well as certain inherent characteristics as suicide is the outcome of behaviours related to intent, will and anticipation and is also associated with personality traits, temperament and character and also involves important social dynamics [139]. At this point, the majority of animal models of suicide can only investigate specific isolated traits associated with suicidal behaviours in humans, including sleep disorders (sleep deprivation), anxiety (exposure to open unprotected space, light-dark box, elevated plus maze), despair (inescapable situation, forced swimming, tail suspension), anhedonia (reduced consumption of palatable sweet solutions), irritability (reaction to uncomfortable stimuli), impulsiveness (delayed reinforcement), aggression (resident intruder test), hopelessness (learned helplessness), acute stress (exposure to inescapable place), social stress (social disruption model) and chronic stress (long-term exposure to unavoidable uncomfortable stimuli) [139]. However, there are efforts to establish more specifically suicide-related animal models, and a fruitful approach in achieving animal models of suicidal behaviour would build models of well-known endophenotypes of suicidal behaviour [139]. The arrested flight model may be one ecologically valid animal model of suicidal behaviour which builds on three critical mechanisms thought to lead to suicide in humans, i.e. defeat, no escape and no rescue. The developers of the model hypothesised that suicidal behaviour encompasses reaction to stressful situations with perception of defeat associated with perception of no escape and no rescue, incorporating helplessness as no rescue and hopelessness as no escape in the model [139, 185].

32.1.2 Suicidal Phenotypes

One of the major difficulties in researching suicidal behaviour is that it involves complex and possibly multiple phenotypes built on various components as already seen in case of animal models, and it also shows a large overlap with psychiatric disorders making it difficult to separate suicide-related and underlying illness-related phenomena and genes in suicide research. Therefore, dissecting suicide-associated phenomena into smaller and better characterisable endophenotypes is an essential step in suicide research. Up to this point, the most extensively investigated suicidal endophenotypes include aggression/impulsiveness and cortisol-social stress response [139]. Impulsiveness, however, is still too wide to be studied in human studies or animal models but is decomposable into measures related to risk assessment, decision making, reward sensitivity and emotional regulation, all of which have previously been related to separate distinct genetic and neurobiological correlates [139]. Given the known role of stress in suicidal behaviour also expressed in the stress-diathesis model, and the role of the HPA-axis in stress response, it can be conceived that genetic variants implicated in the reaction to stress may moderate the influence of stress on behaviour [139, 145].

The other side of searching for suicide-related phenotypes involves understanding and separating the different complex manifestations of suicidal

behaviour. Suicide is expressed along a spectrum, spanning from suicidal ideation through suicide gestures, low-lethality suicide attempts, high lethality suicide attempts to completed suicide, and these different phenotypical manifestations of suicide seem to involve different psychodynamic, genetic as well as neurobiological background; therefore, it is essential to separate between these phenomena in all levels of suicide research.

32.1.3 The Genetic Background of Suicidal Behaviour

32.1.3.1 Twin, Family and Adoption Studies

Family, adoption and twin studies equally support the genetic background of suicidal behaviour [172]. It has long been observed both clinically and scientifically that suicidal behaviour aggregates in families [17, 27, 181], and it has also been found that relatives of suicide attempters and completers also carry a higher risk of suicidal behaviour, although it was still of question whether this genetic predisposition is one for suicide or one for psychiatric illnesses [26]. Studies increasingly indicate that although there is a partly overlapping genetic predisposition, genetic factors increasing risk of suicide are different from those in the background of psychiatric disorders indicating that genetic predisposition for suicides is at least in part likely to be transmitted through a separate, independent pathway from family transmission for susceptibility for mental disorders at [26, 45, 85]. It was also found that genetic transmission of suicidal behaviour is related to genetic transmission of personality traits associated with suicidal behaviour such as aggression [26].

Suicides are difficult to study in twin and adoption settings because suicide is a relatively infrequent phenomenon, and therefore mainly individual case reports are available. There are epidemiological studies concerning the concordance in twins in case of suicide attempts, suggesting that genes at least partially account for the observation that suicide runs in families also showing higher concordance rates for monozygotic than dizygotic twins [48, 60, 146, 147] even after adjusting for such important risk factors as personality traits, traumatic life events, psychiatric illness history and sociodemographic factors and concluding that genetic liability may account for an about 30–55 % variance of severe suicidal behaviour [158, 162], but heritability estimates strongly depend on environmental risk burden [78, 179]. It must also be mentioned that in the aggregation of suicides in families, besides heritability, social learning, social contagion and imitation may also play a role [40]. The importance of genetic factors influencing suicide was also complemented by adoption studies [183]. Altogether, family, twin and adoption studies univocally emphasise the role of an underlying genetic predisposition to suicidal behaviour independently or at least in part independently of a genetic liability to psychiatric illness and also suggesting a role of gene x environment interactions in the emergence of suicidal behaviour [48].

32.1.3.2 Genetic Studies of Suicidal Behaviour

Suicide and the components of suicidal behaviour appear to have strong genetic determinants. Several studies investigated the genetic background of possible endophenotypes associated with suicidal behaviour, mostly that of an impulsive-aggressive intermediate phenotype [171] indicating that higher impulsive-aggressive behaviour is a shared characteristic of suicidal behaviours across various psychiatric diagnoses [41, 104]. It was also shown that impulsive-aggressive traits in families at least partly explain the familial aggregation of suicidal behaviour [26, 85].

Several case-control association studies aimed at identifying the effect of individual genetic variants on susceptibility for suicidal behaviour with inconclusive results. Several genes were identified mainly including those related to pathways which are known to play a role in aggressive and impulsive behaviour, such as the serotonergic system. Generally, investigating genetic associations of suicidal behaviour faces the well-known difficulties of

studies searching for genetic associations in complex diseases in psychiatry, namely, phenotype and endophenotype heterogeneity and misclassification, insufficient power, population stratification and genetic and environmental interactions [48]. Furthermore, epigenetic changes have also been implicated in the emergence of suicidal behaviour, as epigenetic effects have been noted in phenomena associated with suicidal behaviour such as the development of affective disorders, stress reactivity, or early childhood adversities [105] and DNA methylation in certain genes including GABAA and glucocorticoid receptor genes may also have an effect on the expression of suicidal behaviour [105, 106, 138].

While earlier single or candidate gene approaches were frequent, and several genetic predisposing factors were identified, more recently, researchers propose the involvement of whole gene families or certain pathways; for example, in one study, genes located on the 4p locus were found to be related to completed suicide in males [115]. However, GWAS studies and mRNA expression studies are needed to verify significance of previous individual reports and to identify biological relevance of the findings [54].

GWAS Studies in Suicidal Behaviour

In case of complex polygenic traits such as suicidal behaviour, different genes can lead to the same phenotypic manifestations; therefore, a pathway or network genome-wide association approach is the most likely to produce relevant results [157]. So far, eight GWAS studies were published regarding suicidality, the majority of which investigate suicide as a treatment side effect [157]. Several of these studies shed further light on SNPs in genes already implicated in psychiatric disorders or such psychological phenomena which are possibly related to suicide; however, the exact role of the identified genetic variants in suicidal behaviour is yet to be understood.

One study investigating treatment-emergent suicidal ideation in major depression in the STAR*D cohort identified one SNP (PAPLN, papilin or proteoglycan-like sulfated glycoprotein) with genome-wide significance and one SNP (IL28RA or interleukin 28 receptor alpha) as suggestive, in addition to a 4-SNP multivariate model including GRIA3 (AMPA 3 ionotropic glutamate receptor) and GriK2 (kainite 2 ionotropic glutamate receptor) showing a ‘reasonable fit’ [91]. Another treatment-emergent suicidal ideation study did not confirm SNPs identified in the previous study and found no significant associations; however, 79 suggestive SNPs were identified [157], and after independent replications, nine independent SNP associations remained, six of which were annotated to five genes (TMEM 138, CTNNA3, RHEB, CYBASC3, AIMI) with the three other SNPs having intergenic location [108]. A study of suicidal ideation worsening during treatment identified one SNP (rs11143230) with suggestive associations for several others (in KCNIP4 and near ELP3) and several genes (including NTRK2, CCK, YWHAE, SCN8A, CRHR2) [135].

A further study investigating suicidality score in depressed patients found no genome-wide significant results, but seven SNPs from three regions were reported as suggestive, and a gene-wide association analysis for 33 candidate genes identified suggestive associations in case of 5HTR1A, CCK, RGS18, NTRK2 and SCN8A, some of which have earlier been investigated in relation to suicide and suicide-related endophenotypes [149].

A few GWAS studies also targeted actual suicidal behaviour. A study investigating lifetime suicide attempts in bipolar subjects with a two-stage analysis failed to find consistent associations, but a combined meta-analysis identified rs300774 located in a large D block containing three genes SH3YL1, ACP1 and FA150B [186], but another GWAS in suicide attempters [113] brought up no significant SNPs. The only GWAS study on completed suicide identified 58 suggestively associated SNPs, 22 of which were located in or near 19 known genes also pointing to four different pathways and nine of them also showing RNA expression alterations [55]. The main finding of this study was the role of the CD44 gene showing neuroimmunological effects in suicidal behaviour.

In general, only very few genome-wide findings were reproduced so far, but novel candidate genes were identified [157].

Gene Expression Studies

There have been a widening array of studies focusing on alterations in gene expression in suicide completers yielding important results on the involvement of several genes in different areas.

In investigating the limbic system in suicide victims, important patterns of changes in gene expression were identified, the majority of which affected the hippocampus and the amygdala, and genes strongly overrepresented among these results included those involved in transcriptional regulation and metabolism (APLP2, BACE1, SYT4, ADCY8, GABRA1, GABRB1) [152]. In another study performing gene expression analyses in 17 cortical and subcortical regions, the highest number of suicide-specific alterations was observed in prefrontal cortical areas and in the hippocampus [153]. Alterations of synaptic neurotransmission and intercellular signalling were also found including global alterations in glutamatergic and GABAergic genes [90, 153]. A further analysis of differentially expressed genes in the orbitofrontal cortex of suicide victims identified nine transcripts pointing to enrichment in central nervous system development, cell adhesion, cell proliferation regulation and nerve impulse transmission [167]. However, gene expression studies in suicides with different psychiatric diagnoses such as bipolar disorder or schizophrenia indicate that at the molecular level disorder-specific pathways may dominate over common pathways [54].

Metasystem Approach to Suicidal Behaviour

In one study aiming at overviewing the neurobiology of suicidal behaviour as one metasystem, 212 candidate genes were extracted using previous studies encompassing different neurobiological components hypothesised, implicated or shown in the entire suicidal behaviour research field [158] including also GWAS, mRNA expression and proteomic studies [158]. The identified genes were evenly dispersed along the whole genome reflecting an increasing number of candidate genes each year especially during the last decade, with 75 % of genes implicated since 2004 and 50 % since 2010 [158]. In this study, protein-protein interactions (PPIs) between proteins encoded by suicidal behaviour candidate genes were investigated in a large PPI network to identify a network of biochemical functions mediated by these proteins in the background of suicidal behaviour. The study identified a subset of proteins encoded by candidate genes of suicidal behaviour forming a core suicide behaviour interactome, the majority of which genes have already been shown to be upregulated or downregulated in different brain areas of suicide victims. These core suicidal behaviour interactome genes were found to form different neurosystems pointing to the necessity of an intersystem approach [158]. Gene functions identified in this study included molecular functions related to receptor binding in 31 % of genes, enzyme binding in 21 % and transmembrane signalling receptor activity in 20 %; or, from another aspect, biological processes related to neural impulse transmission (42 %), transport regulation (35 %) and endogenous stimulus response (34 %) were prevalent [158]. Such studies draw our attention to the importance of incorporating and balancing results from individual studies and to switching to a paradigm where not individual components, but systems and interactions between systems are investigated.

32.1.4 Structural and Functional Brain Changes in Suicide

The investigation of structural and functional alterations in the suicidal brain has a long history. In general, structural and functional neuroimaging changes point to correlations between changes observable in relation to suicidal behaviour and neuropsychological traits of increased impulsiveness and impaired decision making, specifically increased attention towards negative emotion stimuli and lower problem-solving capacities [37, 76].

Structural imaging results usually point to significantly thinner cortex in depressive patients with high risk for suicide in the ventral prefrontal cortex, dorsolateral prefrontal cortex and anterior cingulate cortex with the same regions differentiating between attempters and non-attempters and high and low lethality attempters among suicidal borderlines [35]. Adult suicide attempters were shown to possess decreased grey matter volume in the fronto-striatal-limbic network and decreased rostral anterior cingulate cortex volume in comparison to depressed adults without suicide attempt, while female suicide attempters exhibited bilateral orbitofrontal cortex grey matter volume decreases and greater right amygdala volume compared to controls [111, 182]. A meta-analysis showed that suicide attempts are associated with structural and functional changes in such brain areas which play a role in decision making contributing to reduction of motivational control exerted over salient negative stimuli-induced intentional behaviour [177].

In suicidal subjects also reduction in the size of the posterior third of the corpus callosum and possibly diminished interhemispheric connectivity as well as decreased fractional anisotropy in the left anterior limb of the internal capsule was reported [35].

Adult suicide attempters also exhibit abnormalities in neural circuitry, with high lethality attempters showing decreased activity in the prefrontal cortex and dorsal anterior cingulate cortex compared to lower lethality attempters [124], and adult male suicide attempters had greater activity in the right lateral orbitofrontal cortex coupled with decreased activity in the right superior frontal cortex to angry versus neutral faces indicating altered response to negative emotion [79]. Reduced activation in the medial prefrontal cortex was found in major depressive patients with high lethality suicide attempts compared to those with low lethality [35].

In general, a meta-analysis of 12 imaging studies in suicidal behaviour identified six brain regions where changes in volume or reactivity to emotional and cognitive stimuli were associated with history of suicide, consisting of decreased volumes observable in the left superior temporal gyrus, rectal gyrus, nucleus caudatus and functional correlates including the right cingulate gyrus, with two clusters in the anterior cingulate and one in the posterior cingulate [177]. The implicated brain regions with structural changes are involved in processing negative stimuli-provoked emotions and especially in the punishing aspects of salient events; therefore, these may play a role in mediating planning behaviour based on negative information [178]. The volumetric reductions are complemented with increased activation during emotional tasks and decreased activation during cognitive tasks in the rostral and dorsal anterior cingulate cortex in those with a positive history of suicidal behaviour [178]. Taken together, the results suggest that increased salience related to negative stimuli coupled with inability of controlling maladaptive responses during cognitive responses as a result of structural deficits may be the neurobiological basis of vulnerability towards suicidality [178].

32.1.4.1 Neurotransmitter Systems in Suicidal Behaviour

Serotonergic System

The serotonergic system has received the most attention and was the target of must studies trying to entangle the neurochemical background of suicidal behaviours following the first observations of lower 5-HIAA levels in the cerebrospinal fluid in suicidal patients [13] later confirmed by a meta-analysis [95], given its well-known role both in affective disorders and its association with impulsive-aggressive behaviours [15, 123], as well the potency of antidepressants to act via influencing serotonergic neurotransmission. Serotonergic alterations have since been demonstrated in the ventral prefrontal cortex as well as in the prefrontal cortex, hypothalamus and brainstem of suicide victims. The known association of low serotonergic function as a trait marker of suicidal behaviour [100] is complemented and contradicted by abrupt stimulation of serotonergic function by antidepressants leading rarely to treatment-emergent suicidal behaviour when initiating antidepressant treatment which may have a genetic predisposition in the background [92].

Several markers of serotonergic function have been associated with suicidal behaviour in different populations including a higher number of TPH-immunoreactive neurons and depressed suicides [21, 22], increased TPH2 mRNA expression in the raphe of completed suicidals [16] indicating either a compensatory mechanism to reduced serotonergic neurotransmission or a response to increased stress or an increased number of serotonergic neurons in the dorsal raphe manifested early on in the brain of suicidal subjects [48, 173].

Brain and platelet serotonin receptor and transporter binding studies indicated a decrease in serotonin transporter expressing neurons coupled with a greater serotonin transporter density per neuron in the dorsal raphe and decreased paroxetine binding in the prefrontal and ventrolateral prefrontal cortex of suicide completers [8, 14, 103]. Decreased 5HT1A receptor binding in the raphe pointing to a loss of receptors [7, 101] was also reported in suicide completers, while no differences in 5HT1A, 5HT1B and 5HT2 binding sites in frontal and temporal cortical areas were found. Decreased 5HT1 receptor density and affinity were however reported in the hippocampus and amygdala of suicidals [30, 31]. Another study found increased 5HT1A receptor density in the prefrontal cortex of nonviolent victims [103], while other studies found no such changes [12, 163]. In spite of some inconsistencies, suicide appears to be associated with increased 5HT1A receptors in some cortical areas [129] and especially the raphe nuclei and Brodmann 8 and 9 [54].

Concerning 5HT2A receptors in the brain, there is a significant contradiction with about half of the studies indicating upregulation [129]. Increased platelet 5HT2A receptor binding and number was however consistently observed in suicidal patients [128]. A significant increase of 5HT2A mRNA in the prefrontal cortex of suicidals was also reported [49, 132]. 5HT2C alterations were found concerning pre-mRNA editing and 5HT2C receptor expression in the prefrontal cortex in suicidals [129].

The results of the above studies suggest that postsynaptic serotonergic receptor upregulation observed in the prefrontal cortex of suicide victims may appear as a compensatory reaction to low serotonergic activity [100]. Reduced serotonergic input based on receptor mapping was also found in the orbital prefrontal cortex involved in behavioural inhibition which may account for the aggressive impulsive behaviours associated with suicidality [100].

In addition to the above receptor studies, the short allele of the 5HTTLPR polymorphism of the serotonin transporter gene was consistently found to show association with suicidal behaviour and especially with violent suicide [38, 62].

Dopamine

There is little evidence concerning the role of dopaminergic function in suicidal behaviour [48]. Reduction of dopamine turnover was reported in the nucleus caudatus, putamen and nucleus accumbens in depressed suicide victims [24].

Noradrenaline

Given the role of noradrenaline in stress reactivity, several studies investigated noradrenergic central nervous system alterations in suicide. Initial studies pointed to lower MHPG levels in urine or CSF in suicidal patients although results are contradictory [28, 150, 151]. Results concerning changes in tryptophan hydroxylase in association with suicide are also inconsistent [129]. The majority of studies reported no evidence concerning the change in number of noradrenaline-producing neurons in the locus coeruleus of suicidal subjects [166].

As far as receptor studies related to noradrenergic functions are concerned, increased α1 binding was reported in the prefrontal cortex [6]; however, decreased α1 receptor binding was also found in suicidals in several brain regions including the prefrontal cortex, temporal cortex and nucleus caudatus [67]. Increase in α2 receptor-binding sites in hippocampus and frontal cortex [6, 64] and also in the locus coeruleus [125] in depressed suicides was also found, while another study reported no α2 alterations in suicidal brains [68]. Density and affinity of α2 receptors were found to be increased in the frontal cortex and hypothalamus in depressed suicides [29, 107], and increased α2 sensitivity was also reported in

the frontal cortex of suicidal victims besides upregulation of α2 receptors in the prefrontal cortex [56, 63]. Increased β-receptor binding in the prefrontal cortex and temporal cortex in suicide victims was also reported [9].

A study observing 23 % fewer locus coeruleus neurons and 38 % lower density of locus coeruleus neurons in suicidals indicated that the observed changes in the noradrenergic neurotransmission in suicide may be a result of fewer locus coeruleus noradrenergic neurons [10].

GABA

An increase in the density of GABAergic neurons in the hippocampus and neocortex of depressed suicidals [20] and a significant reduction in neuronal somal size in cortical GABA interneurons in mostly suicidal depressives [140] were reported pointing to widespread GABAergic dysfunction in association with suicide. No difference, however, was found in GABA levels in the brain [88], in GABA transporter 1 binding [165] or GABA A or GABA B receptor binding in suicidals compared to controls [11, 164, 193]. The number of benzodiazepine-binding GABA sites in suicidals was found to be increased in the frontal cortex but not in the temporal cortex [32].

Glutamate

MK-801 binding studies of NMDA receptors indicated no difference in suicidal subjects [71], but affinity of glycine displaceable site binding was found to be reduced in suicidal brains [119]. Decreased potency of zinc to inhibit NMDA MK-801 binding was found in the hippocampus but not in the cortex of brain of suicide victims [120]. No alteration in NMDA binding was reported in suicidals in other studies [127].

AMPA density was found to be increased in the nucleus caudatus of suicidals [118]. Total AMPA binding was increased in the nucleus caudatus [52], and AMPA-binding upregulation was also reported [118]. In a microarray study by Kim et al., high affinity glial glutamate transporter SLC1A3 (glial high affinity glutamate transporter) and GLUL (glutamate-ammonia ligase) were significantly downregulated in the brain of suicidals [86]. Microarray studies in general replicated GABA and glutamatergic dysfunction in suicidal brain [87, 152].

32.1.4.2 Changes Related to Cell Signalling in Suicide

Surface receptor signal transduction disturbance was hypothesised to be a key underlying mechanism in suicidal behaviour leading scientists to investigate possible alterations of protein kinase A (PKA) and C (PKC) enzymes involved in the adenyl cyclase and phosphoinositide pathways, respectively [54]. Significantly decreased PKA activity was identified in the prefrontal cortex of suicide victims [42, 44], and decreased PKA activity was also observed in Brodmann 9 and hippocampus in suicide subjects [43], while another study in teenage victims found such association only in the prefrontal cortex but not in the hypothalamus [130]. Protein and mRNA expression of PKA subunits RIIβ and Cβ were also found to be decreased in the prefrontal cortex of suicidals [44].

PKC binding was also significantly decreased in prefrontal cortex, and PKC activity was decreased in the prefrontal cortex and hippocampus in suicide victims [131].

32.1.4.3 Glial Changes in Suicidal Behaviour

Especially astrocytes and oligodendrocytes have been implicated in suicidal behaviour [48], and microarray studies in cortical tissues of suicide completers repeatedly point to astrocyte-expressed genes including FGFR2/3, SLC1A3 and GLUL also emphasising glutamate involvement and dysfunction mediated by astrocytes [47, 48, 86]. Oligodendrocyte dysfunction was also implicated in suicide [154].

32.1.5 HPA-Axis Alterations in Suicidal Behaviour

There appears to be a strong relationship between HPA-axis dysfunction and suicide. Cortisol non-suppression in response to dexamethasone challenge during dexamethasone suppression test was found to be a strong predictor of completed suicide [33, 75] with ambiguous results in suicide

attempters [38, 74] with both hyper- and hypoactivity of cortisol following dexamethasone challenge probably related to the heterogeneity of both suicidal phenotypes and suicidal aetiology or methodological issues [48].

It was also studied whether HPA dysfunction is related to corticotropin-releasing factor (CRF) changes or alteration in corticoid receptors. A significant decrease in CRF receptor-binding sites was reported in the prefrontal cortex of suicidals [117] as well as a shift in CRFR1/R2 ratio in the pituitary [70]. Increased CRF levels and immunoreactivity were found in the frontopolar cortex of suicidals associated with a decrease in CRFR1 but not CRFR2 mRNA levels indicating increased CRF levels, decreased CRFR1 and unchanged CRFR2 receptors in completed suicide [109].

32.1.6 The Role of Cytokines in Suicidal Behaviour

The immune system has also been postulated to be altered in suicide although results concerning immune dysregulation are limited. Abnormal cytokine activity was found in some studies. Increased IL3 and IL4 mRNA expression was identified in the prefrontal cortex of female victims and increased IL13 mRNA in male victims [170], as well as increased CSF IL6 in suicide attempters [97]. Protein and mRNA expression of IL1beta, IL6 and TNFα were found to be decreased in teenage suicidal prefrontal cortex [133]. As our knowledge concerning the involvement of cytokines in psychiatric states deepens, further understanding of immunological involvement in suicidal behaviour can also be expected.

32.1.7 Alterations of Lipid Metabolism and Cholesterol Levels in Suicide

Lower levels of cholesterol, an important substance in the coproduction of cortisol, were found to be related to violent behaviour [46, 53, 112, 180] and specifically to suicide in several epidemiological studies [46, 116, 134] as well as to suicide attempts in clinical studies [61, 91, 96, 110] especially in case of violent attempts [3] and impulsive personality-associated suicide events [57]. In support of this, an inverse association was demonstrated between central nervous system cholesterol levels in the frontal cortex and violent suicidal behaviour [48]. Cholesterol levels are associated with measures of serotonergic activity in animal studies [77, 100], and cholesterol is also an important component of cell membrane and axonal myelin sheaths playing an important role in central nervous system development and in the adult brain the formation of new synapses, so lowered brain cholesterol levels may be associated with impaired synaptic plasticity [136]. The mechanism behind the association between lower cholesterol levels and suicide is, however, not clear yet [39].

32.1.8 Peripheral Biomarkers of Suicide

One crucial, clinically relevant aspect of understanding the neurobiology of suicide is to identify such peripheral markers which on the short or long term would be able to predict approaching or potential suicidal behaviour. Suicidal behaviour was previously found to be associated with markers in peripheral tissues such as blood cells, plasma, urine and CSF reflecting pathophysiological abnormalities but also pointing to possible diagnostic and even prognostic markers [129]. It would be crucial to find out if peripheral and central anomalies observed in association with suicidal behaviour have clinical implications and could be used as early screening tools. Given the complexity and multiple causality of suicide, it is unlikely that a single biological marker could be sufficient enough for the accurate, sensitive and specific prediction of suicidal behaviour. It should also be considered that suicidal behaviour has state- and trait-like determinants and associated phenomena, and a combination of these is more likely to be able to predict suicidal tendencies in general and approaching suicide. Combination of biological

predictors of state- and trait-like suicidal abnormalities especially when also combined with clinical and behavioural markers may aid the early detection, identification and prediction of approaching suicide [129]. Identifying these alterations and abnormalities in association may also provide novel targets and therapeutic tools in suicide prevention.

Several genes for molecules playing a role in signal transduction and composing receptors, which have been found to be associated with suicide, are also expressed in lymphocytes making white blood cells a useful tool in studying suicide-related gene expression, whereas platelets are useful for studying neurotransmitter functions related to adrenergic and 5HT2A receptors, 5HT transporter, MAO and BDNF [129]. Possible trait-related suicidal markers include decreased CSF 5-HIAA levels and increased platelet 5HT2A receptors, while state-related markers may be DST or decreased serum BDNF levels [129].

A study attempting to identify suicide-relevant biomarkers using a convergent functional genomics approach identified SAT1 (spermidine/spermine N1-acetyltransferase 1) as a top marker, and in addition, PTEN (phosphatase and tensin homologue), MARCKS (myristoylated alanine-rich protein kinase C substrate) and MAP3K3 (mitogen-activated protein kinase kinase kinase 3) were validated in living subjects by prospective as well retrospective analyses [93]. However, in spite of the advancement of identifying peripheral biomarkers, we are equally far away from using suicide predictors and from possible targets of new interventions in preventing suicide.

32.1.9 Newer Directions

In line with the advancement of techniques in science, there are new approaches to studying the neurobiology of suicidal behaviour. Individualised genetic studies, deep sequencing and large-scale methylation studies, micro-RNA analyses and studies on RNA editing are increasingly employed in delineating the background and correlates of suicidal behaviour [48] and will hopefully bring us closer to developing better pharmacological interventions for preventing suicide.

32.2 Pharmacological Prevention of Suicide in Patients with Major Mood Disorders

In spite of the large variety and approaches in research to understand the neurobiological background of suicidal behaviour, we are far from reaching a consensus within the specific fields and also concerning suicide altogether. The most agreed on and most consistently replicated biological marker of suicidal behaviour after decades of research is still low serotonergic function although encouraging results point to glutamatergic and GABAergic dysfunctions as well. The confusion is in part related to phenotypical and aetiological heterogeneity of suicidal behaviour.

In spite of our increasing knowledge concerning the neurobiological background of suicide and its neurochemical components, we do not have specific pharmacological intervention strategies targeting suicide. Instead, already existing medications are used to treat or decrease suicide-risk traits, such as impulsiveness and aggression, or such suicide-related conditions, as mood disorders. The majority of suicides are committed by major mood disorder patients including unipolar and bipolar depressives; therefore, the proper treatment of these conditions contributes to a major reduction in the risk of suicidal behaviour.

There indeed is a great possibility to treat the psychiatric conditions in the background of suicide; however, the key would be recognition and treatment of suicidal risk in those contacting healthcare services whether for mood disorders or other conditions. Current major depressive episode is the most frequent medical condition in case of suicide victims and attempters, especially if previous suicide attempts and adverse life events are simultaneously present, and in case of lack of appropriate treatment or other protective factors [34, 66]. Suicide is the most severe treatment (and nontreatment) outcome in psychi-

atric disorder patients. Suicide accounts for 15–20 % of deaths among those ever hospitalised due to mood disorders, and approximately 5–15 % of unipolar and bipolar depressive patients die by suicide [23, 66]. Within mood disorder patients, bipolar patients, and especially those with a predominant depressive polarity, show an increased risk of suicide [18]. Suicide is also a state- and severity-dependent phenomenon, since the highest risk of both attempted and committed suicide occurs during severe major depressive or mixed affective episodes, and mood disorder patients practically never commit suicide during euthymic and euphoric manic phases [94, 145, 176]. Therefore, one of the major targets of suicide prevention is early recognition of mood disorders, effective treatment of mood episodes as well as estabilising and expanding euthymic periods. In spite of the known association between suicide and affective illness and affective states, approaching suicidality is often not sufficiently recognised, although nearly 66 % of suicide attempters and victims contact their psychiatrists or GPs within 4 weeks before their suicidal act [99, 137]; thus, besides psychiatrists, also GPs and other doctors have a prominent role in discovering and preventing approaching suicidality. In the context of mood disorders, there are several medications available to prevent suicidal behaviour including antidepressants, antipsychotics, antiepileptics and lithium, depending on the illness and illness phase.

32.2.1 The Role of Antidepressants and Antipsychotics in Suicide Prevention in Major Mood Disorders

32.2.1.1 Antidepressants in the Prevention of Suicide in Unipolar Major Depression

Several studies with different designs and approaches consistently point to the role of antidepressants in decreasing suicide rates in affective disorder patients. In the National Institute of Mental Health Collaborative Depression Study [94], 643 unipolar major depressive patients, treated either with fluoxetine or other antidepressants (411, 64 %) or nontreated (232, 36 %), were followed for 6 years to investigate frequency of attempted and committed suicide. Although patients receiving antidepressant treatment reported more previous depressive episodes compared to nontreated participants, those receiving fluoxetine or any antidepressant therapy showed a 56 % and 40 % risk in suicidal behaviours, respectively, indicating a clear and marked effect of antidepressive therapy in reduction of suicidal behaviour [94]. Another study following up 521 unipolar depressive patients receiving SSRI or TCA pharmacotherapy for an average of 2 years (6–132 months) found that rates of all types of suicidal events showed a more than fivefold increase following antidepressant discontinuation, and the increase in the risk of suicidal behaviour was 16-fold considering completed suicides separately, contributing to a 81 % and 77 % overall suicide risk reduction of all types in TCA- and SSRI-treated patients, respectively [188]. In a large-scale 6-month follow-up study investigating the effect of treatment initiation in more than 82,000 antidepressive treatment episodes of SSRI or other antidepressants in 65,103 unipolar depressive or dysthymic patients with unipolar major depressive disorder or dysthymic disorder, suicide attempt risk was 2.5 times higher in the month preceding antidepressant initiation and showed a progressive decline [156]. A naturalistic, 34–38-year follow-up study of 186 formerly hospitalised unipolar depressive patients receiving long-term antidepressant, antipsychotic and/or lithium pharmacotherapy showed a significant 2.5-fold decrease in suicide rate and these patients lived longer compared to patients receiving no treatment [4]. Register-based observational cohort studies similarly indicate that formerly hospitalised unipolar depressive patients continuing antidepressant treatment exhibit a marked decrease in the rate of completed suicides compared to those discontinuing the medication [159, 168].

Furthermore, there is a progressive and significant decrease of suicide rates in depressive patients observable from the ‘pretreatment era’ (1900–1939), through the ‘ECT era’ (1940–

1959) to the 'antidepressant era' (1960–1992) of 6.3, 5.7 and 3,3./1,000 patient per year, respectively [121] clearly indicating that antidepressive medications have a preventive role in suicide in case of patients with major depression, and the pronounced decline in suicide rates in several countries paralleling a recently increasing antidepressant utilisation also supports the suicide protective effect of antidepressants even at the level of general population [98]. Studies indicate that antidepressant treatment of all patients diagnosed with depression would prevent more than one third of deaths from suicide, independently of age, gender and suicide attempt history [36]. In spite of all these results in several countries, antidepressant exposure is not adequate to produce an undoubted reduction in suicide rates possibly because of prescription of inadequate doses or lack of adherence.

Furthermore, a non-significant increase of suicidal behaviour was found in unipolar patients receiving antidepressant monotherapy in comparison to placebo-treated patients in a meta-analysis of phase II-phase III randomised controlled clinical trials, excluding the most severe and acutely suicidal patients [82–84, 99, 144]. In a meta-analysis of 702 RCTs including more than 87,000 depressive and other psychiatric patients, there was a significant increase with an OR of 2.28 in attempted suicide in SSRI-treated patients compared to placebo; rate of completed suicide, however, was not significantly different [50]. Comparing suicide risk associated with different antidepressant treatments, fluoxetine and sertraline showed lower associated risk compared to paroxetine although most antidepressants did not differ in accordance to FDA meta-analysis pointing to lower risks for sertraline and fluoxetine compared to other antidepressive medications [174]. In case of paediatric depression, an analysis of 25 outpatient antidepressant trials including more than 4,000 patients reported a rate of 3.2 % of patients attempting suicide on antidepressant treatment compared with 1.7 % taking placebo, with no completed suicides [184], while reanalysing placebo-controlled studies of fluoxetine and venlafaxine yielded no observation of an increase in suicide in young people [58], and in a 5-year study investigating suicide in Denmark in the 10–17-year age group, none of the 42 suicide completers received SSRI treatments in the 2 weeks preceding their suicide [161].

Several factors explain the controversy between the suicide-decreasing effects of antidepressants in real-life studies and the suicide-provoking effects seen in some randomised controlled trials. First of all, severely ill, actively suicidal depressives are excluded from antidepressant studies just as bipolar I and bipolar II depressives are too [23]. A challenging paradox of psychiatry and psychopharmacology is that antidepressants prevent suicidal behaviour among severely ill, suicidal 'real-life' unipolar depressives [4, 59, 94, 156, 188], but they can induce such behaviour rarely in less severe, actually non-suicidal 'trial life' unipolar depressives, particularly in the youngest age cohorts [50, 82, 83, 141, 184]. Therefore, these studies are not adequate for drawing conclusions on the possible relationship between antidepressant use and the emergence of suicidal behaviour. Patients at the time of committing their suicidal act while taking antidepressant medication are usually in an activated state. Furthermore, recent findings indicate that this relatively small increase in suicidal behaviour could be related to the depression-worsening potential of antidepressant monotherapy, unprotected by mood stabilisers or atypical antipsychotics, in (mostly young) depressives with subthreshold bipolarity and in unrecognised bipolar depressives [126, 175]. About 30–40 % of DSM-IV-diagnosed unipolar major depressive patients show clinically significant intradepressive hypomanic or manic symptoms contributing to mixed depression as well as such other basic clinical features including family history of bipolar disorder, early onset, bipolar conversion, psychiatric comorbidity, etc., that are characteristic and external validators for bipolar disorder [2, 5, 51, 194]. Nonresponse or worsening of depression and hypomanic and manic switches during antidepressant treatment are significantly more frequent in threshold or subthreshold bipolar depression than unipolar depression [126, 143, 155, 175]. Therefore, the

reported slightly elevated risk of suicidal behaviour in unipolar patients on antidepressive pharmacotherapy may be a consequence of the depression-worsening potential of antidepressant monotherapy in subthreshold and mixed bipolar depressive patients included into these trials falsely diagnosed as unipolar patients [142, 143].

An analysis of 57 placebo-controlled major depression and anxiety disorder paroxetine trials including almost 15,000 patients showed no difference between paroxetine and placebo regarding suicidal behaviour, but analysing major depressive patients separately, the incidence of suicidal behaviour was significantly greater for paroxetine than for placebo (0.32% vs. 0.05%, OR = 6.7) particularly among young adults aged 30 years or less. In the non-depression clinical trial database ($N = 8{,}931$), the rate of suicidal behaviour was identical in the paroxetine and placebo groups [89]. The rare worsening of depression in some patients during antidepressive pharmacotherapy may be related to the emergence of a mentally overstimulated, excited, agitated, anxious, early onset depressive mixed state [2, 126]. A reanalysis of 12 adult, 4 geriatric, and 4 youth fluoxetine RCTs and 21 adult venlafaxine trials showed that both antidepressants decreased suicidal ideation and suicidal behaviours for adult and geriatric major depressive patients, and this protective effect was mediated by decreases in depressive symptomatology in response to psychopharmacological treatment.

In spite of the isolated cases of newly emergent suicide explained above, in everyday clinical practice, benefits of antidepressants highly outweigh this risk, and appropriate use of antidepressants effectively treats and protects depressed patients from suicide [141]. Suicide risk is the highest in the first 10–14 days of newly initiated antidepressive treatment, while antidepressant action is not observable yet; patients with depressive disorder, especially in case where suicide risk is high, should always be closely observed [73, 156]. In the first few weeks of the treatment, appropriate care is always necessary and co-medication with other psychopharmacons is also frequently required. In addition, newer data indicate that a combination of genetic markers may be useful in identifying patients at increased risk of suicide during antidepressant treatment [108].

32.2.1.2 Antidepressants and Antipsychotics in Suicide Prevention in Bipolar Disorders

Antidepressants have very limited value in the long-term treatment of bipolar disorders because of their mood-destabilising effects and should only be applied as an adjunct therapy in case of acute bipolar depression [2, 126, 148, 175]. While in the treatment of bipolar disorder a meta-analysis has not shown the superiority of antidepressants compared to placebo or other current standard treatment [155], hypomanic or manic switches during SSRI treatment are significantly lower than in the case of TCAs, and mood stabilisers contribute to further reduction in the risk of mood switching [25, 114, 148]. The risk of suicidal behaviour in bipolar patients during long-term treatment is highest in those receiving antidepressant and antipsychotic monotherapy and lowest in patients with mood stabiliser monotherapy, while combination therapy with mood stabilisers and either antidepressants or antipsychotics showed an intermediate risk similar to that in bipolar patients during the period 'off' mood stabilisers [190–192] suggesting that a combination of antidepressants or antipsychotics with mood stabilisers markedly reduces (although does not eliminate) the elevated risk of suicide in patients on antidepressant or antipsychotic monotherapy, and mood stabiliser monotherapy is the only treatment strategy that provides the best result in this respect. Therefore, when treating breakthrough depression during long-term management of bipolar patients, supplementary antidepressant and first-generation antipsychotic medication should be administered for as short time as possible and only as a supplement to mood stabilisers, keeping the mood stabiliser monotherapy as the backbone of the long-term treatment.

In contrast to typical antipsychotics, some atypical antipsychotics, including quetiapine and olanzapine plus fluoxetine combination, show acute antidepressive effects, while some, includ-

ing quetiapine, olanzapine and aripiprazole, possess long-term mood-stabilising effects in bipolar disorders [187].

32.2.2 Lithium and Suicide Prevention

32.2.2.1 Lithium in the Prevention of Suicide in Unipolar Major Depression

The suicide protective effect of lithium is not limited to bipolar disorder, where it is mainly indicated as treatment. A meta-analysis of eight studies including 329 patients with a mean exposure for 4.6 years and 6.3 years without lithium treatment indicated that with lithium treatment overall completed and attempted suicide risk in recurrent unipolar major depressive patients was 88 % lower compared to no lithium treatment, indicating the suicide risk reduction efficacy of lithium also in unipolar patients [69]. Furthermore, although the mechanism of action is not clear either regarding this mood stabilising or its antisuicidal effect, the antisuicidal capacity of lithium seems to be at least partly independent of its phase-prophylactic effect, as in a study in 167 recurrent unipolar and bipolar mood disorder patients with at least one previous suicide attempt; during long-term lithium prophylaxis, there was a significant reduction of attempted suicide in poor responders and moderate responders as well (70 % and 78 %, respectively) [1]. Therefore, even in the absence of response to lithium, in case of patients showing a high risk of suicide, lithium therapy should be retained and combined with other mood stabiliser therapy.

32.2.2.2 Lithium and Antiepileptic Mood Stabilisers in the Prevention of Suicide in Bipolar Disorders

Lithium is not only effective in the treatment and prevention of manic episodes, but in combination with antidepressants, it significantly decreases risk of hypomanic or manic switch from depressive phases [25, 66, 114]. In a comprehensive review of 45 randomised, controlled and open clinical studies including 85,229 person-years of risk exposure, an approximately 80 % decrease in the risk of attempted and completed suicides was found in bipolar or unipolar patients on long-term lithium treatment reducing levels close to what is observable in the general population, and incidence ratio of attempts to suicides increased 2.5 times with lithium treatment indicating reduced lethality of suicidal acts [19]. A Danish linkage cohort study analysing more than 13,000 lithium users, purchasing lithium at least twice, was associated with a 0.44 reduced rate of suicide compared to the rate when purchasing lithium only once, and rate of suicide decreased with the increasing number of lithium prescriptions [81]. Generally, results of several studies indicate that lithium has a strong antisuicidal effect markedly exceeding its prophylactic efficacy [168] which may be related to the anti-aggressive activity of lithium due to its serotonin-agonist effect [1, 66]. Lithium levels in drinking water were also found to be significantly negatively associated with suicide mortality in a study in Japan and Austria [78, 122].

Other phase-stabilising agents besides lithium also proved to have a suicide risk-lowering effect. A retrospective chart review of 140 bipolar outpatients on continuous lithium, valproate or carbamazepine treatment for a minimum of 6 months during a 23-year period showed a more than twofold reduction in nonlethal suicidal behaviour during therapy compared to after discontinuation of mood stabilisers, and frequency of nonlethal suicidal behaviour was not different during treatment with lithium, compared to valproate or carbamazepine [189]. A Danish national register-based study analysed the association between continuous mood stabiliser treatment and suicide among all 5,926 patients discharged from psychiatry as an in- or outpatient between 1995 and 2000 in Denmark with an ICD-10 bipolar disorder diagnosis. Patients who continued treatment with various mood stabilisers including lithium, divalproex, lamotrigine, oxcarbazepine and topiramate showed a significant decrease in completed suicide rate compared to patients who purchased mood stabilisers only once, and the rate of suicide decreased consistently with the number of additional prescriptions similar to a

previously quoted study, and long-term treatment with lithium and antiepileptic mood stabilisers was associated with similar reduction in suicide mortality [160].

However, in other studies, lithium proved to have an outstanding antisuicidal effect compared to other mood-stabilising agents. In a naturalistic, retrospective chart review of 405 bipolar patients followed for a mean of 3 years, risk of suicidal behaviour was analysed indicating that mood stabiliser monotherapy with lithium, divalproex or carbamazepine reduced the risk of suicidal behaviour by more than 90 %, with similar benefits for lithium and antiepileptic mood stabilisers [191]. A population-based retrospective real-world observational cohort study including more than 20,000 bipolar I and II patients compared risk of suicide and suicide attempts during lithium, divalproex and carbamazepine and no treatment for a mean follow-up of 2.9 years and found a 42 % reduction in completed suicides in lithium-treated patients compared to non-treated subjects, among whom milder and non-suicidal cases were most likely to be overrepresented. However, risk of suicide deaths after adjustment for age, gender, comorbid disorders and concomitant psychotropic use and suicide attempts resulting in hospitalisation was 2.7 times and 1.8 times higher during divalproex and 1.5 times and 2.9 times higher during carbamazepine than during lithium treatment [65], which is in good agreement with earlier study results reporting lower risk of suicidal behaviour among patients on lithium treatment for bipolar disorder among those receiving treatment with carbamazepine [168].

Conclusion

Understanding the neurobiology and pharmacology of suicide would bear important implications for better prediction, prevention and treatment of suicidal behaviour. Although our in-depth knowledge on the neuroanatomical, neurochemical and genetic associations of suicidal behaviour is increasing, we are still very far away both from establishing a comprehensive view and understanding on the neurobiology of the emergence of suicidal behaviour and from building clinically viable pharmacological tactics based on these findings to treat suicidality. However, we do have some pharmacological tools already at this point which lead to very marked and significant reduction in suicidal behaviour in mood disorder patients during continued treatment, although the mechanism of action of these treatment options with respect to suicidality is not always fully understood. Also, we are still very far away from taking advantage of all other already existing possibilities in the treatment of suicidal behaviour.

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33 Antidepressant and Anticonvulsant Drugs as Adjuvant Analgesics in Chronic Pain

Manuel Sebastián-Aldeanueva, Francisco López-Muñoz, José Antonio Guerra, and Cecilio Álamo

33.1 Introduction

Pain is a constant in human history. We are born, live, and die with pain; thus, defining it seems unnecessary. However, many definitions have been proposed in an attempt to delineate the different components present in pain. Probably the most widely accepted is that of the International Association for the Study of Pain [36] that defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [45]. This suggests that pain is a subjective experience and that the mere presence of physical injury may or may not adequately explain it [68].

In general, adjuvant analgesics are a very heterogeneous group of drugs with a primary indication other than pain, but with analgesic properties in some painful conditions [42]. Although they can be used alone, for example, antidepressants in postherpetic neuralgia, they are usually coadministered with analgesics (opioids or NSAIDs). The term "coanalgesic" is sometimes used synonymously in this setting. The use of adjuvants, associated with different analgesics, has been widely recognized in the analgesic ladder [64]. In addition, some of these agents can act as analgesics primary as a treatment option for management of specific pain [55], e.g., gabapentin for neuropathic pain or antidepressants in postherpetic neuralgia. Using an adjuvant analgesic is important to the success in effective pain management.

On other occasions, adjuvants are agents used to reduce adverse effects of analgesics, such as antiemetics or laxatives with opioids, or to treat symptoms associated with pain, as anxiolytic or hypnotic. These adjuvants don't have analgesic properties and escape the objectives of this work, so it will not be treated in this chapter.

M. Sebastián-Aldeanueva
Primary Care Administration of Soria, Soria, Spain

F. López-Muñoz (✉)
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal
e-mail: francisco.lopez.munoz@gmail.com; flopez@ucjc.edu

J.A. Guerra
Department of Pharmacology, Pharmacy Faculty, Complutense University, Madrid, Spain

C. Álamo
Department of Biomedical Sciences (Pharmacology Area), Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain

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33.2 Wide Classification of Adjuvant Analgesics

Major classes of adjuvant analgesics include antidepressant and antiepileptic drugs (AED). On the other hand, there are some therapeutic groups which are useful in the treatment of pain, even though they lack direct analgesic properties, as corticosteroids, alpha-2 adrenergic agonists, N-methyl-D-aspartate (NMDA) receptor antagonists, gamma-amino-butyric acid (GABA) agonists, benzodiazepines, neuroleptics, muscle relaxants, bisphosphonates, cannabinoids, psychostimulants, anticholinergics, calcitonin, radiopharmaceuticals, and octreotide (Table 33.1). In addition, local anesthetics as topical analgesics are also considered as adjuvants [38, 42] (Table 33.1).

In this chapter, we will describe antidepressant and antiepileptic drugs as adjuvant analgesics.

33.3 Adjuvant Antidepressant Drugs

In the pharmacological armamentarium, there are a large number of substances with antidepressant activity. Several classifications of antidepressants

Table 33.1 Classification of principal adjuvant analgesics

Therapeutic group	Drugs	Principal indication for pain
Antidepressants	*TCAs*: amitriptyline, nortriptyline, desipramine	Multipurpose analgesics
	SSRIs: paroxetine, citalopram	
	SNRIs: venlafaxine, duloxetine, milnacipran	
	DRI: bupropion	
Antiepileptics	Gabapentin, pregabalin, carbamazepine, oxcarbazepine, lamotrigine, topiramate, tiagabine, levetiracetam, zonisamide, phenytoin, valproic acid	
Corticosteroids	Dexamethasone, prednisone	
α2 adrenergic agonists	Clonidine, tizanidine	
NMDA receptor antagonists	Ketamine, dextromethorphan, memantine,	Neuropathic pain
GABA agonists	Baclofen	Musculoskeletal pain
Local anesthetics	Lidocaine, mexiletine	Neuropathic pain
Topical analgesics	Capsaicin, lidocaine, lidocaine/prilocaine	
Benzodiazepines	Diazepam, lorazepam, clonazepam	Musculoskeletal pain
Conotoxins	Ziconotide	Neuropathic pain
Muscle relaxants	Cyclobenzaprine, carisoprodol, methocarbamol, metaxalone, orphenadrine	Musculoskeletal pain
Antipsychotics	Olanzapine	
Bisphosphonates	Pamidronate, zoledronic acid, clodronate	Bone pain
RANKL inhibitor	Denosumab	
Other adjuvant analgesics		
Psychostimulants	Methylphenidate, modafinil	
Anticholinergics	Hyoscine, glycopyrrolate	
Calcitonin		

Modified by Lussier et al. [42]
TACs tricyclic antidepressants, *SSRIs* selective serotonin reuptake inhibitors, *SNRIs* serotonin and norepinephrine reuptake inhibitors, *DRI* dopamine release inhibitor, *NMDA* N-methyl-D-aspartate, *GABA* gamma-aminobutyric acid, *RANKL* receptor activator of nuclear factor kappa-B ligand

Table 33.2 Biochemical classification of antidepressant drugs

Mechanism of action	Acronym	Principals drugs as adjuvants
5-HT and NA reuptake inhibitors with blocking action of diverse receptors	TCAs	Imipramine, amitriptyline, nortriptyline, desipramine
NA reuptake inhibitors with blocking action of diverse receptors	Second generation	Maprotiline
Antagonists of alpha-2 autoreceptors	Second generation	Mianserin
Selective DA reuptake inhibitors	DRI	Bupropion
Selective 5-HT reuptake inhibitors	SSRIs	Fluoxetine, paroxetine, sertraline, citalopram
Antagonists of alpha-2 auto- and hetero-receptors and 5-HT2; 5-HT3 receptors	NaSSA	Mirtazapine
NA and 5-HT reuptake inhibitors	SNRIs	Venlafaxine, duloxetine
Selective NA reuptake inhibitors	NRI	Reboxetine

Modified from López-Muñoz and Álamo [40]
TACs tricyclic antidepressants, *SSRIs* selective serotonin reuptake inhibitors, *SNRIs* serotonin and norepinephrine reuptake inhibitors, *DRI* dopamine release inhibitor, *NRI* norepinephrine reuptake inhibitor

have been proposed according to different criteria: chemical structure, chronological clinical introduction, etc. However, from a theoretical and practice perspective, the more appropriate classification is that used their biochemical profiles (Table 33.2).

The exact mechanism of the analgesic action of antidepressants is uncertain. In general, these agents are very heterogeneous from the biochemical point of view, so it can be considered "dirty" drugs. However, their efficacy can be related to central blockade of central nervous system (CNS) monoamine uptake, particularly serotonin and/or norepinephrine. This is a common characteristic to all antidepressant. This ability to increase rates of monoamines in the synaptic cleft at both supraspinal and spinal levels reinforces descending inhibitory pathways. This effect is postulated as the main mechanism of the analgesic effect of antidepressants [40].

In addition, some antidepressants can block, to varying degrees, a number of other receptor involved in pain processing including α-adrenergic, H1-histaminergic, and N-methyl-D-aspartate (NMDA) receptors. They may also have blocking effects on calcium and sodium channels and be weakly stimulatory at μ-opioid receptors [24]. There is also some evidence that TCAs potentiate the endogenous opioid system [31].

The relation of pain with depression is common, and some authors have argued that the analgesic effect of antidepressants is secondary to the relief of comorbid depression. This hypothesis is possible when the agent is used in antidepressant doses in the treatment of somatoform pain, but does not explain its effectiveness in somatic headaches, experimental or acute pain, or in pain in nondepressed patients or at doses lower than those commonly used in the treatment of depression. Furthermore, the analgesic effect precedes antidepressant activity [21, 41].

On the other hand, it has been postulated that the analgesic effect of antidepressants is secondary to a sedative action. However, most sedative agents are not more analgesics and higher doses of these antidepressants, which cause more sedation, nor are more efficient as analgesics. Also, some authors think analgesic activity of antidepressants as a placebo effect. However, this hypothesis is not supported, since, in the majority of controlled studies, the effect of the antidepressant was superior to placebo [21, 41].

TCAs are believed to have independent analgesic effects as well as an ability to relieve the depressive symptoms associated with chronic pain. Studies have shown that the dose of TCAs required for analgesia frequently is lower than the dose needed to manage depression, with a faster onset of analgesic action in comparison to antidepressant action [7]. On the other hand, the receptorial profile of antidepressants is

responsible for bad tolerability and some adverse effects of these agents [2, 3, 43].

Antidepressants, both from the experimental and clinician point of view, are the adjuvant analgesics most studied. Only 2 years after the clinical introduction of the first TCAs, imipramine, Paoli et al. [53] described its utility as an analgesic in a preliminary report. Five decades later, there are multiple experimental and clinical studies published on this topic. Amitriptyline has been the most widely studied TCA in chronic pain, although some TCAs, including doxepin, imipramine, nortriptyline, desipramine, and milnacipran, also have been used with variable success [22, 23, 34, 35, 47, 48]. However, many of clinical studies with these antidepressants in pain treatment lack adequate methodological characteristics to allow general conclusions based on the evidence. For this reason, few antidepressants have official indication as analgesics. Amitriptyline is approved in some countries for the treatment of neuropathic pain, imipramine in chronic pain, and duloxetine, a SNRI, in diabetic neuropathic pain and fibromyalgia [21].

Due to the lack of head-to-head trials to guide treatment choices, one approach to estimate the relative efficacy of analgesic agents in randomized clinical trials (RCTs) is to use the number needed to treat (NNT), the number of patients that need to be treated with a certain drug to provide one additional patient with at least 50 % pain relief relative to the comparator group. The NNT is used to estimate treatment efficacy, recognizing that there are limitations to this methodology including variability in RCTs (e.g., crossover versus parallel design) and the short-term nature of most RCTs [51].

Despite the widespread use of antidepressants in the treatment of pain, inadequate response to drug treatments constitutes a substantial unmet need in patients with neuropathic pain. Modest efficacy, large placebo responses, heterogeneous diagnostic criteria, and poor phenotypic profiling probably account for moderate trial outcomes [28].

Analgesic properties of TCAs are manifested fundamentally in *neuropathic pain* (NeP). The combined NNTs for TCAs in the management of painful diabetic neuropathy and postherpetic neuralgia were 2.1 and 2.8, respectively [51]. However, some recent Cochrane review found little evidence to support the use of nortriptyline [23], imipramine [34], or desipramine [35] to treat the neuropathic pain. Furthermore, amitriptyline is commonly used to treat neuropathic pain conditions, and it is recommended as a first-line treatment in many guidelines as also questioned. Thus, a recent Cochrane review found low-quality evidence in the NeP treatment with amitriptyline. However, these results have to be balanced against decades of successful treatment in many people with neuropathic pain. Amitriptyline should continue to be used as part of the treatment of neuropathic pain, but only a minority of people will achieve satisfactory pain relief [48].

Similar conclusions can be done with amitriptyline in *fibromyalgia* [47]. However, despite the limited quality of the data, consistent with some clinical guidelines, this antidepressant, used for short periods of time, is the drug with the most solid evidence in fibromyalgia [4, 56]. Pregabalin, duloxetine, milnacipran, and amitriptyline are the current first-line prescribed agents but have had a mostly modest effect [33].

Some SSRIs as citalopram, paroxetine, and escitalopram, but not fluoxetine, appear to have a weak analgesic effect in the management of NeP, and the combined NNT for all studies was 6.8. Thus SSRIs can be considered as fourth line of NeP treatment [51].

The SNRI duloxetine is approved in the United States for the treatment of diabetic neuropathic pain (DNP) and fibromyalgia. The NNT for duloxetine varies from 1.3 to 5.1 in DNP patients. Venlafaxine was also shown to be effective in reducing pain intensity in diabetic neuropathic patients with an NNT between 2.2 and 5.1. TCAs are also an alternative for DNP with an NNT of 1.3 [59].

The Special Interest Group on Neuropathic Pain (NeuPSIG) of the International Association for the Study of Pain did a systematic review and meta-analysis of randomized, double-blind studies of oral and topical pharmacotherapy for neuropathic pain. For SNRIs, mainly including

duloxetine, combined NNTs were 6.4 and lower for TCAs [28]. However, results of duloxetine in patients with central NeP due to spinal cord injury or stroke were negative. Duloxetine was the first antidepressant medication to be approved for the treatment of neuropathic pain specifically associated with DNP [44]. Duloxetine is a frequently preferred option in neuropathic pain management because it is efficacious and well tolerated. Duloxetine is an FDA-approved drug for the treatment of fibromyalgia. In addition, venlafaxine has shown efficacy in trials involving painful diabetic neuropathy and mixed painful polyneuropathy [51].

Milnacipran is the newest SNRI available that has a strong selectivity for norepinephrine reuptake [22, 39]. Some RCTs have shown milnacipran to be highly effective in the treatment of fibromyalgia. When a systematic review of RCTs was performed in fibromyalgia patients receiving milnacipran 100–200 mg daily, it was found to be effective for a minority in the treatment of pain, providing moderate levels of pain relief (at least 30 %) to about 40 % of participants, compared with about 30 % with placebo [19]. Milnacipran is the only antidepressant other than duloxetine that is FDA approved for treatment of fibromyalgia pain, but with a modest efficacy.

Another classic field of action of antidepressants has been chronic headaches and migraines [20, 52]. Although all tricyclic antidepressants appear to have some efficacy in migraine [37], this appears to be independent of the antidepressant action, and patients did not need to be depressed for them to work. However the selective serotonin reuptake inhibitors appear not to be generally effective in migraine [46] and in a significant number of patients make them worse. The role of the SNRIs is unclear. TCAs are the treatment of first choice in tension-type headache, where in fact very few treatments have been shown to be effective [58].

Furthermore, antidepressants are widely used to treat *painful chronic rheumatic* conditions, but, contrary to neuropathic pain, little is known about their true analgesic properties and value in these situations. In a systematic review from 140 papers, a weak analgesic effect is observed for chronic low back pain, with an efficacy level close to that of analgesics. Antidepressant drugs, particularly TCAs and SNRIs, have analgesic effects in chronic rheumatic painful states in which analgesics and NSAIDs are not very efficient, such as fibromyalgia and chronic low back pain. TCAs, even at low doses, have analgesic effects equivalent to those of SNRIs, but are less well tolerated. Duloxetine appears efficacious and tolerable for the treatment of chronic pain associated with osteoarthritis [17]. SSRIs have modest analgesic effects, but higher doses are required to achieve analgesia. There is no clear evidence of an analgesic effect in osteoarthritis, but studies have poor methodological quality [54].

Although few clinical trials have specifically evaluated these drugs for *cancer pain*, some clinical trials, as well as clinical experience, generally support their analgesic effects [42]. In cancer pain, antidepressant can show effectiveness if there is a neuropathic component. When depression is present, the use of antidepressant dose is necessary. If there are no depressive symptoms, the analgesic efficacy can occur with lower doses. On the other hand, it is noteworthy that TCAs, in addition to its own analgesic ability, can potentiate morphine and other opioids effects [32].

In general, mixed reuptake inhibitors such as TCAs or duloxetine are more effective than selective agents, emphasizing the importance of both serotonergic and noradrenergic pathways in pain control. It is significant that these antidepressants were both effective in depressive and in patients without concomitant depression. In addition, among the TCAs in respondent pain patients, plasmatic levels are lower than the antidepressant concentration in depressive patients [21].

Although TCAs have reasonable analgesic properties, they are also associated with multiple undesirable adverse effects that vary depending on the individual agent. Adverse effects include anticholinergic effects, particularly dry mouth, antihistaminergic effects, alpha-1 adrenergic receptor blockade, and cardiac effects. Cardiac side effects are important as they may preclude the use of these drugs in patients with cardiac conduction

disturbances or recent infarction [2, 30]. Guidelines from the International Association for the Study of Pain (IASP) recommend prescribing TCAs with caution in patients with ischemic cardiac disease or ventricular conduction abnormalities, limiting the dosages to less than 100 mg per day when possible and obtaining a screening electrocardiogram for patients older than 40 years [26].

33.4 Adjuvant Antiepileptic Drugs

The history of the treatment of pain with anticonvulsants was born in 1942 with the successful use of phenytoin in the treatment of trigeminal neuralgia [8]. Since then, antiepileptic or anticonvulsant drugs have confirmed its usefulness in clinical pictures other than epilepsy, such as the treatment of bipolar disorder, impulse control disorders, eating disorders, and especially in the management of different painful conditions. These agents are widely used in pain clinics to treat neuropathic pain. At this moment, there is good bibliographic support for use of antiepileptic drugs in the treatment of postherpetic neuralgia, trigeminal neuralgia, and painful diabetic neuropathy [6]. This has led to their use in other neuropathic pain conditions such as poststroke pain, phantom limb pain, and pain following spinal injury, although the published evidence for their use in these conditions is less robust.

Antiepileptic drugs work in a number of different ways, all of which have relevance to their effect on pain [57]. Some drugs have more than one mechanism of action. Phenytoin was the first antiepileptic employed in the treatment of neuropathic pain, but it has a more experimental and historical than clinical interest. The coefficient benefit risk of phenytoin is not adequate, especially in the elderly. This drug is an enzyme inducer with multiple potential interactions, and the frequency and intensity of their adverse effects make it inacceptable for the treatment of pain [2]. Currently there is no sufficient evidence justifying the use of phenytoin in neuropathic pain or fibromyalgia [11].

Carbamazepine is another classical anticonvulsant chemically related to TCAs with a central and peripheral mechanism of action. Carbamazepine decreases the excitability of trigeminal nucleus and produces a reduction of conductance to Na^+ and K^+, by modulation of voltage-gated sodium channel. Carbamazepine diminished the characteristic ectopic activity of A-delta and C fibers in neuropathic pain, without altering the physiological nerve propagation [14]. Carbamazepine remains the drug of first choice for idiopathic trigeminal neuralgia (TN). Anecdotally, it may also be useful in the management of glossopharyngeal neuralgia [70]. However, the characteristics of the clinical trials in neuropathic pain do not meet the current criteria of quality necessary, so is not recommended for the management of this pain type [51, 65, 67]. In addition, due to their interactions and their adverse effects, carbamazepine may be used only when they have failed other medications [2].

Oxcarbazepine is an anticonvulsant drug closely related to carbamazepine and is reportedly better tolerated. The guidelines on trigeminal neuralgia management that have been agreed and jointly published by the American Academy of Neurology and the European Federation of Neurological Societies recommend carbamazepine (CBZ) and oxcarbazepine (OXC) as the first-choice medical treatments in patients with TN. However, does no exits evidence from randomized controlled trials to determine the efficacy or safety of oxcarbazepine for other kinds of neurophatic pain [72].

Lamotrigine has been studied in a variety of peripheral and central NeP conditions, with variable results. Lamotrigine works by blocking tetrodotoxin-resistant sodium channels and inhibition of glutamate release from presynaptic neurons. Lamotrigine has been shown to have efficacy in neuropathic pain not related to cancer [60, 61, 71]. It has been shown to improve pain associated with diabetic neuropathy, multiple sclerosis, spinal cord injury, central poststroke pain, polyneuropathy, complex regional pain syndrome, and refractory TN [63]. However, large, high-quality, long-duration studies reporting clinically useful levels of pain relief for individual

participants provided no convincing evidence that lamotrigine is effective in treating neuropathic pain and fibromyalgia at doses of about 200–400 mg daily [51]. Given the availability of more effective treatments including antiepileptic and antidepressant medicines, lamotrigine does not have a significant place in therapy based on the available evidence [66, 67].

Gabapentin and pregabalin, the gabapentinoids, are both efficacious in treating neuropathic pain. Gabapentin and pregabalin are structural analogous of GABA, but none of the two acts on GABA-A or GABA-B receptors. These agents interact with the alpha-2-delta subunit of presynaptic voltage-gated calcium channels and modulate calcium entry in the neuron, so it inhibits the release of various neurotransmitters, as glutamate and aspartate, diminishing the excitatory transmission in the dorsal horn. This is the mechanism of its antiepileptic, anxiolytic, and analgesic action [41].

These agents have been studied in large clinical trials, although mainly in the management of painful diabetic neuropathy and postherpetic neuralgia. Gabapentin is considered as first-line treatment for neuropathic pain syndromes, due to its favorable side effect profile and no major drug interactions [18, 50, 62]. Gabapentin has been shown to be well tolerated and efficacious for the treatment of neuropathic pain in multiple placebo-controlled, randomized clinical trials [1, 15, 16, 25, 50, 69] The drug is frequently used for postherpetic neuralgia, painful diabetic neuropathy, and cancer-associated neuropathic pain. The combined NNTs for gabapentin in the management of painful polyneuropathy and postherpetic neuralgia were 6.4 and 4.3, respectively. The addition of gabapentin has also shown a synergistic effect in patients already receiving opioids for cancer pain, where the combination is more effective than either drug alone [29]. Poor oral bioavailability and nonlinear pharmacokinetics with dose escalation are a few limitations encountered with the use of gabapentin, which makes it difficult to predict appropriate therapeutic dose and requires a prolonged titration time period [9, 10].

Pregabalin, a drug structurally similar to gabapentin, was developed to overcome the issues of low potency and nonlinear pharmacokinetics encountered with gabapentin [12]. In addition, pregabalin has higher affinity for the presynaptic calcium channel. It is widely used in treatment of neuropathic pain when gabapentin is not tolerated [18, 49]. Four studies have shown that pregabalin provides significant pain relief and improved quality of life in painful diabetic neuropathy [13], and some clinical trials have shown efficacy in postherpetic neuralgia [5]. The combined NNTs for pregabalin in the management of painful diabetic neuropathy and postherpetic neuralgia were 4.5 and 4.2, respectively [27]. As opposed to gabapentin, the pregabalin dose may be more rapidly escalated.

Side effects of antiepileptic drugs reported in clinical trials usually relate to acute toxicity. Careful dose titration may minimize the likelihood of adverse events. Information regarding longer-term adverse events can be derived in part from the use of these drugs for the treatment of epilepsy. Side effects of the antiepileptic drugs are usually those affecting the CNS and gastrointestinal and hematological systems. Minor adverse events associated with antiepileptic drug use are common but do not always lead to discontinuation of therapy. There are insufficient data to make robust comparisons between drugs regarding very rare adverse events. The practical prescription of these drugs is also influenced by a number of important pharmacokinetic issues including variable oral absorption, induction of hepatic enzymes, and extensive protein binding. Clinicians must be aware of the many interactions that these drugs have with other medications.

33.5 Conclusions About Adjuvant Use in Chronic Pain

Pain, especially chronic and persistent pain, is not just a nociceptive physical experience but also involves affect, cognition, behavior, or social relations. Thus a multimodal treatment should take into account all these aspects. In most cases, total pain alleviation is not possible with pharmacological treatment, so it can often only be reduced

to a more tolerable level. The analgesic efficacy of antidepressants appears to be independent of the existence or not of depression. The analgesic effect may occur at lower doses and plasma concentrations than necessary to relieve depression. Similarly, the analgesic effect starts before the antidepressant effect.

Recent evidence-based therapeutic guidelines have recommended the use of TCAs and SNRIs as therapeutic tools of first choice in the treatment of neuropathic pain. The highest levels of therapeutic evidence in the treatment of fibromyalgia are obtained with TCAs, there is more experience with amitriptyline, and with duloxetine that is better tolerability. With the SSRIs' evidence is lower, it is postulated that antidepressants that act on noradrenergic and serotonergic mechanisms are more effective than acting only by inhibiting a monoamine. Antidepressants are an alternative to be considered when the usual analgesics have failed.

On the other hand, there are clinical studies that highlight the effectiveness of anticonvulsant agents in the control of chronic pain, especially neuropathic pain, but also cancer pain. Gabapentinoids, gabapentin and pregabalin, appear to be similar in their mechanisms of action and side effect profiles and allow for more rapid titration than antidepressant agents and in general have few drug interactions. Pregabalin carries the advantage of twice-daily dosing and linear pharmacokinetics relative to gabapentin. These agents, together with TCAs and SNRIs, are first line of treatment for NeP.

However, there is no universal medication that would alleviate the pain without the tribute of adverse effects. Many of the adjuvants have analgesic activity only in special clinical situations, so that they require to be defined properly. For the majority of drugs, there is clinical "evidence" only in some syndromes, so extrapolation to other clinical entities should be done with caution. The complexity of chronic pain recommends a multimodal approach. In addition, it is necessary to know the advantages and disadvantages and the possible interactions between analgesics, adjuvants, and other medications. Periodically, the medication should be re-evaluated to see the possibility to delete it, or, in any case, adjust the dosage.

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34 Genetic Polymorphisms of Cytochrome P450 and Antidepressants

Ana Isabel Wu-Chou, Yu-Li Liu, and Winston W. Shen

Abbreviations

ADR	Adverse drug reaction
Anti-TB	Anti-tuberculosis
CYP 1A2	Cytochrome P450 1A2
CYP 2B6	Cytochrome P450 2B6
CYP 2C19	Cytochrome P450 2C19
CYP 2C9	Cytochrome P450 2C9
CYP 2D6	Cytochrome P450 2D6
CYP 3A4	Cytochrome P450 3A4
CYP 3A5	Cytochrome P450 3A4
CYP450	Cytochrome P450
EM	Extensive metabolizer
FDA of the US	Food and Drug Administration of the United States
IM	Intermediate metabolizer
PM	Poor metabolizer
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricyclic antidepressant
UM	Ultrarapid metabolizer

A.I. Wu-Chou, MD
Department of Psychiatry, Wan Fang Medical Center, Taipei Medical University, 111, Section 3, Hsin Long Road, Taipei 116, Taiwan
e-mail: ana.wu@alumni.duke.edu

Y.-L. Liu, PhD
Center for Neuropsychiatric Research, National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 350, Taiwan

Department of Pharmacology, China Medical University, Taichung, Taiwan
e-mail: ylliou@nhri.org.tw

W.W. Shen, MD (✉)
Department of Psychiatry, Wan Fang Medical Center, Taipei Medical University, 111, Section 3, Hsin Long Road, Taipei 116, Taiwan

Department of Psychiatry, College of Medicine, Taipei 110, Taipei, Taiwan
e-mail: Shenwinw@gmail.com

34.1 Introduction

Depression is one of the leading causes of disability, and an estimated 350 million people live with this disorder around the world. Disabilities affect people's ability to work and to form relationships and ultimately may lead to death, with about one million people committing suicide every year as reported by the World Health Organization (www.who.int/mental_health/management/depression/flyer_depression_2012.pdf). The introduction of antidepressants has greatly revolutionized the outcome of many patients diagnosed with depression by improving their quality of life, but only about one-third of the patients achieve remission on a first antidepressant trial while the rest have to receive subsequent antidepressants that require longer treatments, resulting in more days of functioning

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loss and greater socioeconomic costs. To understand the mechanisms of antidepressant action and individual factors (such as genetic variations) that affect antidepressant response is important clinically. With the advent of the Human Genome Project, the ideal of individualized or personalized medicine became a promising field in medicine. Even the Food and Drug Administration (FDA) of the United States of America has included information about genetic testing on package inserts of more than 100 medications and at least of nine antidepressants. The emerging field of pharmacogenetics holds the promise to give physicians tools to make treatment decisions based on predictive genetic testing and to predict treatment efficacy and adverse drug reactions (ADRs) individually.

Since the three previous reviews in the 1990s [1–3], the number of pharmacogenetic studies has grown rapidly. But given the complexity of our brain physiology and biochemistry, current knowledge of this field is only beginning to evolve [4]. Therefore, no clear recommendations for the use of pharmacogenetic testing to guide treatment are available (www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm).

Our genetic makeup determines much of our response to medications through genes that encode for metabolizing enzymes, receptors, transporters, etc. Although factors such as drug compliance, drug-drug interactions, and environmental factors also affect treatment response, there are several examples where different genetic constitutions largely influence on therapeutic outcomes. Substantial evidences show that hepatic enzymes responsible for the metabolism of drugs play a significant role in determining treatment response but evidence of polymorphisms that affect drug transporters and drug targets with concrete clinical significance is scarcer. For example, warfarin is a metabolized enzyme of cytochrome P450 family, and the effect of *CYP 2C9* polymorphisms on the therapeutic effects of warfarin has been extensively studied. The US FDA has even added this information about how variations in the *CYP 2C9* gene affect both drug responses and may predict the maintenance dose of warfarin in the drug's package insert. Other examples include significant clinical effects of genetic polymorphisms for drugs such as omeprazole (*CYP 2C19*), warfarin and phenytoin (*CYP 2C9*), and nortriptyline, codeine, and sparteine (*CYP 2D6*) [5].

Patients are usually treated with antidepressants for several months, and genetic variations that affect drug elimination are especially important in chronic dosing because they determine drug serum steady-state levels. The major drug elimination pathways in the human body are renal excretion and metabolic transformation. The cytochrome P450 is a superfamily of enzymes responsible for 80 % of all phase I drugs [2, 6], and it has been reported that individualized *CYP P450* gene-based treatment is important in 10–20 % of all drug therapies [7]. The importance of *CYP P450* pharmacogenetics in drug development and clinical practice is obvious. In this book chapter, we will give background information on pharmacogenetics, review the evidences of clinically important genetic polymorphisms of cytochrome P450 in antidepressant treatments one by one, and summarize the state of arts of this topic at the end of this chapter.

34.2 Background on Pharmacogenetics

"Pharmacogenetics" is the classic term used to refer to the study of how genetics affects drug response. Nonetheless, pharmacogenetics is a term more recently introduced and has a broader definition. With the goal of individualizing therapies, pharmacogenomics is referred to the analysis of the entire genomes across populations and the identification of genes responsible for drug response. On one hand, individualized therapies can translate into increasing the number of responders and decreasing the number of patients who suffer from ADRs. The advantage of genetic testing over the other types of laboratory testing is their ability to predict situations before treatment starts. For example, before genetic markers for carbamazepine were discovered [8], the only clue clinicians had for the assessment of risk of Stevens-Johnson syndrome was family history

[9]. On the other hand, pharmacogenetics also plays an important role in drug development by helping improve drug efficacy and reduce the number of patients needed for successful drug therapy.

"Genetic polymorphism" in its broadest definition refers to the presence of at least two genetic variants in a population that occurs at a significant frequency (usually at a cutoff value of 1 %). Polymorphisms may include single-nucleotide polymorphisms, repetitions, insertions, or deletions of genes that result in different types of individuals who respond to drug treatment differently. For example, enzymes of the CYP P450 family are highly polymorphic, and these variations can increase, reduce, and even abolish enzyme activity. Individuals with two alleles that code for "normal" enzyme function are the "wild-type" or homozygous extensive metabolizer (EM); those with two variant alleles that result in inactive enzymes are termed poor metabolizer (PM). In between these two, there is usually an immediate metabolizer (IM) phenotype that results usually from the presence of one variant and one normal allele. EMs and IMs together are sometimes termed "EMs" in studies that use phenotypes to classify individual's metabolizing status.

34.3 P450 Isozymes Related to Antidepressant Metabolism

The CYP P450 enzymes are a family of heme-dependent monooxygenases largely found in liver cells [1, 2], but also present in extrahepatic tissues such as the small intestine and brain. They are responsible for the metabolism of xenobiotics, drugs, and carcinogens and classified into families and subfamilies according to amino acid sequences [1, 2]. Enzymes belonging to families one to three are the most clinically relevant with about 90 % of drug and xenobiotic metabolism carried out by six main enzymes: *CYPs 1A3*, *2C9*, *2C19*, *2D6*, *2E1*, and *3A4* [1]. Of these six enzymes, *2D6* and *3A4* are responsible for the majority of drug metabolism [10, 11]. To note, many factors besides genetic variation may affect the expression of CYP enzymes, e.g., diet, hormone levels, drug-drug interactions, etc. CYP expression also varies across ethnicities and biological ages [12].

The naming of the different CYP polymorphisms is done according to the Cytochrome P450 Allele Nomenclature Committee (www.cypalleles.ki.se), and the enzymes most studied for their effects in drug metabolism include *CYPs 2C9*, *2C19*, *2D6*, and *1A2* [1–3]. These enzymes are highly polymorphic and these affect individuals' phenotypes. Here, we are reviewing individual hepatic isozymes that play roles in phase I biotransformation of antidepressants.

34.4 CYP 2D6

CYP 2D6 is the most studied gene. Although it accounts for only 2–4 % of all hepatic CYP enzymes [13], it is responsible for the metabolism of about 25 % of all drugs including many antidepressants [14]. It is located on chromosome 22q13.1 and is highly polymorphic with over 100 allelic variants (www.cypalleles.ki.se/cyp2d6.htm). Strong inhibitors of the CYP 2D6 include quinidine, numerous SSRIs (citalopram, fluoxetine, paroxetine), and bupropion; sertraline and duloxetine also inhibit the enzyme though to a lesser extent. CYP 2D6 is less inducible compared with the other CYP450 isoenzymes.

Different CYP 2D6 enzymatic activities result in four phenotypes: PMs with no active alleles, IMs with one or two partially active alleles, EMs with one or two active alleles, and ultrarapid metabolizers (UMs) with more than two active alleles. Some articles reported an association between the polymorphisms and clinical response [15, 16], as well as antidepressant-related ADRs [17–19]. PMs complain of more ADRs at lower antidepressant dosages [20–22], whereas UM patients need higher doses to reach therapeutic plasma concentrations [23] and fail to respond to antidepressant treatment [24].

Clinically significant interethnic variation is seen in the prevalence of the different alleles. In Caucasian populations, 71 % of 2D6 allele code

for functional enzymes with *2D6*4* and *2D6 *5* accounting for most of the nonfunctional enzymes. Asians metabolize 2D6-metabolized drugs more slowly mainly because of the presence of a higher frequency of *CYP2D6*10*, an allele that produces an unstable enzyme with reduced metabolic activity [25]. Replications of the enzyme result in a UM phenotype and is seen in 29 % of black Ethiopians [26] and only in 1–2 % of Swedish Caucasians [27].

Polymorphisms of *CYP 2D6* have been reported to affect serum levels of many antidepressants. For example, one of the most studied antidepressants in this category is paroxetine, which many articles report an association between the number of active alleles and plasma levels of the drug at both single dose [28] and at steady plasma state [29–31]. Similar results are reported for venlafaxine [17, 32, 33], fluoxetine [34], citalopram [35, 36], mirtazapine [37], and various TCAs [38–40]. Although some investigators have found no differences between IMs and EMs [41, 42], these inconsistent findings may be explained by the involvement of other compensatory CYP450 isozymes and differences among different ethnic groups. Dosage recommendations of different antidepressants in accordance to *CYP 2D6* genotype have been reported elsewhere [43].

Understanding the genetic makeup of *CYP 2D6* is useful in personalizing dose-escalation regimens for antidepressants with low therapeutic margin like the tricyclic antidepressants (TCAs). Most TCAs are metabolized by 2D6, and PMs of 2D6 are found to have higher plasma levels of antidepressants compared with EMs and potentially more likely to experience ADRs that are dose dependent. For example, EMs in a study [44] have been found to have the lowest risk of amitriptyline ADRs and toxicities. Similarly, PMs treated with TCAs have higher chance of switching to other antidepressants within 45 days compared with Ems [45, 46]. In contrast, the *2D6* genotype had no overt effects on the discontinuation of mirtazapine and paroxetine [47]. Therefore, gaining knowledge on the metabolizer status of 2D6 is more relevant in individuals receiving TCAs than in patients on other classes of antidepressants.

34.5 CYP 2C19

CYP 2C19 is a member of the *CYP 2C* gene cluster located on chromosome 10q24.1-q24.3 with 47 allelic variants. In the field of pharmacogenomics of antidepressants, *CYP 2C19* is the second most studied gene after *CYP2D6*. This enzyme is involved in the metabolism of several antidepressants such as SSRIs (sertraline [48], escitalopram [49], and citalopram [50]), venlafaxine, and various TCAs [51]. This isozyme is strongly inhibited by fluvoxamine and moclobemide and induced by carbamazepine. Most studies on *CYP2 C19* are focused on antidepressant pharmacokinetics, and only a few studies have investigated the possible associations between the polymorphisms and clinical outcomes.

The most common alleles are the 1* (normal activity), *2 and *3 (decreased or no activity), and *17 (increased activity). EMs carry at least one *1 allele while PMs are homozygous for 2* and 3* alleles. Among Europeans and Asians, *CYP 2C19*2* is responsible for the majority of the PM phenotypes [51]. The frequency of PMs is particularly high among Asian and Pacific Islander populations (20 %), whereas only about 3–5 % of Caucasians are PMs [52].

The *17 allele is associated with increased enzyme activity, and individuals with this polymorphism are postulated to metabolize drugs faster than individuals who are heterozygous or homozygous of the *2C19*1* [53]. For example, they have 42 % lower mean plasma levels of geometric escitalopram and compared with wild type, *2C19*1/*1*, but these results have not been replicated in a smaller, open-label study with healthy sample [54]. Another interesting study showed that adults without 2C19 activity have lower levels of depression than EMs [55]. Although the mechanism for this finding remains unknown, in vitro studies have suggested that 2C19 is involved in the biotransformation of SSRIs [51]. Individuals who are heterozygous 2C 19*1/*17, *2/ *17, or *3/*17 tend to be EMs [56].

Among SSRIs, many published articles show that the metabolism of citalopram is affected by *CYP 2C19* polymorphisms, whereas PMs of this enzyme have higher plasma levels of the

antidepressant compared with EMs [36, 50, 57]. One study also showed that variants of *CYP 2C19* are associated with citalopram therapeutic response. Although similar results in drug plasma levels have been reported for escitalopram [58–61], no difference in the mean left rotation:right rotation (S:R) ratios of citalopram and desmethylcitalopram is found at steady state between PMs and EMs [62]. But CYP 2C19 does not affect serum sertraline's levels [48]. CYP 2C19 is also involved in the metabolism of several TCAs. PMs of the enzyme have higher plasma levels and metabolic ratio (MR) of amitriptyline compared with *CYP 2C19**1/*1 in both Japanese [63] and Caucasian subjects [64]. Conversely, EMs (*17) are associated with decreased MR [35]. Similar results indicate that the involvement of 2C19 has been reported for imipramine [39, 65, 66] and trimipramine [67]. *CYP 2C19* has also been reported to affect serum levels of the MAOI moclobemide and that probe drugs such as omeprazole and moclobemide inhibit each other's metabolism in EMs [68, 69]. But plasma levels of moclobemide are not associated with therapeutic efficacy, but are correlated to an increased risk for ADRs in PMs [70].

Although the US FDA declared that CYP 2D6 is a valid biomarker for psychotropic drug submissions in 2005 and approved the Roche AmpliChip CYP450 for determining *CYP 2D6* and *CYP 2C19* genotypes, the evidence for the application of *CYP 2C19* pharmacogenetics in clinical practice remains inconclusive. But the clinical significance of *2C19* polymorphisms is likely to be demonstrated when the metabolism of other *CYP* isoforms is decreased. For example, PM of both *CYP 2C19* and CYP 2D6 is associated with a citalopram half-life two to three times longer than the normal controls and thus more severe ADRs such as gastrointestinal disturbances and restlessness [71].

34.6 CYP 2C9

CYP 2C9 is located on chromosome 10q24.2 and is the most abundant of all CYP2C isoforms, accounting for about a third of the total hepatic P450 protein. Although sharing up to 90 % of the amino acid sequence with *CYP 2C19*, 2C9 has its own substrate specificities. Several variants resulting in decreased enzymatic activity have been described, with *2 and *3 being reported to be the most common.

CYP 2C9 gene has been relatively less studied in antidepressant pharmacogenetics. Polymorphisms of the enzyme, together with *CYP 2D6* variants, have been reported to affect plasma levels of fluoxetine [72] and to influence oral clearance of trimipramine but not at a statistically significant level [67]. Another case-control study had found that *CYP 2C9* genotypes do not affect the plasma levels of several antidepressants including SSRIs (citalopram, fluvoxamine, paroxetine, sertraline, etc.), TCAs (amitriptyline, clomipramine, doxepin, etc.), and other antidepressants (mirtazapine, venlafaxine) [73].

34.7 CYP 1A2

CYP 1A2 is a well-conserved gene located on chromosome 15q24.1 and is involved in the metabolism of multiple drugs. Many of them are also substrates for other CYP isoenzymes. It is one of the major CYP450 enzymes and accounts for about 13 % of all CYP hepatic protein [74]. CYP 1A2 is highly inducible by smoking, and a UM phenotype results from polymorphisms (*1F) affecting gene transcription in the presence of exogenous inducers such as tobacco [75]. CYP 1A2 is involved in the metabolism of some antidepressants, and UMs may have reduced plasma drug levels. One study showed that 1A2 polymorphisms (rs4646425; rs2472304; rs2470890) are associated with dose and response to paroxetine at week 4 of treatment [76], whereas two other reports showed that smoking, but not *CYP 1A2* genotype, influences plasma levels of fluvoxamine [77, 78]. Similar to fluvoxamine, smoking has been found to produce lower plasma levels of trazodone instead of *CYP 1A2* genotype [79]. Another study also showed that CYP 1A2 EMs experience more severe escitalopram ADRs in the early stages of treatment [80]. CYP 1A2 is also responsible for the metabolism of the newer antidepressant agomelatine, a melatonin receptor agonist, and the use of this drug is contraindicated in patients taking strong CYP 1A2 inhibitors [81].

Interindividual variation in *CYP 1A2* activity is extensive, and no polymorphism to predict metabolic phenotypes has yet been conclusively identified [82]. To some extent, interindividual variability is possibly due to environmental factors or epigenetic phenomena. But many epidemiological studies have tried to link specific polymorphisms with susceptibilities to different diseases such as myocardial infarction, various types of cancers, dyskinesias, etc.

34.8 CYP 3A4/CYP 3A5

CYP3A4 and CYP3A5 together account for the metabolism of about half of the drugs that go through phase 1 biotransformation in hepatic CYP isozymes. Their activities may overlap so they can both compensate for each other's decreased activity. They are also easily induced when exposed to substrates so that polymorphisms may have less influence in therapeutic response. The CYP3A4 is potently inhibited by grapefruit juice and various drugs such as antivirals, antibiotics (erythromycin, chloramphenicol), antifungals, cimetidine, as well as cardiovascular medications (verapamil, diltiazem, etc.). Potent inducers of this enzyme include steroids, anti-TB medications (rifampicin), anticonvulsants (carbamazepine, phenytoin), barbiturates, as well as antiviral drugs (efavirenz and nevirapine) [81].

CYP3A4 are the most abundant enzymes in the P450 family and metabolize more than 50 % of drugs [82–84]. Activity levels of CYP 3A4 can vary up to 60-fold, and it has been reported that 40–60 % of this variability is determined by genetic makeup [85]. Although several variant alleles that result in different enzymatic activities have been described for this isoenzyme, the mutations are seen at low population frequencies and cannot fully explain the interindividual variation in *CYP3A4* activity [86].

34.9 CYP2B6

The CYP2B6 isozyme is highly polymorphic, and variants that result in reduced expression and activity include the alleles *CYP2B6**6, *CYP2B6**16, *CYP2B6**18, and *CYP2B6**28. The first one is common in several populations with frequency of 20–30 % while the others are more common in Black populations. Studies on *2B6*, albeit few, are mostly focused on the metabolism of bupropion. A study using healthy subjects showed that carriers of the *4 allele have a 1.66-fold higher serum levels of bupropion compared with those of the wild-type *1 [87]. Similar results have been confirmed in an in vitro study [88].

34.10 Summary of P450 Isozymes Related to Antidepressant Treatments

Table 34.1 is a summary table of studies on P450 isozymes according to antidepressant substrates. Differing in tables of the previous reviews [1–3], this table contains much more studies on P450 isozymes, of which many Oriental studies from Japan, Korea, and Taiwan have been added.

Table 34.1 List of studies on P450 isozymes according to antidepressant substrates

CYP450	Antidepressant substrates	Relevant pharmacokinetic studies [reference number]
2D6	Paroxetine	Yoon et al. (2000) [28] Ueda et al. (2006) [29] Sawamura et al. (2004) [30] Charlier et al. (2003) [31] Ozdemir et al. (1999) [41] Gex-Fabry et al. (2008) [42] Murphy et al. (2003) [47]
	Venlafaxine	Fukuda et al. (1999) [32] Nichols et al. (2009) [33] Shams et al. (2006) [17]
	Fluoxetine	LLerena et al. (2004) [34]
	Citalopram	de Vos et al. (2011) [35] Fudio et al. (2010) [36]
	Mirtazapine	Lind et al. (2009) [37] Murphy et al. (2003) [47]
	Various TCAs	Morita et al. (2000) [38] Schenk et al. (2008) [39] Kirchheiner et al. (2004) [43] Steimer et al. (2005) [44] Mulder et al. (2005) [45] Bijl et al. (2008) [46]

(continued)

Table 34.1 (continued)

CYP450	Antidepressant substrates	Relevant pharmacokinetic studies [reference number]
2C19	Sertraline	Rudberg et al. (2008a) [48]
	Escitalopram	Rudberg et al. (2008b) [49] Sim et al. (2006) [53] Ohlsson Rosenborg et al. (2008) [54] Tsai et al. (2010) [58] Jin et al. (2010) [59] Noehr-Jensen et al. (2009) [60] Rudberg et al. (2006) [61]
	Citalopram	Yin et al. (2006) [50] Fudio et al. (2010) [36] Yu et al. (2003) [57] Carlsson et al. (2005) [62]
	Moclobemide	Yu et al. (2001) [68] Cho et al. (2002) [69]
	TCAs	Tsai et al. (2010) [58] Jin et al. (2010) [59]; Noehr-Jensen et al. (2009) [60] Rudberg et al. (2006) [61] Carlsson et al. (2001) [62] Charlier et al. (2003) [31]; de Vos et al. (2011) [35]
2C9	Fluoxetine TCAs Others (venlafaxine, mirtazapine)	van der Weide et al. (2005) [64] Carlsson et al. (2001) [62] Morinobu et al. (1997) [65]
1A2	Paroxetine Fluvoxamine Escitalopram	Lin et al. (2010) [76] Suzuki et al. (2011) [77] Katoh et al. (2010) [78] Kuo et al. (2013) [80]

Conclusion

The field of psychiatric pharmacogenomics is growing fast and is made evident by the vast scientific literature cited in this chapter. There are many more studies that we could not include. Table 34.1 provides a summary of relevant studies according to CYP family. We are now closer than ever before in the understanding of CYP450's functional properties and its gene expression and how these lead to interindividual variability and affect genotype-phenotype correlations and, finally, how these elements all come together and affect clinical practice. A recent study in 2013 showed that P450 polymorphisms can be useful in guiding clinicians in making medication decisions and that the guided group showed improved patient outcomes [89]. But despite these exciting findings, some studies have found no association between genotype and clinical efficacy [42, 44, 90] while those who report positive associations tend to have smaller sample sizes or report results that have not been replicated [24, 50, 91]. The apparent contradiction found across studies may be explained by both (A) differences in depression phenotypes [6] and (B) a lack of direct association between antidepressant plasma levels and treatment responses [92, 93]. Plasma levels of antidepressants may differ from the levels at target site due to differences among individuals in their transport systems across the blood-brain barrier [94, 95].

There is still a long way to go for incorporating knowledge on pharmacogenetics into a routine daily practice for many reasons. Some scholars concern in medical, ethical, and legal issues discussed elsewhere [96] while others concern about the fact that conclusive clinical utility and practicality of CYP genotyping have yet to be drawn. To implement pharmacogenomics in daily practice, suitable infrastructure such as testing facility and educated personnel need to be delineated and accepted universally.

Although this book chapter has stressed on the effects of CYP variants on antidepressants, it is important to keep in mind that other factors – both genetic and nongenetic – affect drug treatment response. Among genetic factors to also consider are genes of the serotonin transporter and receptor, whereas important nongenetic factors include possible drug-drug interactions and environmental factors [97, 98].

The US FDA approved a pharmacogenetic kit, AmpliChip CYP450 Test (Roche Molecular System), which may help validate studies and establish guidelines for personalized depression treatment. Future studies can evaluate for how antidepressant pharmacogenomics can predict which individuals are more likely to experience ineffective or non-efficacious treatments, and new consensus should address how to integrate this vast knowledge into clinical work.

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35

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Abbreviations

ABCs	ATP-binding cassette transporter family
AD	Alzheimer's disease
ADH	Alcohol dehydrogenase
ADRs	Adverse drug reactions
ALDH	Aldehyde dehydrogenase
BBB	Blood–brain barrier
BCRP	Breast cancer resistance protein
BER	Base excision repair
BMI	Body mass index
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CYPs	Cytochrome-P450 enzymes
DPD	Dihydropyrimidine dehydrogenase
EMs	Extensive metabolizers
FKBP5	FK506-binding protein 5
GST	Glutathione S-transferase
HMT	Histamine methyltransferase
HTR2A	5-Hydroxytryptamine receptor 2A
IMs	Intermediate metabolizers
MAOIs	Monoamine oxidase inhibitors
MDD	Major depressive disorder
MRI	Magnetic resonance imaging
MRP4	Multidrug resistance-associated protein 4
NAT	N-acetyltransferases
NQO1	NADPH-quinone oxidoreductase
OAT	Organic anion transporters
PepTs	Peptide transporters
PMs	Poor metabolizers
rDD	Recurrent depression
SLC	Solute carrier transporter family
SLCO	Solute carrier organic transporter family
SSRIs	Selective serotonin reuptake inhibitors
STs	Sulfotransferases
TCAs	Tricyclic antidepressants
TPH2	Tryptophan hydroxylase 2
TPMT	Thiopurine-methyltransferase
UDP-GT	UDP-glucuronosyltransferases
UGTs	Uridine 5′-triphosphate glucuronosyl transferases
UMs	Ultra-rapid metabolizers

R. Cacabelos (✉)
Chair of Genomic Medicine, Camilo José Cela University, Madrid, Spain

EuroEspes Biomedical Research Center, Institute of Medical Science and Genomic Medicine, Bergondo, Corunna 15165, Spain
e-mail: rcacabelos@euroespes.com

C. Torrellas
EuroEspes Biomedical Research Center, Institute of Medical Science and Genomic Medicine, Bergondo, Corunna 15165, Spain

F. López-Muñoz
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal

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35.1 Introduction

Depression is a major problem of mental health in the community with a prevalence of 5–10 % for females and 2–5 % for males and a lifetime risk of 10–25 % in women and 5–12 % in men. Antidepressants are among the most prescribed drugs in the USA and the EU. The most important classes of antidepressants are the selective serotonin reuptake inhibitors (SSRIs), serotonin–norepinephrine reuptake inhibitors (SNRIs), tricyclic and tetracyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and noradrenergic and serotonergic modulators [1].

Since the pioneering works of Bönicke and Reif, Carson and coworkers, Kalow and Staron, and Motulsky in the 1950s and the introduction of the concept of "pharmacogenetics" by Vogel in 1959, Sjöqvist and coworkers soon made clear in 1967–1973 that the metabolism of tricyclic antidepressants was genetically controlled [2–5]. Over 500 papers corroborated this assumption during the past half century. However, pharmacogenetics is still in its infancy, and its concept has evolved into a broader spectrum after the completion of the human genome project [6–8]. At the present time, pharmacogenomics relates to the application of genomic technologies, such as genotyping, gene sequencing, gene expression, genetic epidemiology, transcriptomics, proteomics, metabolomics, and bioinformatics, to drugs in clinical development and on the market, applying the large-scale systematic approaches of genomics to speed up the discovery of drug response markers, whether they act at the level of drug target, drug metabolism, or disease pathways. For the past decade, several books have been published illustrating the progress of pharmacogenomics [3, 9–14], culminating in the first World Guide for Drug Use and Pharmacogenomics published in 2012 [1].

While antidepressants are widely used to treat major depressive disorder and anxiety disorders, only half of the patients will respond to antidepressant treatment and only a third of patients will experience a remission of symptoms [15]. Both pharmacodynamics and pharmacokinetic properties of antidepressants and other CNS drugs are highly dependent upon pharmacogenomic factors [16]. In comparison with other pharmacological categories, the basic pharmacogenetics of antidepressants is relatively well known in vivo and in vitro. Most antidepressants are metabolized via CYP enzymes. CYP variants may potentially influence the metabolism of major antidepressants: amitriptyline, amoxapine, citalopram, clomipramine, desipramine, doxepin, duloxetine, escitalopram, fluoxetine, fluvoxamine, imipramine, isocarboxazid, L-tryptophan, maprotiline, minaprine, mirtazapine, moclobemide, nefazodone, nortriptyline, paroxetine, phenelzine, protriptyline, reboxetine, sertraline, tranylcypromine, trazodone, trimipramine, and venlafaxine [1]. However, the genes involved in the pharmacogenomic response to antidepressant drugs may fall into five major categories: (i) genes associated with the pathogenesis of depression (disease-specific genes, pathogenic genes), (ii) genes associated with the mechanism of action of drugs (mechanistic genes), (iii) genes associated with drug metabolism (metabolic genes), (iv) genes associated with drug transporters (transporter genes), and (v) pleiotropic genes involved in multifaceted cascades and metabolic reactions [4, 16–19]. Recent studies indicate that the prescription of antidepressants by trial and error, neglecting the pharmacogenetic profile of the patients, is subjected to an error rate over 60 % with the consequent problems in efficacy and safety [20, 21].

The therapeutic lessons obtained from pharmacogenetics in the past, as pointed out by Meyer in 2004 [2], can be the following:

(i) All drug effects vary from person to person and all drug effects are influenced by genes.
(ii) Most drug responses are multifactorial.
(iii) Genetic polymorphisms of single genes, including mutations in coding sequences, gene duplications, gene deletions, and regulatory mutations, affect numerous drug-metabolizing enzymes, including several cytochrome-P450 enzymes (*CYPs*), N-acetyltransferases (*NAT*), thiopurine-methyltransferase (*TPMT*), and UDP-glucuronosyltransferases (*UDP-GT*); individuals that possess these polymorphisms are at risk of experiencing documented adverse reactions or inefficacy of drugs at usual doses.
(iv) Genetic polymorphisms of drug targets and drug transporters are increasingly recog-

nized (receptors, ion channels, growth factors) as causing variation in drug responses.
(v) Several targets respond to treatment only in subgroups of patients who carry sensitizing mutations of these targets.
(vi) The frequency of variation of drug effects, whether multifactorial or genetic, varies considerably in ethnically defined populations.
(vii) Application of response-predictive genetic profiles on clinical outcomes has, so far, been done mostly in academic centers and has not yet reached clinical practice [2].

To gain expertise in the pharmacogenomics of a particular disease, it is necessary to understand the principles of five basic steps: (a) the genetics of the disorder to be studied in all its modalities (Mendelian genetics, susceptibility genetics, mitochondrial genetics, epigenetic phenomena, genome–environment interactions), (b) structural and functional genomics, (c) proteomics, (d) metabolomics, and (e) pharmacogenomics. The development of new compounds or retesting of old drugs by using pharmacogenetic strategies encompass the following steps in a multidisciplinary fashion:

(a) Genetic screening (genotyping) of single genes to identify major gene targets
(b) Analysis of genetic variation to differentiate populations
(c) Structural and functional genomic analyses including genetic clusters and haplotypes
(d) Analysis of genotype–phenotype correlations to characterize major phenotypes as therapeutic targets associated with a particular gene or a cluster of genes involved in a metabolic pathway
(e) Implementation of basic and clinical pharmacogenomic procedures for drug development [16, 22–24]

35.2 Pathogenic Genes

The molecular mechanisms underlying major depressive disorder (MDD) are largely unknown. Heritability of major depressive disorder is estimated to be at 0.36–0.70 [25], with the relative risk of the disorder four to eight times greater in relatives of probands [26]. Over 1000 different genes distributed across the human genome have been screened for major depression for the past decade, and less than 100 genes remain potentially associated with depression in different populations (Table 35.1). Classic genes conventionally associated with MDD include *FKBP5* (FK506-binding protein 5)(6p21.31) (major depressive disorder and accelerated response to antidepressant drug treatment) [27], *TPH2* (tryptophan hydroxylase 2) (12q21.1) (susceptibility to unipolar depression and susceptibility to ADHD-2) [28], *MDD1* (major depression disorder 1)(12q22–q23.2) [29], *HTR2A* (5-hydroxytryptamine receptor 2A)(13q14.2) (response to citalopram therapy in major depressive disorder; susceptibility to alcohol dependence, anorexia nervosa, obsessive–compulsive disorder, schizophrenia, and seasonal affective disorder) [30], and *MDD2* (major depressive disorder 2, unipolar depression 2) (15q25.3–q26.2) [31]. Cytogenetic analysis also revealed that patients with a balanced translocation t(1;11)(q43;q21) may be at risk of suffering MDD and other mental disorders [32]. Heterozygous mutations in the *DCTN1* gene on chromosome 2p13 have been associated with Perry syndrome, an autosomal dominant neurodegenerative disorder classically characterized by adult-onset parkinsonism and depression, frontotemporal dementia, and progressive supranuclear palsy. *DCTN1* gene variants can also cause distal motor neuronopathy type VIIB and confer increased susceptibility to amyotrophic lateral sclerosis [33, 34].

Many other genes may be involved in the pathogenesis of depression [35, 36] (Table 35.1). New players, such as epigenetic changes, alterations in mtDNA related to oxidative stress, and modifications in telomere length, may also contribute to alter neuronal mechanisms involved in depressive conditions [37]. SNPs of genes involved in DNA repair, such as genes encoding three glycosylases (*hOGG1*, *MUTYH*, and *NEIL1*) (c.977C > G – *hOGG1* (rs1052133), c.972G > C – *MUTYH* (rs3219489), and c.*589G > C – *NEIL1* (rs4462560)), particularly in the base excision repair (BER) pathway, may also modulate the risk of recurrent depression (rDD). The C/C genotype and allele C of the c.*589G > C

Table 35.1 Selected genes potentially associated with mood disorders

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
1p22		*CMM4*	Melanoma, cutaneous malignant, 4	608035	Melanoma
1p31	582.07 kb	*PDE4B*	Phosphodiesterase 4B, cAMP specific	600127	Susceptibility to schizophrenia
1p32-p31	1255.76 kb	*DAB1*	Disabled homolog 1 (drosophila)	603448	Susceptibility to autism
1p33-p32	3.00 kb	*ARTN*	Artemin	603886	
1p34	41.52 kb	*HDAC1*	Histone deacetylase 1	601241	
1p34.1	11.23 kb	*MUTYH*	mutY homolog (*E. coli*)	604933	Adenomas, multiple colorectal
					Colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas
					Gastric cancer, somatic
1p34-p33	233.00 kb	*GRIK3*	Glutamate receptor, ionotropic, kainate 3	138243	Susceptibility to schizophrenia
1p35-p34	19.00 kb	*FAAH*	Fatty acid amide hydrolase	602935	Susceptibility to drug abuse
1p36		*CMM*	Cutaneous malignant melanoma/dysplastic nevus	155600	
1p36.23	60.52 kb	*PER3*	Period homolog 3 (drosophila)	603427	
1p36.3	20.33 kb	*MTHFR*	Methylenetetrahydrofolate reductase (NAD(P)H)	607093	Developmental delay
					Neural tube defects, folate sensitive
					Susceptibility to schizophrenia
					Susceptibility to increased mortality risk in men in middle and old age
					Susceptibility to recurrent abortus in early pregnancy
					Susceptibility to cardiovascular disease
1p36.3-p34.3	2.00 kb	*HTR1D*	5-hydroxytryptamine (serotonin) receptor 1D	182133	
1p36-p35	14.27 kb	*HTR6*	5-hydroxytryptamine (serotonin) receptor 6	601109	Susceptibility to schizophrenia
1q21	84.00 kb	*BCL9*	B-cell CLL/lymphoma 9	602597	
1q21	10.42 kb	*GBA*	Glucosidase, beta, acid	606463	Gaucher disease, types perinatal lethal, I, II, and III
					Parkinson's disease 20, early onset
					Susceptibility to Lewy body dementia
1q21-q22	66.10 kb	*NTRK1*	Neurotrophic tyrosine kinase, receptor, type 1	191315	Hereditary sensory and autonomic neuropathy, type IV
					Medullary thyroid carcinoma, familial
					Insensitivity to pain, congenital, with anhidrosis
1q21-q23	2.30 kb	*CRP*	C-reactive protein, pentraxin-related		Susceptibility to systemic lupus erythematosus (SLE)
					Susceptibility to cardiovascular disease

1q23.3	300.23 kb	*NOS1AP*	Nitric oxide synthase 1 (neuronal) adaptor protein	605551	Susceptibility to schizophrenia
					Susceptibility to variation of the QT interval (cardiac repolarization)
					Susceptibility to sudden cardiac death
1q23.3	8.23 kb	*RGS4*	Regulator of G-protein signaling 4	602516	Schizophrenia 9
1q24.3	37.45 kb	*FMO1*	Flavin-containing monooxygenase 1	136130	Susceptibility to sporadic amyotrophic lateral sclerosis
1q25	160.08 kb	*PLA2G4A*	Phospholipase A2, group IVA (cytosolic, calcium dependent)	600522	PLA2G4A deficiency
1q25.2-q25.3	8.61 kb	*PTGS2*	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	600262	Susceptibility to development of prostate cancer
					Susceptibility to aspirin resistance
1q31-q32	4.89 kb	*IL10*	Interleukin 10	124092	Susceptibility to hepatocellular carcinoma
					Susceptibility to HIV-1,
					Susceptibility to acute myocardial infarction
					Susceptibility to Wegener's granulomatosis
					Susceptibility to sudden infant death with infectious cause
					Susceptibility to severe malarial anemia
					Susceptibility to leprosy
1q31-q32	118.41 kb	*PTPRC*	Protein tyrosine phosphatase, receptor type, C	601577	Severe combined immunodeficiency
					Susceptibility to multiple sclerosis
					Susceptibility to Graves' disease
1q32.1	7.86 kb	*CHI3L1*	Chitinase 3-like 1 (cartilage glycoprotein-39)	601525	Susceptibility to schizophrenia
1q42.1	414.46 kb	*DISC1*	Disrupted in schizophrenia 1	605210	Schizophrenia 12
					Susceptibility to autism spectrum disorders
					Susceptibility to attention-deficit/hyperactivity disorder (ADHD)
1q42.1		*DISC2*	Disrupted in schizophrenia 2 (nonprotein coding	606271	Susceptibility to autism spectrum disorders
1q42.1	37.00 kb	*TSNAX*	Translin-associated factor X	602964	Susceptibility to autism spectrum disorders
1q43	791.60 kb	*RYR2*	Ryanodine receptor 2 (skeletal)	180902	Arrhythmogenic right ventricular dysplasia 2
					Ventricular tachycardia, catecholaminergic polymorphic, 1
2p11.2	10.23 kb	*TGOLN2*	Trans-golgi network protein 2	603062	

(continued)

Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
2p13.1	30.93 kb	*DCTN1*	Dynactin 1 (p150, glued homolog, drosophila)	601143	Lower motor neuron disease, dynactin type
					Perry syndrome
					Susceptibility to amyotrophic lateral sclerosis
					Distal motor neuropathy type VIIB
2p15-p13	34.00 kb	*SLC1A4*	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	600229	
2p16.3	78.41 kb	*RTN4*	Reticulon 4	604475	Susceptibility to dilated cardiomyopathy
2p22-p21	26.40 kb	*CAD*	Carbamoyl phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase	114010	
2p23.3	99.00 kb	*ADCY3*	Adenylate cyclase 3	600291	
2q11.2	76.00 kb	*NPAS2*	Neuronal PAS domain protein 2	603347	
2q14	7.03 kb	*IL1B*	Interleukin 1, beta	147720	Gastric cancer risk after *H. pylori* infection
					Susceptibility to schizophrenia
					Susceptibility to chronic kidney disease and periodontitis
					Susceptibility to diabetes
2q14.2	16.00 kb	*IL1RN*	Interleukin 1 receptor antagonist	147679	Interleukin 1 receptor antagonist deficiency
					Susceptibility to chronic kidney disease and periodontitis
					Susceptibility to ankylosing spondylitis
2q22-q23	8.34 kb	*NR4A2*	Nuclear receptor subfamily 4, group A, member 2	601828	Parkinson's disease 22
2q24	8.95 kb	*TBR1*	T-box, brain, 1	604616	
2q31	44.46 kb	*GAD1*	Glutamate decarboxylase 1 (brain, 67 kDa)	605363	Symmetrical spastic cerebral palsy, nonprogressive
2q32	4.19 kb	*DLX1*	Distal-less homeobox 1	600029	Chromosome 2q interstitial deletion, including 2q31.1
2q32	27.00 kb	*INPP1*	Inositol polyphosphate-1-phosphatase	147263	
2q33	6.17 kb	*CTLA4*	Cytotoxic T-lymphocyte-associated protein 4	123890	Susceptibility to Graves' disease (locus 5)
					Autoimmune lymphoproliferative syndrome, type V
					Diabetes mellitus, insulin dependent, 12
					Hashimoto's thyroiditis
					Systemic lupus erythematosus
					Susceptibility to celiac disease
2q33.3-q34	1162.91 kb	*ERBB4*	V-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	600543	Amyotrophic lateral sclerosis 19

2q34	75.67 kb	*CREB1*	cAMP-responsive element-binding protein 1	123810	
2q34-q35	310.06 kb	*MAP2*	Microtubule-associated protein 2	157130	
2q37.3	352.78 kb	*HDAC4*	Histone deacetylase 4	605314	Brachydactyly-mental retardation syndrome
					Chromosome 2q subtelomeric deletion syndrome
2q37.3	44.53 kb	*PER2*	Period homolog 2 (drosophila)	603426	Familial advanced sleep phase syndrome
3p21.2	11.55 kb	*GRM2*	Glutamate receptor, metabotropic 2	604099	
3p21.3	160.25 kb	*LARS2*	Leucyl-tRNA synthetase 2, mitochondrial	604544	Perrault syndrome 4
3p22.1	58.79 kb	*MOBP*	Myelin-associated oligodendrocyte basic protein	600948	
3p22-p21.3	8.35 kb	*CCK*	Cholecystokinin	118440	Susceptibility to obesity
3p25	126.16 kb	*HRH1*	Histamine receptor H1	600167	
3p25	19.21 kb	*OXTR*	Oxytocin receptor	167055	
3p25	187.67 kb	*SYN2*	Synapsin II	600755	
3p25.3	16.73 kb	*OGG1*	8-oxoguanine DNA glycosylase	601982	Renal cell carcinoma, clear cell, somatic
3p26	5.83 kb	*BHLHE40*	Basic helix–loop–helix family, member e40	604256	
3p26.1-p25.1	880.29 kb	*GRM7*	Glutamate receptor, metabotropic 7	604101	Susceptibility to age-related hearing impairment
					Susceptibility to attention-deficit/hyperactivity disorder (ADHD)
3q12	244.14 kb	*ABI3BP*	ABI family, member 3 (NESH)-binding protein	606279	
3q12-q13.3	38.01 kb	*NR1I2*	Nuclear receptor subfamily 1, group I, member 2	603065	Susceptibility to ulcerative colitis
3q13.1-q13.2	98.18 kb	*GAP43*	Growth-associated protein 43	162060	
3q13.2-q21	164.00 kb	*ADCY5*	Adenylate cyclase 5	600293	Dyskinesia, familial, with facial myokymia
3q13.3	50.34 kb	*DRD3*	Dopamine receptor D3	126451	Essential tremor hereditary 1
					Susceptibility to schizophrenia
3q13.3	272.47 kb	*GSK3B*	Glycogen synthase kinase 3 beta	605004	
3q22.1		*TF*	Transferrin	190000	Atransferrinemia
4p12	145.59 kb	*GABRA2*	Gamma-aminobutyric acid (GABA) A receptor, alpha 2	137140	Susceptibility to alcohol dependence
4p15.2	45.00 kb	*PI4K2B*	Phosphatidylinositol 4-kinase type 2 beta	612101	
4p16	1.00 kb	*ADRA2C*	Adrenergic, alpha-2C-, receptor	104250	Susceptibility to congestive heart failure

(continued)

Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
4p16	33.42 kb	*WFS1*	Wolfram syndrome 1 (wolframin)	606201	Neurosensory deafness 14
					Neurosensory deafness 38
					Neurosensory deafness 6
					Wolfram syndrome
					Juvenile-onset diabetes
4p16.1	2.38 kb	*DRD5*	Dopamine receptor D5	126453	Primary cervical dystonia
4p16.3	41.20 kb	*CPLX1*	Complexin 1	605032	
4q12	114.34 kb	*CLOCK*	Clock homolog (mouse)	601851	Susceptibility to adult attention-deficit/hyperactivity disorder
4q12	26.93 kb	*REST*	RE1-silencing transcription factor	600571	
4q22	1341.00 kb	*PDLIM5*	PDZ and LIM domain 5	605904	
4q23	15.05 kb	*ADH1B*	Alcohol dehydrogenase 1B (class I), beta polypeptide	103720	Susceptibility to esophageal squamous cell carcinoma (ESCC)
4q31	8.00 kb	*NPY2R*	Neuropeptide Y receptor Y2	162642	
4q31.1	363.00 kb	*NR3C2*	Nuclear receptor subfamily 3, group C, member 2	600983	
4q31.3-q32	20.87 kb	*NPY1R*	Neuropeptide Y receptor Y1	162641	
4q35.1	21.73 kb	*MTNR1A*	Melatonin receptor 1A	600665	Susceptibility to circadian rhythm sleep disorders
					Susceptibility to recurrent calcium nephrolithiasis
					Susceptibility to attention-deficit/hyperactivity disorder (ADHD)
					Susceptibility to polycystic ovary syndrome
5p13	5p13.2	*SLC1A3*	Solute carrier family 1 (glial high-affinity glutamate transporter), member 3	600111	Episodic ataxia, type 6
5p13	19.93 kb	*IL7R*	Interleukin 7 receptor	146661	Severe combined immunodeficiency, lacking T lymphocytes
5p13.1-p12	24.03 kb	*GDNF*	Glial cell-derived neurotrophic factor	600837	Central hypoventilation syndrome
					Susceptibility to Hirschsprung's disease
5p15.3	52.64 kb	*SLC6A3*	Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	126455	Parkinsonism-dystonia, infantile
					Attention-deficit/hyperactivity disorder (ADHD)
5q11.2-q13	1.00 kb	*HTR1A*	5-hydroxytryptamine (serotonin) receptor 1A	109760	Periodic fever, menstrual cycle dependent
5q12	924.76 kb	*PDE4D*	Phosphodiesterase 4D, cAMP specific	600129	Acrodysostosis
					Susceptibility to stroke
5q23	13.76 kb	*HBEGF*	Heparin-binding EGF-like growth factor	126150	Susceptibility to diphtheria

5q31.1	323.35 kb	*GRIA1*	Glutamate receptor, ionotropic, AMPA 1	138248	
5q31.1	53.05 kb	*HSPA4*	Heat shock 70 kDa protein 4	601113	
5q31.1	152.17 kb	*TRPC7*	Transient receptor potential cation channel, subfamily C, member 7	605692	Susceptibility to amyotrophic lateral sclerosis-parkinsonism/dementia complex
5q31.2	6.00 kb	*HINT1*	HINT1 histidine triad nucleotide-binding protein 1	601314	Neuromyotonia and axonal neuropathy, autosomal recessive
5q31.3	455.83 kb	*NR3C1*	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	138040	Cortisol resistance, primary
5q31-q33	203.00 kb	*HTR4*	5-hydroxytryptamine (serotonin) receptor 4	602164	
5q32	70.35 kb	*CAMK2A*	Calcium/calmodulin-dependent protein kinase II alpha	114078	
5q33.3	69.05 kb	*ADRA1B*	Adrenergic, alpha-1B-, receptor	104220	
5q34	52.77 kb	*GABRA1*	Gamma-aminobutyric acid (GABA) A receptor, alpha 1	137160	Epileptic encephalopathy, early infantile, 19
					Susceptibility to epilepsy, childhood absence, 4
					Susceptibility to epilepsy, juvenile myoclonic, 5
					Susceptibility to schizophrenia
5q35.1	3.49 kb	*DRD1*	Dopamine receptor D1	126449	Susceptibility to schizophrenia
					Susceptibility to alcoholism
					Susceptibility to nicotine dependence
					Susceptibility to autism spectrum disorder in male-only
6p21	65.00 kb	*MDGA1*	MAM domain containing glycosylphosphatidylinositol anchor 1	609626	Susceptibility to schizophrenia
6p21.3	111.82 kb	*GRM4*	Glutamate receptor, metabotropic 4	604100	
6p21.3	2.76 kb	*TNF*	Tumor necrosis factor	191160	Susceptibility to asthma
					Susceptibility to dementia vascular
					Susceptibility to malaria cerebral
					Susceptibility to migraine without aura
					Susceptibility to septic shock
					Susceptibility to rheumatoid arthritis
					Susceptibility to systemic lupus erythematosus

(continued)

Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
6p21.3	6.00 kb	*HLA-DQB1*	Major histocompatibility complex, class II, DQ beta 1	604305	Diabetes mellitus insulin-dependent 1
					Susceptibility to celiac disease
					Susceptibility to multiple sclerosis 1
6p21.3		*HLA-DRB1*	Major histocompatibility complex, class II, DR beta 1	142857	Diabetes mellitus insulin-dependent 1
					Susceptibility to multiple sclerosis 1
					Susceptibility to pemphigoid
					Susceptibility to rheumatoid arthritis
					Susceptibility to sarcoidosis 1
6p21.31	155.35 kb	*FKBP5*	FK506 binding protein 5	602623	
6p21.31	30.96 kb	*GABBR1*	Gamma-aminobutyric acid (GABA) B receptor, 1	603540	Susceptibility to obsessive–compulsive disorder
					Susceptibility to nasopharyngeal carcinoma
					Susceptibility to obstructive sleep apnea syndrome
6p22.1	15.34 kb	*MOG*	Myelin oligodendrocyte glycoprotein	159465	Narcolepsy 7
6p22.3	140.26 kb	*DTNBP1*	Dystrobrevin-binding protein 1	607145	Hermansky–Pudlak syndrome 7
					Schizophrenia 3
6p24-p22		*SCZD3*	Schizophrenia disorder 3	600511	Schizophrenia
6q13	1.00 kb	*HTR1B*	5-hydroxytryptamine (serotonin) receptor 1B	182131	Susceptibility to attention-deficit/hyperactivity disorder with preferential transmission of paternal alleles
6q14-q15	26.18 kb	*CNR1*	Cannabinoid receptor 1 (brain)	114610	Susceptibility to anorexia nervosa
					Susceptibility to obesity related phenotypes
					Susceptibility to hebephrenic schizophrenia
6q16.3-q21	671.05 kb	*GRIK2*	Glutamate receptor, ionotropic, kainate 2	138244	Mental retardation autosomal recessive 6
6q21	35.04 kb	*HDAC2*	Histone deacetylase 2	605164	
6q21	4.76 kb	*CD24*	CD24 molecule	600074	Susceptibility to systemic lupus erythematosus
6q23	148.81 kb	*SGK1*	Serum/glucocorticoid regulated kinase 1	602958	Susceptibility to type 2 diabetes
6q23.2	1.00 kb	*TAAR6*	Trace amine associated receptor 6	608923	Susceptibility to schizophrenia
6q24-q25	236.37 kb	*OPRM1*	Opioid receptor, mu 1	600018	Susceptibility to idiopathic generalized epilepsy or absence epilepsy
					Susceptibility to drug dependence
6q25	8.97 kb	*VIP*	Vasoactive intestinal peptide	192320	Susceptibility to idiopathic pulmonary arterial hypertension

6q25.1	412.78 kb	*ESR1*	Estrogen receptor 1	133430	Estrogen resistance
					Susceptibility to atherosclerosis
					Susceptibility to migraine
					Susceptibility to myocardial infarction
6q25.3	14.21 kb	*SOD2*	Superoxide dismutase 2, mitochondrial	147460	Microvascular complications of diabetes 6
					Susceptibility to familial idiopathic dilated cardiomyopathy in Japanese
					Susceptibility to exudative age-related macular degeneration
					Susceptibility to Behçet disease
7p12	188.31 kb	*EGFR*	Epidermal growth factor receptor	131550	Inflammatory skin and bowel disease, neonatal, 2
					Susceptibility to non-small cell lung cancer
7p12.2	107.02 kb	*DDC*	Dopa decarboxylase (aromatic L-amino acid decarboxylase)	107930	Aromatic L-amino acid decarboxylase deficiency
7p12.3	449.25 kb	*ABCA13*	ATP-binding cassette, subfamily A (ABC1), member 13	607807	Susceptibility to schizophrenia
7p14.3	48.16 kb	*CRHR2*	Corticotropin-releasing hormone receptor 2	602034	
7p15.1	7.00 kb	*NPY*	Neuropeptide Y	162640	Susceptibility to schizophrenia
7p15.3		*SP4*	Sp4 transcription factor	600540	
7q21.11	408.88 kb	*PCLO*	Piccolo (presynaptic cytomatrix protein)	604918	Pontocerebellar hypoplasia, type 3
7p21.2	693.64 kb	*DGKB*	Diacylglycerol kinase, beta 90 kDa	604070	
7q21.1	27.21 kb	*CYP3A4*	Cytochrome P450, family 3, subfamily A, polypeptide 4	124010	Susceptibility to prostate cancer
					Adverse drug reactions/toxicities when drugs extensively metabolized by CYP3A4
7q21.12	210.00 kb	*ABCB1*	ATP-binding cassette, subfamily B (MDR/TAP), member 1	171050	Susceptibility to inflammatory bowel disease
					Susceptibility to renal epithelial tumors
7q21.1-q21.2	220.96 kb	*GRM3*	Glutamate receptor, metabotropic 3	601115	Schizophrenia 20
7q21-q22	8.51 kb	*TAC1*	Tachykinin, precursor 1	162320	
7q22	517.73 kb	*RELN*	Reelin	600514	Epilepsy, familial temporal lobe, 7
					Lissencephaly 2

(continued)

Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
7q22.1	3.06 kb	*VGF*	VGF nerve growth factor inducible	602186	
7q31	476.70 kb	*KCND2*	Potassium voltage-gated channel, Shal-related subfamily, member 2	605410	
7q31.3	568.34 kb	*CADPS2*	Ca++-dependent secretion activator 2	609978	Susceptibility to autistic-like phenotypes
7q34-q35	191.05 kb	*TBXAS1*	Thromboxane A synthase 1 (platelet)	274180	Ghosal hemato-diaphyseal dysplasia
					Thromboxane A synthase deficiency
7q36.1	14.00 kb	*HTR5A*	5-hydroxytryptamine (serotonin) receptor 5A	601305	
7q36.3	116.78 kb	*VIPR2*	Vasoactive intestinal peptide receptor 2	601970	
8p11.2	39.65 kb	*CHRNB3*	Cholinergic receptor, nicotinic, beta 3	118508	Susceptibility to heavy alcohol use
					Susceptibility to tobacco dependence
8p11.21	15.84 kb	*CHRNA6*	Cholinergic receptor, nicotinic, alpha 6	606888	Susceptibility to heavy alcohol use
					Susceptibility to tobacco dependence
8p11.21	47.50 kb	*SFRP1*	Secreted frizzled-related protein 1	604156	
8p11.2-p11.1	3.00 kb	*DKK4*	Dickkopf homolog 4 (Xenopus laevis)	605417	
8p12	3.00 kb	*ADRB3*	Adrenergic, beta-3-, receptor	109691	Non-insulin dependent diabetes and/or morbid obesity
8p12	57.70 kb	*FGFR1*	Fibroblast growth factor receptor 1	136350	Hartsfield syndrome
					Hypogonadotropic hypogonadism 2 with or without anosmia
					Jackson-Weiss syndrome
					Osteoglophonic dysplasia
					Pfeiffer syndrome
					Trigonocephaly 1
8p12	1125.74 kb	*NRG1*	Neuregulin 1	142445	Schizophrenia 6
					Susceptibility to late-onset alzheimer's disease with psychoses
					Susceptibility to Hirschsprung's disease
8p12	3.01 kb	*PLAT*	Plasminogen activator, tissue	173370	Thrombosis, recurrent
					Susceptibility to ischemic stroke
8p21	19.54 kb	*CHRNA2*	Cholinergic receptor, nicotinic, alpha 2 (neuronal)	118502	Epilepsy, nocturnal frontal lobe, type 4

8p21	5.89 kb	*FGF17*	Fibroblast growth factor 17	603725	Hypogonadotropic hypogonadism 20 with or without anosmia
8p21	80.06 kb	*FZD3*	Frizzled family receptor 3	606143	Susceptibility to schizophrenia
8p21	5.33 kb	*NEFM*	Neurofilament, medium polypeptide	162250	Susceptibility to Parkinson's disease
8p21		*SCZD6*	Schizophrenia disorder 6	603013	Schizophrenia
8p21.1	61.00 kb	*FBXO16*	F-box protein 16	608519	
8p21.2	117.26 kb	*ADRA1A*	Adrenergic, alpha-1A-, receptor	104221	
8p21.3		*ATP6V1B2*	ATPase, H+ transporting, lysosomal 56/58 kDa, V1 subunit B2	606939	
8p21.3	84.00 kb	*PPP3CC*	Protein phosphatase 3, catalytic subunit, gamma isozyme	114107	Susceptibility to schizophrenia
8p21.3	20.00 kb	*SLC18A1*	Solute carrier family 18 (vesicular monoamine), member 1	193002	
8p22	9.34 kb	*FGF20*	Fibroblast growth factor 20	605558	Susceptibility to Parkinson's disease
					Renal hypodysplasia/aplasia 2
8p22	9.97 kb	*NAT2*	N-acetyltransferase 2 (arylamine N-acetyltransferase)	612182	N-acetyltransferase 2 (arylamine N-acetyltransferase) deficiency
8p22-p21	80.27 kb	*DPYSL2*	Dihydropyrimidinase-like 2	602463	Alzheimer's disease
8p22-p21.3	107.09 kb	*PCM1*	Pericentriolar material 1	600299	Thyroid carcinoma, papillary
					Atypical chronic myeloid leukemia
					T-cell lymphoma
8p23	134.66 kb	*ARHGEF10*	Rho guanine nucleotide exchange factor (GEF) 10	608136	Susceptibility to ischemic stroke
					Slowed nerve conduction velocity
8p23-p21	5.64 kb	*EGR3*	Early growth response 3	602419	
8q11.2	25.00 kb	*OPRK1*	Opioid receptor, kappa 1	165196	Susceptibility to opiate addiction
					Susceptibility to alcohol dependence
8q13	2.00 kb	*CRH*	Corticotropin-releasing hormone	122560	Congenital isolated adrenocorticotropic hormone deficiency
8q21.13-q21.3	28.00 kb	*IMPA1*	Inositol(myo)-1(or 4)-monophosphatase 1	602064	
8q23-q24	5.00 kb	*PENK*	Proenkephalin	131330	Susceptibility to opiate dependence
9p11.1	5.96 kb	*ALDH1B1*	Aldehyde dehydrogenase 1 family, member B1	100670	
9p12	12.29 kb	*BAG1*	BCL2-associated athanogene	601497	Susceptibility to Alzheimer's disease

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Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
9p13.1	215.54 kb	*CNTNAP3*	Contactin-associated protein-like 3	610517	
9p13.3	4.69 kb	*CREB3*	cAMP-responsive element-binding protein 3	606443	
9p24	32.71 kb	*VLDLR*	Very low-density lipoprotein receptor	192977	Cerebellar hypoplasia and mental retardation with or without quadrupedal locomotion 1
9q22.1	355.04 kb	*NTRK2*	Neurotrophic tyrosine kinase, receptor, type 2	600456	Obesity, hyperphagia, and developmental delay
9q31.1	169.23 kb	*GRIN3A*	Glutamate receptor, ionotropic, N-methyl-D-aspartate 3A	606650	Susceptibility to schizophrenia
					Susceptibility to Alzheimer's disease
9q34	22.98 kb	*DBH*	Dopamine beta-hydroxylase (dopamine beta-monooxygenase)	609312	Dopamine beta-hydroxylase deficiency, congenital
9q34	11.22 kb	*TOR1A*	Torsin family 1, member A (torsin A)	605204	Dystonia 1, idiopathic torsion
					Dystonia, early onset atypical, with myoclonic features
					Susceptibility to sporadic idiopathic dystonia
9q34.3	29.61 kb	*GRIN1*	Glutamate receptor, ionotropic, N-methyl D-aspartate 1	138249	Susceptibility to schizophrenia
					Mental retardation, autosomal dominant 8
10p11.23	88.25 kb	*GAD2*	Glutamate decarboxylase 2 (pancreatic islets and the brain, 65 kDa)	138275	Stiff-man syndrome
10p12.2	179.00 kb	*PIP5K2A*	Phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	603140	
10q21	707.23 kb	*ANK3*	Ankyrin 3, node of Ranvier (ankyrin G)	600465	Mental retardation, autosomal recessive, 37
					Susceptibility to late-onset alzheimer's disease
					Susceptibility to autism spectrum disorders (ASDs)
10q22.3		*SCZD11*	Schizophrenia susceptibility locus, chromosome 10q related	608078	Schizophrenia
10q24	50.00 kb	*CYP2C9*	Cytochrome P450, family 2, subfamily C, polypeptide 9	601130	Adverse drug reactions/toxicities when drugs extensively metabolized by CYP2C9
					Warfarin resistance
10q24	90.00 kb	*CYP2C19*	Cytochrome P450, family 2, subfamily C, polypeptide 19	124020	Adverse drug reactions/toxicities when drugs extensively metabolized by CYP2C19

10q24	6.87 kb	*C10orf2*	Chromosome 10 open reading frame 2	606075	Infantile-onset spinocerebellar ataxia
					Mitochondrial DNA depletion syndrome 7 (hepatocerebral type)
					Perrault syndrome 5
					Progressive external ophthalmoplegia, autosomal dominant, 3
10q24-q26	3.65 kb	*ADRA2A*	Adrenergic, alpha-2A-, receptor	104210	Susceptibility to attention-deficit/hyperactivity disorder (ADHD)
10q24-q26	2.86 kb	*ADRB1*	Adrenergic, beta-1-, receptor	109630	Heart failure
10q25	36.38 kb	*SLC18A2*	Solute carrier family 18 (vesicular monoamine), member 2	193001	Susceptibility to alcoholism
					Susceptibility to Parkinson's disease
					Susceptibility to schizophrenia
11p11.2	36.13 kb	*CRY2*	Cryptochrome 2 (photolyase like)	603732	Altered sleep patterns
11p13	67.16 kb	*BDNF*	Brain-derived neurotrophic factor	113505	Wilms tumor–aniridia–genitourinary anomalies–mental retardation syndrome
					Central hypoventilation syndrome, congenital
					Susceptibility to memory impairment
					Susceptibility to anorexia nervosa
11p13-p12	168.35 kb	*SLC1A2*	Solute carrier family 1 (glial high-affinity glutamate transporter), member 2	600300	Amyotrophic lateral sclerosis
11p15	109.49 kb	*ARNTL*	Aryl hydrocarbon receptor nuclear translocator like	602550	Susceptibility to type 2 diabetes
					Infertility
					Problems with gluconeogenesis and lipogenesis
					Altered sleep patterns
11p15.2	5.00 kb	*CALCA*	Calcitonin-related polypeptide alpha	114130	
11p15.3-p14	19.77 kb	*TPH1*	Tryptophan hydroxylase 1	191060	Susceptibility to schizophrenia
					Susceptibility to addictions
					Anger-related traits
					Somatic anxiety
11p15.4	2.28 kb	*ADM*	Adrenomedullin	103275	
11p15.5	3.40 kb	*DRD4*	Dopamine receptor D4	126452	Personality trait of novelty seeking
					Autonomic nervous system dysfunction
					Attention-deficit/hyperactivity disorder

(continued)

Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
11q13	7.78 kb	*MEN1*	Multiple endocrine neoplasia I	613733	Adrenal adenoma, somatic
					Angiofibroma, somatic
					Carcinoid tumor of the lung
					Lipoma, somatic
					Multiple endocrine neoplasia 1
					Parathyroid adenoma, somatic
11q13.3	6.66 kb	*GAL*	Galanin prepropeptide	137035	
11q14-q21		*SCZD2*	Schizophrenia disorder 2	603342	Schizophrenia
11q21-q22	13.16 kb	*MTNR1B*	Melatonin receptor 1B	600804	Susceptibility to diabetes mellitus, type 2
11q22.2-q22.3	20.87 kb	*IL18*	Interleukin 18 (interferon-gamma-inducing factor)	600953	Susceptibility to asthma
					Susceptibility to Behçet's disease
					Susceptibility to systemic lupus erythematosus (SLE)
11q22.3	325.94 kb	*GRIK4*	Glutamate receptor, ionotropic, kainate 4	600282	
11q23	85.00 kb	*ALG9*	Asparagine-linked glycosylation 9, alpha-1,2-mannosyltransferase homolog (*S. cerevisiae*)	606941	Congenital disorder of glycosylation, type 1 L
11q23	65.68 kb	*DRD2*	Dopamine receptor D2	126450	Dystonia, myoclonic
11q23.1	41.00 kb	*HTR3B*	5-hydroxytryptamine (serotonin) receptor 3B	604654	
11q23.1	317.19 kb	*NCAM1*	Neural cell adhesion molecule 1	116930	
12p12	418.61 kb	*GRIN2B*	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	138252	Epileptic encephalopathy, early infantile, 27
					Mental retardation, autosomal dominant 6
12p12.1	4.89 kb	*BHLHE41*	Basic helix–loop–helix family, member e41	606200	Short sleeper
12p12.2	57.92 kb	*SLCO1C1*	Solute carrier organic anion transporter family, member 1C1	613389	Fatigue
12p12.2-p11.2	87.48 kb	*ARNTL2*	Aryl hydrocarbon receptor nuclear translocator-like 2	614517	
12p13	63.19 kb	*NTF3*	Neurotrophin 3	162660	
12p13	7.18 kb	*GNB3*	Guanine nucleotide-binding protein (G protein), beta polypeptide 3	139130	Susceptibility to hypertension, essential
12p13.3	27.16 kb	*CACNA1C*	Calcium channel, voltage-dependent, L type, alpha 1C subunit	114205	Brugada syndrome 3
					Timothy syndrome

12q12-q13	33.04 kb	*TIMELESS*	Timeless homolog (drosophila)	603887	Prostate cancer
					Lung cancer
					Breast cancer
					Mental disorders
12q13	217.29 kb	*SCN8A*	Sodium channel, voltage gated, type VIII, alpha subunit	600702	Cerebellar atrophy, ataxia, mental retardation
					Epileptic encephalopathy, early infantile 13
12q14	4.97 kb	*IFNG*	Interferon, gamma	147570	Aplastic anemia
12q21	99.05 kb	*PAWR*	PRKC, apoptosis, WT1, regulator	601936	
12q21.1	93.00 kb	*TPH2*	Tryptophan hydroxylase 2	607478	Susceptibility to attention-deficit/hyperactivity disorder 7
12q22-q23	4.46 kb	*DUSP6*	Dual-specificity phosphatase 6	602748	Hypogonadotropic hypogonadism 19 with or without anosmia
12q22-q23.2		*MDD1*	Major depressive disorder 1	608520	
12q23	90.13 kb	*APAF1*	Apoptotic peptidase-activating factor 1	602233	
12q23-q24.1	102.46 kb	*CRY1*	Cryptochrome 1 (photolyase like)	601933	Altered sleep patterns
12q24	20.85 kb	*DAO*	D-amino-acid oxidase	124050	Schizophrenia
					Amyotrophic lateral sclerosis 13
12q24	53.18 kb	*P2RX7*	Purinergic receptor P2X, ligand-gated ion channel, 7	602566	
12q24.11	69.87 kb	*ATP2A2*	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	108740	Acrokeratosis verruciformis
					Darier–White disease
12q24.12	8.47 kb	*FAM109A*	Family with sequence similarity 109, member A	614239	
12q24.2	60.62 kb	*CAMKK2*	Calcium/calmodulin-dependent protein kinase 2, beta	615002	
12q24.2-q24.31	148.60 kb	*NOS1*	Nitric oxide synthase 1 (neuronal)	163731	Susceptibility to infantile pyloric stenosis and to asthma
					Susceptibility to enuresis
13q14.11	181.00 kb	*DGKH*	Diacylglycerol kinase, eta	604071	
13q14-q21	63.48 kb	*HTR2A*	5-hydroxytryptamine (serotonin) receptor 2A	182135	Susceptibility to alcohol dependence
					Susceptibility to anorexia nervosa
					Susceptibility to obsessive–compulsive disorder
					Susceptibility to schizophrenia
13q32		*SCZD7*	Schizophrenia disorder 7	603176	Schizophrenia

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Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
13q32-33	25.00 kb	*DAOA*	D-amino-acid oxidase activator	607408	Schizophrenia 7
14q22.1-q22.2	60.88 kb	*GCH1*	GTP cyclohydrolase 1	600225	Dystonia 5, progressive, with diurnal variations
					Guanosine triphosphate cyclohydrolase 1 deficiency
					Malignant hyperphenylalaninemia
14q22.3	9.76 kb	*OTX2*	Orthodenticle homeobox 2	600037	Agnathia-otocephaly 2
					Chromosome 14q deletion, encompassing band q22
					Microphthalmia, isolated 2
					Microphthalmia syndromic 5
					Pituitary hormone deficiency, combined, 6
					Retinal dystrophy, early onset, with or without pituitary dysfunction
14q23.2	111.52 kb	*ESR2*	Estrogen receptor 2 (ER beta)	601663	Osteoporosis
					Susceptibility to Alzheimer's disease in women
					Susceptibility to premature coronary artery disease
14q24.2-q24.3	14.00 kb	*DIO2*	Deiodinase, iodothyronine, type II	601413	Susceptibility to mental retardation
					Susceptibility to osteoarthritis
					Susceptibility to hypertension
14q24.3	3.46 kb	*FOS*	FBJ murine osteosarcoma viral oncogene homolog	164810	Apoptotic cell death
14q31	155.84 kb	*GALC*	Galactosylceramidase	606890	Galactosylceramide beta-galactosidase deficiency
					Krabbe disease
14q32.1-q32.2	39.53 kb	*BDKRB2*	Bradykinin receptor B2	113503	
14q32.32	26.39 kb	*AKT1*	V-akt murine thymoma viral oncogene homolog 1	164730	Proteus syndrome
					Breast cancer, somatic
					Colorectal cancer, somatic
					Cowden syndrome 6
					Ovarian cancer, somatic
					Susceptibility to schizophrenia
15q11.2-q12	11.00 kb	*GABRA5*	Gamma-aminobutyric acid (GABA) A receptor, alpha 5	137142	

15q11.2-q12	230.24 kb	*GABRB3*	Gamma-aminobutyric acid (GABA) A receptor, beta 3	137192	Susceptibility to epilepsy, childhood absence, 5
					Angelman syndrome
					Prader–Willi syndrome
					Nonsyndromic orofacial clefts
					Autism spectrum disorder 4
15q13	108.70 kb	*SLC12A6*	Solute carrier family 12 (potassium/chloride transporters), member 6	604878	Agenesis of the corpus callosum with peripheral neuropathy
15q14-q15	555.13 kb	*RYR3*	Ryanodine receptor 3	180903	Susceptibility to severe epileptic encephalopathies
15q15		*SCZD10*	Schizophrenia disorder 10 (periodic catatonia)	605419	Schizophrenia 10
15q22	153.67 kb	*ADAM10*	ADAM metallopeptidase domain 10	602192	Kitamura reticulate acropigmentation
					Susceptibility to Alzheimer's disease 18
15q22.2	741.02 kb	*RORA*	RAR-related orphan receptor A	600825	
15q24.1	6.00 kb	*CYP1A2*	Cytochrome P450, family 1, subfamily A, polypeptide 2	124060	Adverse drug reactions/toxicities when drugs extensively metabolized by CYP1A2
5q24.2	8.26 kb	*NEIL1*	Nei endonuclease VIII-like 1 (E. coli)	608844	
15q25	381.80 kb	*NTRK3*	Neurotrophic tyrosine kinase, receptor, type 3	191316	Medulloblastoma
					Secretory breast carcinoma
15q25	18.50 kb	*POLG*	Polymerase (DNA directed), gamma	174763	Ataxia-myopathy syndrome 1
					Mitochondrial neurogastrointestinal encephalopathy
					Occipital lobe epilepsy with nystagmus
					Neuronal degeneration of childhood with liver disease, progressive
					Sensory ataxic neuropathy, dysarthria, and ophthalmoparesis
					Progressive external ophthalmoplegia, autosomal dominant
					Progressive external ophthalmoplegia, autosomal recessive
15q25.3-q26.2		*MDD2*	Major depressive disorder 2	608691	
16p13.2	429.35 kb	*GRIN2A*	Glutamate receptor, ionotropic, N-methyl D-aspartate 2A	138253	Epilepsy, focal, with speech disorder and with or without mental retardation
16p13.3	153.00 kb	*ADCY9*	Adenylate cyclase 9	603302	
16p13.3	3.22 kb	*PGP*	Phosphoglycolate phosphatase	172280	
16q12.2	50.56 kb	*SLC6A2*	Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	163970	Orthostatic intolerance

(continued)

Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
16q22.1		*HP*	Haptoglobin	140100	Anhaptoglobinemia
					Hypohaptoglobinemia
16q22.1	17.23 kb	*NQO1*	NAD(P)H dehydrogenase, quinone 1	125860	Susceptibility to benzene toxicity
					Poor survival after chemotherapy for breast cancer
					Susceptibility to leukemia, post-chemotherapy
16q24.3	2.00 kb	*MC1R*	Melanocortin 1 receptor (alpha melanocyte-stimulating hormone receptor)	155555	Melanoma, cutaneous malignant, 5
					UV-induced skin damage
17p13	11.01 kb	*ARRB2*	Arrestin, beta 2	107941	
17p13.1	14.00 kb	*ALOX12*	Arachidonate 12-lipoxygenase	152391	
17p13.1	29.84 kb	*DLG4*	Disks, large homolog 4 (drosophila)	602887	Susceptibility to nicotinic dependence
17p13.1	11.97 kb	*PER1*	Period homolog 1 (drosophila)	602260	Susceptibility to cancer
17p13.3	55.72 kb	*YWHAE*	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	605066	Small-cell lung cancer
					Chromosome 17p13 deletion, distal to PAFAH1B1 involving YWHAE
					Chromosome 17p13.3 microduplications
					Miller–Dieker lissencephaly syndrome
17q11.2	7.94 kb	*NR1D1*	Nuclear receptor subfamily 1, group D, member 1	602408	
17q11.2	39.58 kb	*SLC6A4*	Solute carrier family 6 (neurotransmitter transporter, dopamine), member 4	182138	Sudden infant death syndrome
					Aggressive behavior in Alzheimer's disease patient
					Anxiety-related personality traits
					Obsessive–compulsive disorder
17q11.2-q12	1.93 kb	*CCL2*	Chemokine (C-C motif) ligand 2	158105	Susceptibility to mycobacterium tuberculosis
					Susceptibility to spina bifida
17q12	97.00 kb	*PPP1R1B*	Protein phosphatase 1, regulatory (inhibitor) subunit 1B	604399	
17q12-q22	51.55 kb	*CRHR1*	Corticotropin-releasing hormone receptor 1	122561	Chromosome 17q21.31 microdeletion syndrome
					Chromosome 17q21 microduplication syndrome
17q21	11.00 kb	*CNP*	2′,3′-cyclic nucleotide 3′ phosphodiesterase	123830	
17q21	46.89 kb	*HDAC5*	Histone deacetylase 5	605315	Colon cancer

17q21.1	134.00 kb	*MAPT*	Microtubule-associated protein tau	157140	Dementia, frontotemporal, with or without parkinsonism
					Pick disease
					Supranuclear palsy
					Susceptibility to Parkinson's disease
					Chromosome 17q21.31 microdeletion syndrome
					Chromosome 17q21 microduplication syndrome
17q21.3		*ERDA1*	Expanded repeat domain, CAG/CTG 1	603279	
17q21.31	75.17 kb	*STAT3*	Signal transducer and activator of transcription 3 (acute-phase response factor)	102582	Hyper IgE syndrome 2
					Autoimmune disease, multisystem, infantile onset
17q21.32	12.88 kb	*TBX21*	T-box 21	604895	Asthma and nasal polyps
17q23.3	21.32 kb	*ACE*	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	106180	Stroke, hemorrhagic
					Renal tubular dysgenesis
					Susceptibility to Alzheimer's disease
					Microvascular complications of diabetes 3
					Susceptibility to myocardial infarction
					Left ventricular hypertrophy (Caucasians), cardiomyopathy, myocardial infarction
17q25	2.55 kb	*AANAT*	Arylalkylamine N-acetyltransferase	600950	Susceptibility to delayed sleep phase syndrome
18p		*MAFD1*	Major affective disorder 1	125480	
18p		*SCZD8*	Schizophrenia disorder 8	603206	Schizophrenia
18p11	7.00 kb	*ADCYAP1*	Adenylate cyclase-activating polypeptide 1 (pituitary)	102980	
18p11.2	49.00 kb	*IMPA2*	Inositol(myo)-1(or 4)-monophosphatase 2	605922	Febrile convulsions, familial, 6
18p11.21	3.06 kb	*CHMP1B*	Chromatin-modifying protein 1B	606486	
18p11.22	26.89 kb	*NAPG*	N-ethylmaleimide-sensitive factor attachment protein, gamma	603216	
18p11.22	31.72 kb	*NDUFV2*	ADH dehydrogenase (ubiquinone) flavoprotein 2, 24 kDa	600532	Hypertrophic cardiomyopathy with encephalopathy
					Mitochondrial complex I deficiency
18q12.3	126.25 kb	*PIK3C3*	Phosphoinositide-3-kinase, class 3	602609	

(continued)

Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
18q21	70.73 kb	*ME2*	Malic enzyme 2, NAD(+)-dependent, mitochondrial	154270	Susceptibility to idiopathic generalized epilepsy
18q21.1	367.48 kb	*TCF4*	Transcription factor 4	602272	Pitt–Hopkins syndrome
					Chromosome 18q distal deletion
19p13.1	26.00 kb	*AKAP8*	A kinase (PRKA) anchor protein 8	604692	
19p13.11	40.24 kb	*PDE4C*	Phosphodiesterase 4C, cAMP specific	600128	
19p13.2	48.98 kb	*PDE4A*	Phosphodiesterase 4A, cAMP specific	600126	
19p13.2-p13.1	41.35 kb	*NOTCH3*	Notch 3	600276	Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy
					Hereditary multi-infarct dementia
					Infantile myofibromatosis-2
					Myofibromatosis, infantile 2
19p13.3	44.47 kb	*LDLR*	Low-density lipoprotein receptor	606945	Hypercholesterolemia, familial
					LDL cholesterol level QTL2
19p13.3	4.00 kb	*NRTN*	Neurturin	602018	
19p13.3		*PSPN*	Persephin	602921	
19p13.3-p13.2	15.78 kb	*ICAM1*	Intercellular adhesion molecule 1	147840	Susceptibility to malaria cerebral
					Candidiasis familial chronic mucocutaneous 1
19q13	5.70 kb	*KLK8*	Kallikrein-related peptidase 8	605644	
19q13.1	21.67 kb	*MAG*	Myelin-associated glycoprotein	159460	
19q13.1	153.87 kb	*RYR1*	Ryanodine receptor 1 (skeletal)	180901	Susceptibility to malignant hyperthermia
					Central core disease
					Minicore myopathy with external ophthalmoplegia
					King–Denborough syndrome
					Neuromuscular disease, congenital, with uniform type 1 fiber

19q13.2	3.61 kb	*APOE*	Apolipoprotein E	107741	Alzheimer's disease associated with *APOE-4*
					Familial dysbetalipoproteinemia, hyperlipoproteinemia type III
					Age-related macular dystrophy 2
					Sea-blue histiocyte disease
					Macular degeneration, age related
					Susceptibility to myocardial infarction
					Lipoprotein glomerulopathy
19q13.2	67.48 kb	*GRIK5*	Glutamate receptor, ionotropic, kainate 5	600283	
19q13.2	12.00 kb	*GSK3A*	Glycogen synthase kinase 3 alpha	606784	Alzheimer's disease
19q13.2	6.91 kb	*CYP2A6*	Cytochrome P450, family 2, subfamily A, polypeptide 6	122720	Adverse drug reactions/toxicities when drugs extensively metabolized by CYP2A6
					Coumarin resistance
19q13.2	27.10 kb	*CYP2B6*	Cytochrome P450, family 2, subfamily B, polypeptide 6	123930	Adverse drug reactions/toxicities when drugs extensively metabolized by CYP2B6
					Susceptibility to efavirenz central nervous system toxicity
19q13.3	6.82 kb	*DBP*	D site of albumin promoter (albumin D-box)-binding protein	124097	Susceptibility to inflammatory bowel disease
					Susceptibility to chronic obstructive pulmonary disease
19q13.3	3.84 kb	*NTF4*	Neurotrophin 4	162662	Glaucoma, primary open angle, O
19q13.31	27.65 kb	*ATP1A3*	ATPase, Na+/K+ transporting, alpha 3 polypeptide	182350	Dystonia 12
					Alternating hemiplegia of childhood 2
					CAPOS syndrome
20p12.3	15.67 kb	*PROKR2*	Prokineticin receptor 2	607123	Hypogonadotropic hypogonadism 3 with or without anosmia
					Kallmann syndrome 3
20p13	2.17 kb	*AVP*	Arginine vasopressin	192340	Diabetes insipidus, neurohypophyseal type
20q11.2	5.00 kb	*GHRH*	Growth hormone–releasing hormone	139190	Isolated growth hormone deficiency due to defect in GHRF
					Gigantism due to GHRF hypersecretion
21q22.1	22.51 kb	*MRAP*	Melanocortin 2 receptor accessory protein	609196	Glucocorticoid deficiency 2
21q22.13		*MAFD3*	Major affective disorder 3, early onset	609633	

(continued)

Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
21q22.2	103.08 kb	*SYNJ1*	Synaptojanin 1	604297	Parkinson's disease 20, early onset
21q22.3	97.56 kb	*ABCG1*	ATP-binding cassette, subfamily G (WHITE), member 1	603076	
21q22.3	121.65 kb	*PCNT*	Pericentrin	605925	Microcephalic osteodysplastic primordial dwarfism, type II
					Seckel syndrome 7
21q22.3	27.00 kb	*PFKL*	Phosphofructokinase, the liver	171860	Hemolytic anemia due to phosphofructokinase deficiency
21q22.3	92.92 kb	*TRPM2*	Transient receptor potential cation channel, subfamily M, member 2	603749	Susceptibility to amyotrophic lateral sclerosis
					Susceptibility to parkinsonism–dementia
22q11.2	66.00 kb	*GNB1L*	Guanine nucleotide-binding protein (G protein), beta polypeptide 1 like	610778	Cat-eye syndrome
					DiGeorge syndrome
					Derivative 22 syndrome
22q11.21	28.24 kb	*COMT*	Catechol-O-methyltransferase	116790	Susceptibility to panic disorder
					Susceptibility to schizophrenia
22q11.21	108.02 kb	*MAPK1*	Mitogen-activated protein kinase 1	176948	Chromosome 22q11.2 microdeletion syndrome, distal to the DG/VCF typically deleted region
22q11.21	23.00 kb	*PRODH*	Proline dehydrogenase (oxidase) 1	606810	Hyperprolinemia, type I
					Susceptibility to schizophrenia 4
22q11.21	26.89 kb	*TBX1*	T-box 1	602054	Conotruncal heart malformations TBX1 related
					Chromosome 22q11.2 microdeletion syndrome
					DiGeorge syndrome
					Tetralogy of Fallot
					Velocardiofacial syndrome
22q11.23	137.67 kb	*BCR*	Break-point cluster region	151410	Leukemia, acute lymphocytic
					Leukemia, chronic myeloid
					Philadelphia chromosome
					Chromosome 22q11.2 microdeletion syndrome, distal to the DG/VCF typically deleted region
22q11.2-q13.2	15.00 kb	*APOL4*	Apolipoprotein L, 4	607254	Schizophrenia
22q12	6.01 kb	*XBP1*	X-box-binding protein 1	194355	Susceptibility to inflammatory bowel disease
22q12	13.00 kb	*APOL2*	Apolipoprotein L, 2	607252	Schizophrenia

22q12.3	13.11 kb	*YWHAH*	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide	113508	Early-onset schizophrenia
22q13	25.66 kb	*SREBF1*	Sterol regulatory element-binding transcription factors	184756	Susceptibility to obesity
					Susceptibility to type 2 diabetes
22q13.1	2.12 kb	*ATF4*	Activating transcription factor 4 (tax-responsive enhancer element B67)	604064	Susceptibility to schizophrenia
22q13.1	138.80 kb	*CACNG2*	Calcium channel, voltage-dependent, gamma subunit 2	602911	Mental retardation, autosomal dominant 10
					Chronic pain following nerve injury
22q13.1	16.00 kb	*CSF2RB*	Colony-stimulating factor 2 receptor, beta, low affinity (granulocyte macrophage)	138981	Congenital pulmonary alveolar proteinosis 2
					Surfactant metabolism dysfunction, pulmonary, 5
22q13.1	27.00 kb	*CSNK1E*	Casein kinase 1, epsilon	600863	
22q13.1	4.00 kb	*CYP2D6*	Cytochrome P450, family 2, subfamily D, polypeptide 6	124030	Codeine sensitivity
					Debrisoquine sensitivity
					Adverse drug reactions/toxicities when drugs extensively metabolized by CYP2D6
22q13.1	35.00 kb	*SYNGR1*	Synaptogyrin 1	603925	Focal segmental glomerulosclerosis 4
22q13.1	14.46 kb	*APOL1*	Apolipoprotein L, 1	603743	Susceptibility to end-stage renal disease, nondiabetic
					Susceptibility to glomerulosclerosis, focal segmental, 4
22q13.33	25.54 kb	*MLC1*	Megalencephalic leukoencephalopathy with subcortical cysts 1	605908	Megalencephalic leukoencephalopathy with subcortical cysts
Xp11.23	22.89 kb	*HDAC6*	Histone deacetylase 6	300272	Chondrodysplasia with platyspondyly, distinctive brachydactyly, hydrocephaly, and microphthalmia
Xp11.3	91.92 kb	*MAOA*	Monoamine oxidase A	309850	Mental retardation with impulsive behavior
					MAOA/MAOB deletion syndrome
					Brunner syndrome
Xp21.1	46.00 kb	*XK*	X-linked Kx blood group (McLeod syndrome)	314850	McLeod syndrome
					X-linked, recessive disorder
Xq13	243.59 kb	*HDAC8*	Histone deacetylase 8	300269	Cornelia de Lange syndrome 5
					Wilson–Turner mental retardation syndrome

(continued)

Table 35.1 (continued)

Locus	Size	Symbol	Title/gene	OMIM	Other related diseases
Xq13	23.90 kb	*MED12*	Mediator complex subunit 12	300188	Lujan–Fryns syndrome
					Ohdo syndrome, X-linked
					Opitz–Kaveggia syndrome
					FG syndrome 1
Xq13.1	60.64 kb	*DLG3*	Disks, large homolog 3 (drosophila)	300189	Mental retardation, 90
Xq22	16.11 kb	*PLP1*	proteolipid protein 1	300401	Pelizaeus–Merzbacher disease, type 1
					Spastic paraplegia 2
Xq24	358.68 kb	*HTR2C*	5-hydroxytryptamine (serotonin) receptor 2C	312861	Susceptibility to metabolic syndrome in patients with schizophrenia
					Susceptibility to obesity
Xq25	306.67 kb	*GRIA3*	Glutamate receptor, ionotropic, AMPA 3	305915	Mental retardation, X-linked 94
Xq27.3	39.18 kb	*FMR1*	Fragile-X mental retardation 1	309550	Fragile X syndrome
					Premature ovarian failure, fragile-X associated
					Fragile-X tremor ataxia syndrome
Xq27-q28		*MAFD2*	Major affective disorder 2	309200	
Xq28	15.00 kb	*G6PD*	Glucose-6-phosphate dehydrogenase	305900	Favism
					Hemolytic anemia due to G6PD deficiency
Xq28	4.87 kb	*GPR50*	G protein-coupled receptor 50	300207	
Xq28	14.43 kb	*L1CAM*	L1 cell adhesion molecule	308840	X-linked hydrocephalus or stenosis of the aqueduct of Sylvius
					MASA syndrome
					X-linked spastic paraplegia
					Partial agenesis of corpus callosum
					CRASH syndrome
					Hydrocephalus due to aqueductal stenosis
					Hydrocephalus with congenital idiopathic intestinal pseudoobstruction
					Hydrocephalus with Hirschsprung's disease

decrease the risk of rDD occurrence, while the G/G genotype and allele G of the same SNP increase the risk. This polymorphism has a stronger association with early-onset depression (<35 years) than with late-onset depression (≥35 years of age). The G/G genotype of the c.972G > C increases the risk of late-onset rDD, and the combined genotype C/C-C/C of c.977C > G and c.*589G > C significantly reduces the risk of rDD [38].

Genome-wide association studies have suggested a role for a genetic variation in the presynaptic gene *PCLO* in MDD. As with many complex traits, the *PCLO* variant has a small contribution to the overall heritability, and the association does not always replicate. The *PCLO* p.Ser4814Ala missense variant produces mild cellular phenotypes, which do not translate into behavioral phenotypes [39]. Milaneschi et al. [40] examined the polygenic features of MDD and two common clinical subtypes (typical and atypical). MDD subtypes had differential polygenic signatures; typical was strongly associated with schizophrenia, while atypical was additionally associated with body mass index (BMI) and triglycerides. SNP heritability was 32 % for MDD and 38 % and 43 % for subtypes with, respectively, decreased (typical) and increased (atypical) appetite/weight.

35.3 Mechanistic Genes

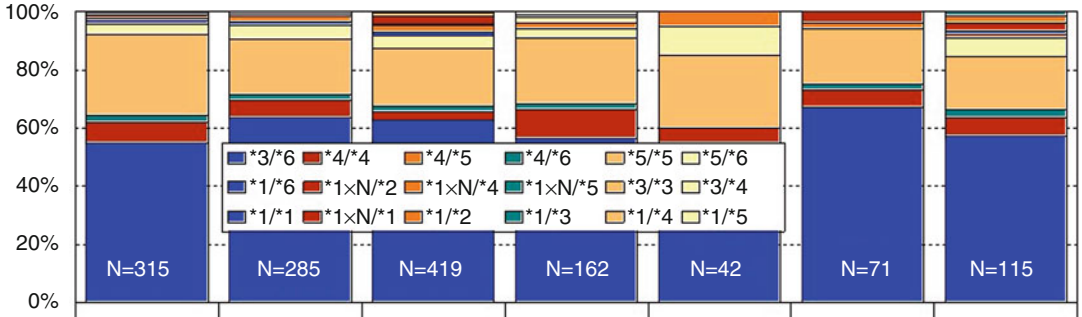
Most genes associated with the mechanism of action of antidepressant drugs encode receptors, enzymes, and neurotransmitters (serotonin, noradrenaline, dopamine, histamine, acetylcholine) on which drugs act as ligands (agonists, antagonists), enzyme modulators (substrates, inhibitors, inducers), or neurotransmitter regulators (releasers, reuptake inhibitors) [4].

35.4 Metabolic Genes

Most pharmacogenetic studies with antidepressants are related to genes involved in drug metabolism. Excellent studies on the metabolism of antidepressants by different authors are collected in the World Guide for Drug Use and Pharmacogenomics [1]. Drug metabolism includes phase I reactions (i.e., oxidation, reduction, and hydrolysis) and phase II conjugation reactions (i.e., acetylation, glucuronidation, sulfation, and methylation). The principal enzymes with polymorphic variants involved in phase I reactions are the cytochrome P450 monooxygenases (CYP3A4/CYP3A5/CYP3A7, CYP2E1, CYP2D6, CYP2C19, CYP2C9, CYP2C8, CYP2B6, CYP2A6, CYP1B1, CYP1A1/2) and other enzymes such as epoxide hydrolase, esterases, NQO1 (NADPH-quinone oxidoreductase), DPD (dihydropyrimidine dehydrogenase), ADH (alcohol dehydrogenase), and ALDH (aldehyde dehydrogenase), and major enzymes involved in phase II reactions include UGTs (uridine 5′-triphosphate glucuronosyl transferases), TPMT, COMT (catechol-O-methyltransferase), HMT (histamine methyltransferase), STs (sulfotransferases), GST-A (glutathione S-transferase A), GST-P, GST-T, GST-M, NAT1 (N-acetyl transferase 1), NAT2, and others. Among these enzymes, CYP2D6, CYP2C9, CYP2C19, and CYP3A4/CYP3A5 are the most relevant in the pharmacogenetics of central nervous system (CNS) drugs in general and antidepressants in particular [1, 4, 17, 18]. Approximately, 18 % of neuroleptics are major substrates of CYP1A2 enzymes, 40 % of CYP2D6, and 23 % of CYP3A4; 24 % of antidepressants are major substrates of CYP1A2 enzymes, 5 % of CYP2B6, 38 % of CYP2C19, 85 % of CYP2D6, and 38 % of CYP3A4; 7 % of benzodiazepines are major substrates of CYP2C19 enzymes, 20 % of CYP2D6, and 95 % of CYP3A4. Most CYP enzymes exhibit ontogenetic-, age-, sex-, circadian-, and ethnic-related differences. The practical consequence of this genetic variation is that the same drug can be differentially metabolized according to the genetic profile/expression during each subject's life-span and that knowing the pharmacogenomic profile of an individual, his/her pharmacodynamic response is potentially predictable to some extent.

35.4.1 CYP2D6

CYP2D6 is a 4.38-kb gene with nine exons mapped on 22q13.2. Four RNA transcripts of 1190–1684 bp are expressed in the brain, liver,

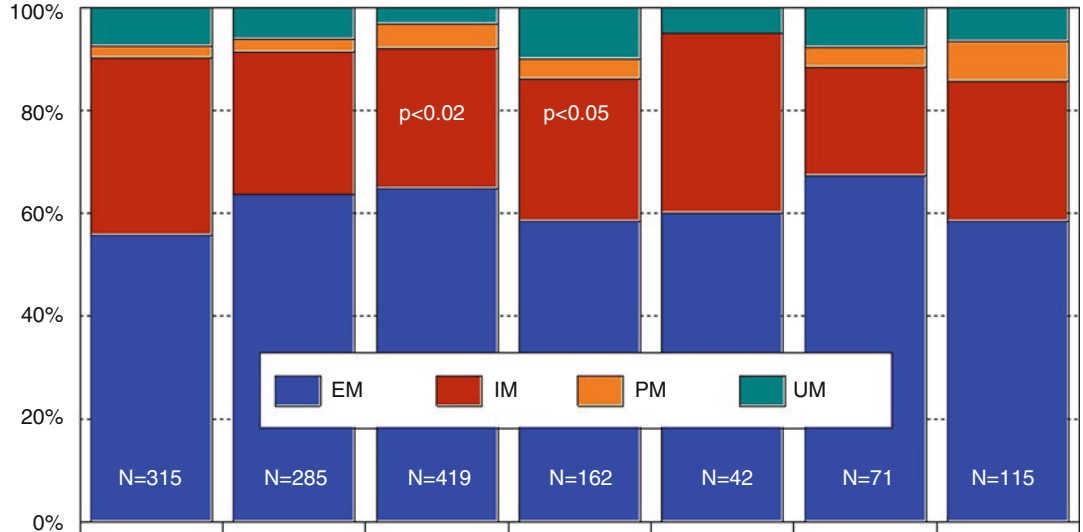


	C	ANX	DEP	PSY	ADHD	EPI	MR
*5/*6	0,46	0,66	0,44	0,99	0	0	0
*5/*5	0	0	0	0	0	0	0
*4/*6	0	0	0	0	0	0	1,3
*4/*5	0,46	0,66	0,88	0,99	0	0	2,6
*4/*4	1,37	0	3,08	0	0	3,85	2,6
*3/*6	0	0	0	0	0	0	1,3
*3/*4	0	0	0,44	1,98	0	0	0
*3/*3	0	0	0	0	0	0	0
*1×N/*5	0	0	0	0	0	0	0
*1×N/*4	0,91	1,99	2,2	1,98	5	1,92	1,3
*1×N/*2	0	0	0	0	0	0	0
*1/*6	0,91	1,32	1,32	0	0	0	0
*1/*5	3,65	4,64	4,41	2,97	10	0	6,49
*1/*4	27,85	19,21	19,82	22,77	25	19,23	18,18
*1/*3	2,28	1,99	1,76	1,98	0	1,92	2,6
*1/*2	0	0	0	0	0	0	0
*1×N/*1	7,31	5,96	3,08	9,9	5	5,77	6,49
*1/*1	54,79	63,58	62,56	56,44	55	67,31	57,14

Fig. 35.1 Distribution of *CYP2D6* genotypes among patients with anxiety (*ANX*), depression (*DEP*), psychosis (*PSY*), ADHD, epilepsy (*EPI*), mental retardation (*MR*), and controls (*C*)

spleen, and reproductive system, where four major proteins of 48–55 kDa (439–494 aa) are identified. This protein is a transport enzyme of the cytochrome P450 subfamily IID or multigenic cytochrome P450 superfamily of mixed-function monooxygenases which localizes to the endoplasmic reticulum and is known to metabolize as many as 25 % of commonly prescribed drugs and over 60 % of current psychotropics. The gene is highly polymorphic in the population. There are 141 *CYP2D6* allelic variants, of which −100C > T, −1023C > T, −1659G > A, −1707delT, −1846G > A, −2549delA, −2613-2615delAGA, −2850C > T, −2988G > A, and −3183G > A represent the ten most important variants. Different alleles result in the extensive, intermediate, poor, and ultra-rapid metabolizer phenotypes, characterized by normal, intermediate, decreased, and multiplied ability to metabolize the enzyme's substrates, respectively. P450 enzymes convert xenobiotics into electrophilic intermediates which are then conjugated by phase II enzymes to hydrophilic derivatives that can be excreted. According to the database of the World Guide for Drug Use and Pharmacogenomics [1], 982 drugs are CYP2D6 related, 371 drugs are substrates, over 300 drugs are inhibitors, and 18 drugs are CYP2D6 inducers.

Among healthy individuals, extensive metabolizers (EMs) account for 55.71 % of the population, whereas intermediate metabolizers (IMs) are 34.7 %, poor metabolizers (PMs) 2.28 %, and ultra-rapid metabolizers (UMs) 7.31 %. Among patients with depression, 64.76 % are EMs, 27.31 % are IMs, 4.85 % are PMs, and 3.08 are UMs (Figs. 35.1 and 35.2). Remarkable interethnic differences exist in the frequency of the PM and UM phenotypes among different societies all over the world. On average, approximately 6.28 % of the world population belongs to the PM category. Europeans (7.86 %), Polynesians (7.27 %), and Africans (6.73 %) exhibit the highest rate of PMs, whereas Orientals (0.94 %) show the lowest rate. The frequency of PMs among Middle



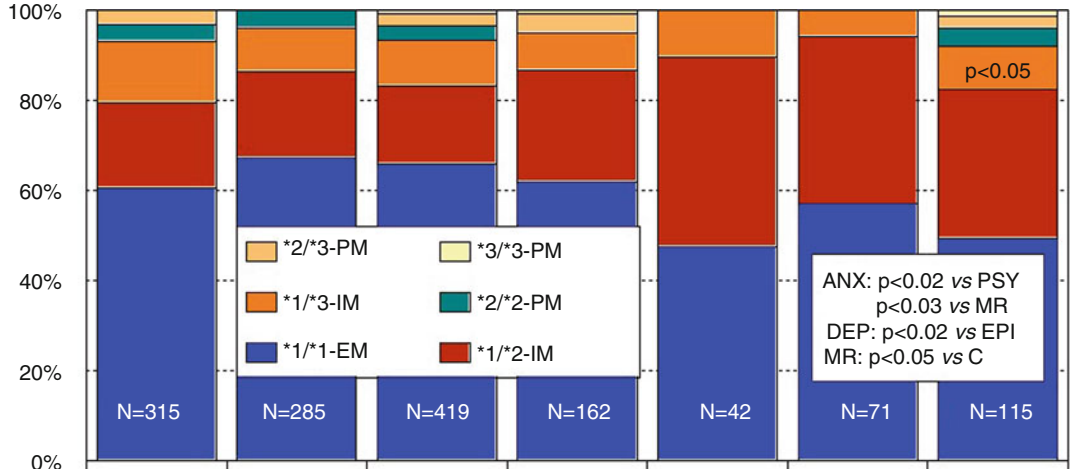
	C	ANX	DEP	PSY	ADHD	EPI	MR
UM	7,31	5,96	3,08	9,9	5	7,69	6,49
PM	2,28	2,65	4,85	3,96	0	3,85	7,79
IM	3,47	27,81	27,31	27,72	35	21,15	27,27
EM	55,71	63,58	64,76	58,42	60	67,31	58,44

Fig. 35.2 Distribution of *CYP2D6* extensive metabolizers (*EM*), intermediate metabolizers (*IM*), poor metabolizers (*PM*), and ultra-rapid metabolizers (*UM*) among patients with anxiety (*ANX*), depression (*DEP*), psychosis (*PSY*), ADHD, epilepsy (*EPI*), mental retardation (*MR*), and controls (*C*)

Eastern populations, Asians, and Americans is in the range of 2–3 %. *CYP2D6* gene duplications are relatively infrequent among Northern Europeans, but in East Africa, the frequency of alleles with duplication of *CYP2D6* is as high as 29 %. In Europe, there is a north–south gradient in the frequency of PMs (6–12 % of PMs in Southern European countries and 2–3 % PMs in northern latitudes) [1, 4, 17]. In a recent study, Bagheri et al. [41] compared the prevalence of the *CYP2D6**10, *4, and 14* alleles in an Iranian population of different ethnicities with those of other populations. The *CYP2D6**4 (G1846A) and *14 (G1758A) allelic frequencies were not detected in different ethnicities, demonstrating the absence of a significant contribution of these alleles in Iranian populations. However, the T/T, C/T, and C/C genotype frequencies of the *CYP2D6**10 allele were significantly different in all Iranian ethnic groups. The frequency of the homozygous T/T variant of the *CYP2D6**10 allele was significantly high in the Lure and low in the Kurd ethnicities. The frequency of the T/T variant of the *CYP2D6**10 allele in central Iran was the highest, while the south of Iran had the lowest frequency. About 39.3 % of subjects (24.3 % homozygous T/T *CYP2D6**10 as PMs and 15 % heterozygous C/T *CYP2D6**10 as IMs) with this genotype are candidates to experience adverse drug reactions (ADRs) with common drugs in Iran.

35.4.2 CYP2C9

CYP2C9 is a gene (50.71 kb) with nine exons mapped on 10q24. An RNA transcript of 1860 bp is mainly expressed in hepatocytes, where a protein of 55.63 kDa (490 aa) can be identified. Over 600 drugs are CYP2C9 related, 311 acting as substrates (177 are major substrates, 134 are minor substrates), 375 as inhibitors (92 weak, 181 moderate, and 102 strong inhibitors), and 41 as inducers of the CYP2C9 enzyme [1]. There are 481 *CYP2C9* SNPs. *CYP2C9-*1/*1* EMs represent 60.56 % of the healthy population; **1/*2*



	C	ANX	DEP	PSY	ADHD	EPI	MR
*3/*3-PM	0	0	0,91	1,03	0	0	1,37
*2/*3-PM	3,28	0	2,74	4,12	0	0	2,74
*2/*2-PM	3,76	4,11	3,2	0	0	0	4,1
*1/*3-IM	13,62	9,59	10,05	8,25	10,53	5,88	9,59
*1/*2-IM	18,78	19,18	17,35	24,74	42,1	37,25	32,88
*1/*1-EM	60,56	67,12	65,75	61,86	47,37	56,86	49,32

Fig. 35.3 Distribution of *CYP2C9* extensive metabolizers (*EM*), intermediate metabolizers (*IM*), and poor metabolizers (*PM*) among patients with anxiety (*ANX*), depression (*DEP*), psychosis (*PSY*), ADHD, epilepsy (*EPI*), mental retardation (*MR*), and controls (*C*)

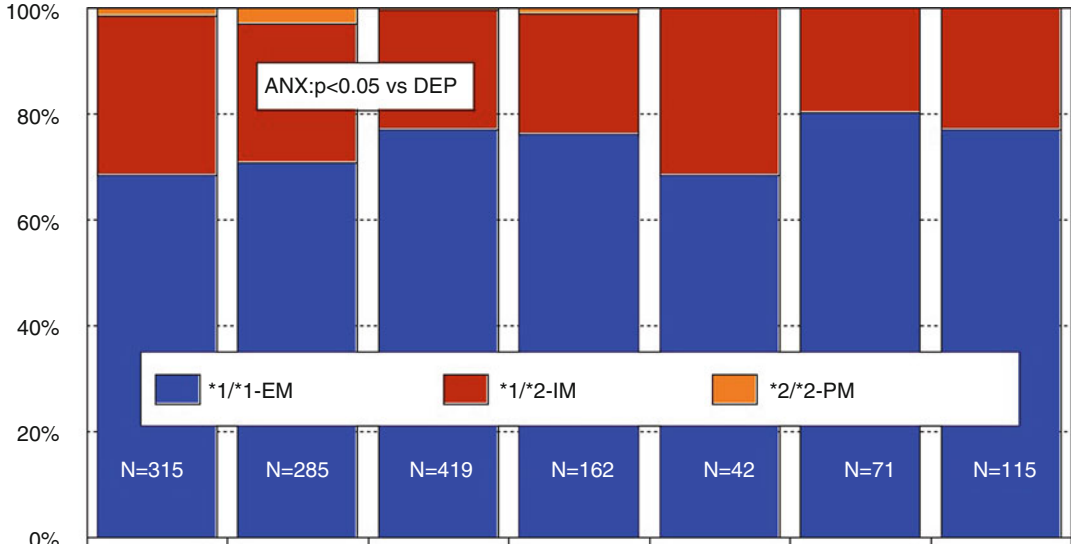
and **1/*3* IMs 18.78 % and 13.62 %, respectively (32.39 % IMs); and **2/*2*, **2/*3*, and **3/*3* PMs, 3.76 %, 3.28 %, and 0 %, respectively (7.04 % PMs). No *CYP2C9-*3/*3* cases have been found in the control population; however, in patients with depression, psychosis, and mental retardation, the frequency of this genotype is 0.91 %, 1.03 %, and 1.37 %, respectively. Significant variation has been found in *CYP2C9* genotypes among diverse brain diseases [1, 4, 17] (Fig. 35.3).

35.4.3 CYP2C19

CYP2C19 is a gene (90.21 kb) with nine exons mapped on 10q24.1q24.3. RNA transcripts of 1901 bp, 2395 bp, and 1417 bp are expressed in liver cells where a protein of 55.93 kDa (490 aa) is identified. Nearly 500 drugs are CYP2C19 related, 281 acting as substrates (151 are major substrates, 130 are minor substrates), 263 as inhibitors (72 weak, 127 moderate, and 64 strong inhibitors), and 23 as inducers of the CYP2C19 enzyme [1]. About 541 SNPs have been detected in the *CYP2C19* gene. The frequencies of the three major *CYP2C19* geno-phenotypes in the control population are *CYP2C19-*1/*1*-EMs 68.54 %, *CYP2C19-*1/*2*-IMs 30.05 %, and *CYP2C19-*2/*2*-PMs 1.41 %. Minor variation has been reported in different CNS disorders [1, 4, 17] (Fig. 35.4).

35.4.4 CYP3A4/CYP3A5

CYP3A4 is a gene (27.2 kb) with 13 exons mapped on 7q21.1. RNA transcripts of 2153 bp, 651 bp, 564 bp, 2318 bp, and 2519 bp are expressed in the intestine, liver, prostate, and other tissues where four protein variants of 57.34 kDa (503 aa), 17.29 kDa (153 aa), 40.39 kDa (353 aa), and 47.99 kDa (420 aa) are identified. The human *CYP3A* locus contains the three *CYP3A* genes (*CYP3A4*, *CYP3A5*, and *CYP3A7*) and three pseudogenes as well as a novel *CYP3A* gene termed *CYP3A43*. The gene encodes a putative protein with between 71.5 % and 75.8 % identity to the other CYP3A proteins. The predominant hepatic form is CYP3A4, but CYP3A5 contributes



	C	ANX	DEP	PSY	ADHD	EPI	MR
*2/*2-PM	1,41	2,91	0,41	1,03	0	0	0
*1/*2-IM	30,05	26,16	22,45	22,68	31,58	19,61	22,97
*1/*1-EM	68,54	70,93	77,14	76,29	68,42	80,39	77,03

Fig. 35.4 Distribution of *CYP2C19* extensive metabolizers (*EM*), intermediate metabolizers (*IM*), and poor metabolizers (*PM*) among patients with anxiety (*ANX*), depression (*DEP*), psychosis (*PSY*), ADHD, epilepsy (*EPI*), mental retardation (*MR*), and controls (*C*)

significantly to the total liver CYP3A activity. This enzyme metabolizes over 1900 drugs, 1033 acting as substrates (897 are major substrates; 136 are minor substrates), 696 as inhibitors (118 weak, 437 moderate, and 141 strong inhibitors), and 241 as inducers of the CYP3A4 enzyme [1]. About 347 SNPs have been identified in the *CYP3A4* gene (*CYP3A4*1A*: wild type), 25 of which are of clinical relevance; in a Caucasian population, 82.75 % are EMs (*CYP3A5*3/*3*), 15.88 % are IMs (*CYP3A5*1/*3*), and 1.37 % are UMs (*CYP3A5*1/*1*). Unlike other human P450s (*CYP2D6*, *CYP2C19*), there is no evidence of a "null" allele for *CYP3A4* [1].

35.4.5 *CYP* Clustering

The construction of a genetic map integrating the most prevalent *CYP2D6+CYP2C19+CYP2C9* polymorphic variants in a trigenic cluster yields 82 different haplotype-like profiles. The most frequent trigenic genotypes are **1*1-*1*1-*1*1* (25.70 %), **1*1-*1*2-*1*2* (10.66 %), **1*1-*1*2-*1*1* (10.45 %), **1*4-*1*1-*1*1* (8.09 %), **1*4-*1*2-*1*1* (4.91 %), **1*4-*1*1-*1*2* (4.65 %), and **1*1-*1*3-*1*3* (4.33 %). These 82 trigenic genotypes represent 36 different pharmacogenetic phenotypes. According to these trigenic clusters, only 26.51 % of the population show a pure 3EM phenotype, 15.29 % are 2EM1IM, 2.04 % are pure 3IM, 0 % are pure 3PM, and 0 % are 1UM2PM (the worst possible phenotype). This implies that only one-quarter of the population processes normally the drugs which are metabolized via CYP2D6, CYP2C9, and CYP2C19 (approximately 60 % of the drugs of current use) [1, 4, 17].

35.5 Transporter Genes

ABC genes, especially *ABCB1* (ATP-binding cassette, subfamily B, member 1; P-glycoprotein-1, P-gp1; multidrug resistance 1, MDR1)(7q21.12), *ABCC1* (9q31.1), *ABCG2* (white1)(21q22.3), and other genes of this family encode proteins which

are essential for drug metabolism and transport. The multidrug efflux transporters P-gp, multidrug resistance-associated protein 4 (MRP4), and breast cancer resistance protein (BCRP), located on endothelial cells lining the brain vasculature, play important roles in limiting movement of substances into and enhancing their efflux from the brain. Transporters also cooperate with phase I/phase II metabolism enzymes by eliminating drug metabolites. Their major features are their capacity to recognize drugs belonging to unrelated pharmacological classes and their redundancy, by which a single molecule can act as a substrate for different transporters. This ensures an efficient neuroprotection against xenobiotic invasions. The pharmacological induction of *ABC* gene expression is a mechanism of drug interaction, which may affect substrates of the upregulated transporter, and overexpression of MDR transporters confers resistance to anticancer agents and CNS drugs [42, 43].

ABCB1 is probably the most important drug transporter in the brain. The *ABCB1* gene maps on 7q21.12 spanning 209.39 kb (29 exons) with the structure of a P-glycoprotein and a Y-box sequence 5′-CTGATTGG-3′ in its cis-regulatory elements. Several transcripts/variants are highly expressed in the adrenal gland, blood–brain barrier (BBB), brain, kidney, liver, placenta, small intestine, and uterus, and low expression is present in many other tissues. These transcripts encode a protein (ABCB1-001: 141.48 kDa; 1280 aa ABCB1-002: 5.89 kDa; 51 aa ABCB1-003: 5.68 kDa; 48 aa ABCB1-201: 2.52 kDa; 22 aa) of the ATP-binding cassette superfamily, subfamily B (MDR/TAP) with two ATP-binding and two transmembrane (2TM) domains (2×6 segments), acting as a transport carrier and a lipid translocase of broad specificity. This is a large transmembrane protein which is an integral part of the BBB and functions as a drug-transport pump transporting a variety of drugs from the brain back into the blood. About 1630 *ABCB1* variants have been identified [1]. Of interest, *ABCB1* has approximately 116 polymorphic sites in Caucasians and 127 in African-Americans with a minor allele frequency greater than 5 %. Some of the most commonly studied variants are 1236C > T, 2677G > A/T, and 3435C > T, and the most commonly studied haplotype involves the 1236, 2677, and 3435 (TTT) SNPs and three intronic SNPs (intron 9, intron 13, intron 14) named *ABCB1*13*. There are many other *ABCB1* variants such as −129C > T (5′-UTR), 61A > G (Asn21Asp), and 1199G > A (Ser400Asn) that have been studied in vivo and in vitro. Variants of the *ABCB1* gene have been associated with a diverse number of diseases and with a great variety of drugs, natural products, and endogenous agents [1]. Over 1270 drugs have been reported to be associated with the Abcb1 transporter protein (P-gp), of which 490 are substrates, 618 are inhibitors, 182 are inducers, and 269 are additional compounds which belong to different pharmacological categories of products with potential Abcb1 interaction [1].

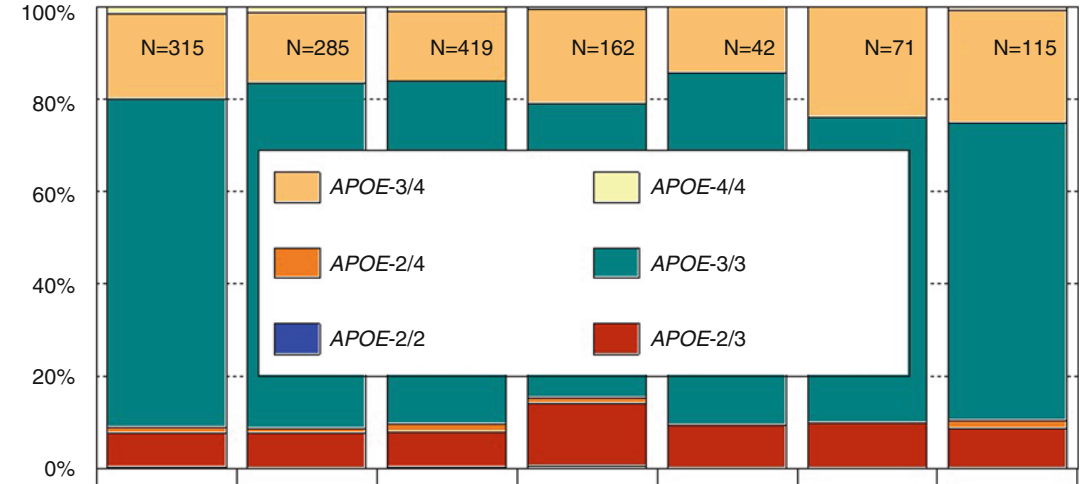
Important for CNS pharmacogenomics are transporters encoded by genes of the solute carrier superfamily (*SLC*) and solute carrier organic (*SLCO*) transporter family, responsible for the transport of multiple endogenous and exogenous compounds, including folate (*SLC19A1*), urea (*SLC14A1*, *SLC14A2*), monoamines (*SLC29A4*, *SLC22A3*), amino acids (*SLC1A5*, *SLC3A1*, *SLC7A3*, *SLC7A9*, *SLC38A1*, *SLC38A4*, *SLC38A5*, *SLC38A7*, *SLC43A2*, *SLC45A1*), nucleotides (*SLC29A2*, *SLC29A3*), fatty acids (*SLC27A1-6*), neurotransmitters (*SLC6A2* (noradrenaline transporter), *SLC6A3* (dopamine transporter), *SLC6A4* (serotonin transporter, SERT), *SLC6A5*, *SLC6A6*, *SLC6A9*, *SLC6A11*, *SLC6A12*, *SLC6A14*, *SLC6A15*, *SLC6A16*, *SLC6A17*, *SLC6A18*, *SLC6A19*), glutamate (*SLC1A6*, *SLC1A7*), and others [17]. Some organic anion transporters (OAT), which belong to the SLC22A family, are also expressed at the BBB and regulate the excretion of endogenous and exogenous organic anions and cations [44]. The transport of amino acids and di- and tripeptides is mediated by a number of different transporter families, and the bulk of oligopeptide transport is attributable to the activity of members

of the *SLC15A* superfamily (peptide transporters 1 and 2 [*SLC15A1* (PepT1) and *SLC15A2* (PepT2)], and peptide/histidine transporters 1 and 2 [*SLC15A4* (PHT1) and *SLC15A3* (PHT2)]). ABC and SLC transporters expressed at the BBB may cooperate to regulate the passage of different molecules into the brain [45]. Polymorphic variants in *ABC* and *SLC* genes may also be associated with pathogenic events in CNS disorders and drug-related safety and efficacy complications [4, 16, 17].

35.6 Pleiotropic Genes

APOE is the prototypical paradigm of a pleiotropic gene with multifaceted activities in physiological and pathological conditions [46, 47] (Fig. 35.5). *APOE* variants are associated with dementia, cardiovascular disorders, and atherosclerosis [1] (Fig. 35.5). There is an accumulation of *APOE*-4 carriers among patients with dementia, either degenerative or vascular [46] (Fig. 35.5). APOE is consistently associated with the amyloid plaque marker for Alzheimer's disease (AD). *APOE-4* may influence AD pathology interacting with APP metabolism and Aβ accumulation, enhancing hyperphosphorylation of tau protein and NFT formation, reducing choline acetyltransferase activity, increasing oxidative processes, modifying inflammation-related neuroimmunotrophic activity and glial activation, altering lipid metabolism, lipid transport, and membrane biosynthesis in sprouting and synaptic remodeling, and inducing neuronal apoptosis [22, 46–49].

An interactive effect of depressive symptoms and *APOE* ε4 allele status on cognitive decline has been shown in old age. Carriers of the *APOE*-4 allele with more depressive symptoms have faster cognitive decline than those with either depression or the *APOE*-4 allele. Current



	C	ANX	DEP	PSY	ADHD	EPI	MR
APOE-4/4	1,59	1,4	0,96	0,62	0	0	0,87
APOE-3/4	18,41	15,09	15,27	20,37	14,29	23,94	24,35
APOE-3/3	71,11	74,74	74,22	63,58	76,19	66,2	64,34
APOE-2/4	1,27	1,05	1,67	1,23	0	0	1,74
APOE-2/3	7,3	7,72	7,64	13,58	9,52	9,86	8,7
APOE-2/2	0,32	0	0,24	0,62	0	0	0

Fig. 35.5 Distribution of *APOE* genotypes in patients with anxiety (*ANX*), depression (*DEP*), psychosis (*PSY*), ADHD, epilepsy (*EPI*), mental retardation (*MR*), and controls (*C*)

depression is associated with poorer speed and memory. A negative effect of the *APOE*-4 allele on speed and memory is found in people older than 60 years of age [50] (Fig. 35.5).

35.6.1 Influence of APOE and ACE on Depression and Anxiety in Dementia

Behavioral disturbances and mood disorders are intrinsic components of dementia associated with memory disorders [51–55]. The appearance of anxiety, depression, psychotic symptoms, verbal and physical aggressiveness, agitation, wandering, and sleep disorders complicates the clinical picture of dementia and adds important problems to the therapeutics of AD and the daily management of patients as well. Under these conditions, psychotropic drugs (antidepressants, anxiolitics, hypnotics, and neuroleptics) are required, and most of these substances contribute to deteriorate cognition and psychomotor functions. Both *APOE*- and *ACE* (Angiotensin I-Converting Enzyme)-related polymorphic variants have been associated with mood disorders [56, 57] and panic disorder [58]. Gender, age, dementia severity, *APOE*-4, and general medical health appear to influence the occurrence of individual neuropsychiatric symptoms in dementia, and medical comorbidity increases the risk of agitation, irritability, disinhibition, and aberrant motor behavior [59]. A positive association between *APOE*-4 and neuropsychiatric symptoms [60] and depressive symptoms in AD has been reported [61], especially in women [62]. In other studies, no association of *APOE*-4 with behavioral dyscontrol (euphoria, disinhibition, aberrant motor behavior, and sleep and appetite disturbances), psychosis (delusions and hallucinations), mood (depression, anxiety, and apathy), and agitation (aggression and irritability) could be found [63]. Some authors did not find association of *APOE*-4 with major depression in AD [64, 65] or in patients with major depression in a community of older adults [66], but an apparent protective effect of *APOE*-2 on depressive symptoms was detected [67]. Others, in contrast, found that *APOE*-4 was associated with an earlier age of onset, but not cognitive functioning, in late-life depression [68]. *Apoe*$^{-/-}$ mice without human *ApoE* or with *APOE*-4, but not *APOE*-3, show increased measures of anxiety [69]. Differences in anxiety-related behavior have been observed between APOE-deficient C57BL/6 and wild type C57BL/6 mice, suggesting that *APOE* variants may affect emotional state [70]. Histamine H3 autoreceptor antagonists increase anxiety measures in wild-type mice, but not in ApoE$^{-/-}$ mice, and ApoE deficient mice show higher sensitivity to the anxiety-reducing effects of the H1 receptor antagonist mepyramine than wild-type mice, suggesting a role of H3-autoreceptor-mediated signaling in anxiety-like symptoms in this AD-related animal model [71].

In humans, *APOE*-4 carriers with deep white matter hyperintensities in magnetic resonance imaging (MRI) show association with depressive symptoms and vascular depression [72]. Reduced caudate nucleus volumes and genetic determinants of homocysteine metabolism accumulate in patients with psychomotor slowing and cognitive deficits [73], and older depressed subjects have persisting cognitive impairments associated with hippocampal volume reduction [74, 75]. Depressive symptoms are also associated with stroke and atherogenic lipid profile [76].

During the past two decades, antipsychotic, antianxiety and cognitive-enhancing effects have been attributed to ACE inhibitors [77, 78]. It has been reported that some ACE inhibitors (captopril, SQ29,852) display similar effects to benzodiazepines in dealing with anxiety-related behaviors in animals [79], and another ACE inhibitor (ceronapril) might be shared with neuroleptic drugs an ability to enhance latent inhibition in learning tasks [77]. One SNP (rs4291) located in the promoter region of the *ACE* gene has been recently associated with unipolar major depression [56].

A multifactorial (combination) treatment has been shown to be extremely effective in reducing anxiety and depression in patients with AD [23]. This therapeutic response was APOE and ACE dependent. At baseline, all *APOE* variants showed a similar anxiety and depression rate, except the *APOE*-4/4 carriers who differed from the rest in significantly lower rates of anxiety and depression (Fig. 35.6). Remarkable changes

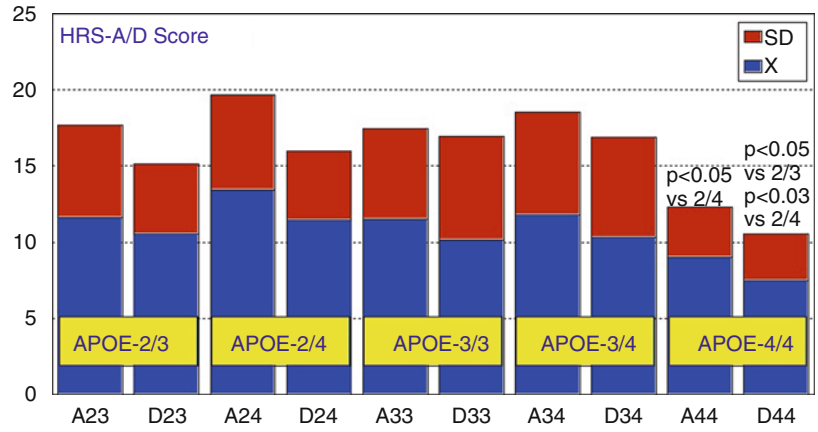


Fig. 35.6 *APOE*-related anxiety (*A*) and depression (*D*) rates (baseline levels) in patients with Alzheimer's disease

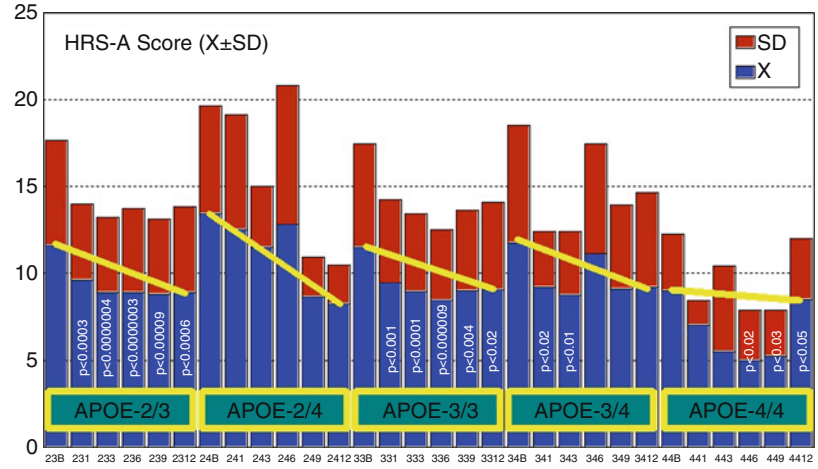


Fig. 35.7 *APOE*-related anxiety rate in patients with Alzheimer's disease treated with a combination therapy

in anxiety were found among different *APOE* genotypes (Fig. 35.7). Practically, all APOE variants responded with a significant diminution of anxiogenic symptoms, except patients with the *APOE*-4/4 genotype who only showed a slight improvement. The best responders were *APOE*-2/4> *APOE*-2/3> *APOE*-3/3> *APOE*-3/4 carriers (Fig. 35.7). The modest anxiolytic effect seen in *APOE*-4/4 patients might be due to the very low anxiety rate observed at baseline. In any case, APOE-4/4 carriers are the worst responders, with results similar to those obtained in cognitive performance [16]; however, the potential influence of *APOE* variants on anxiety and cognition in AD does not show a clear parallelism, suggesting that other more complex mechanisms are involved in the onset of anxiety in dementia. Concerning depression, all APOE genotypes improved their depressive symptoms with treatment except those with the *APOE*-4/4 genotype which worsen along the treatment period, especially after 9 months (Fig. 35.8). The best responders were *APOE*-2/4> *APOE*-2/3> *APOE*-3/3> *APOE*-3/4 carriers, and the worst responder was *APOE*-4/4 (Fig. 35.8).

Patients with each one of the three *ACE*-I/D indel variants are equally anxiogenic (Fig. 35.9) and depressive (Fig. 35.10) at baseline, and all of them favorably respond to the multifactorial protocol by gradually reducing anxiety and depressive symptoms along the 12-month treatment period (Figs. 35.9 and 35.10). The best responders are ACE-I/D followed by *ACE*-D/D and *ACE*-/I, the latter exhibiting the less significant change in anxiogenic parameters (Fig. 35.9); in *ACE*-D/D carriers the anxiolytic response is

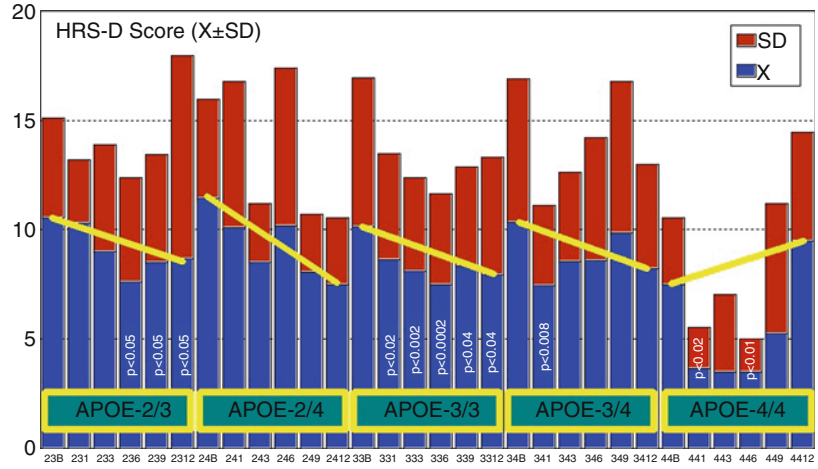
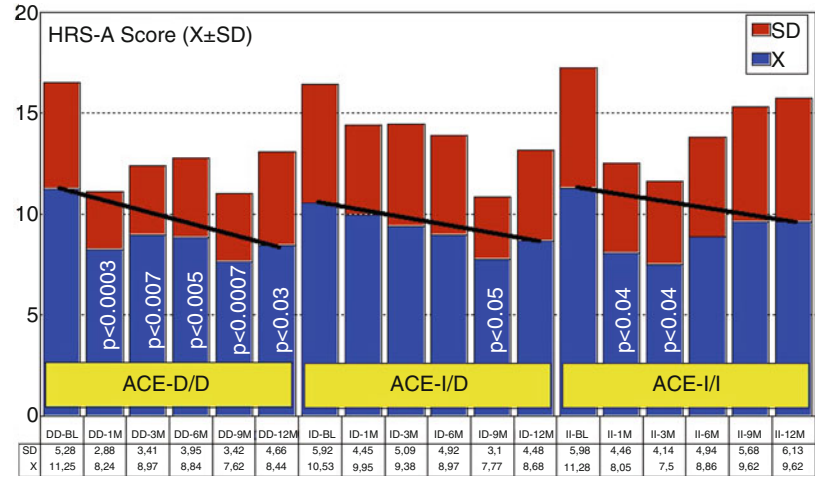
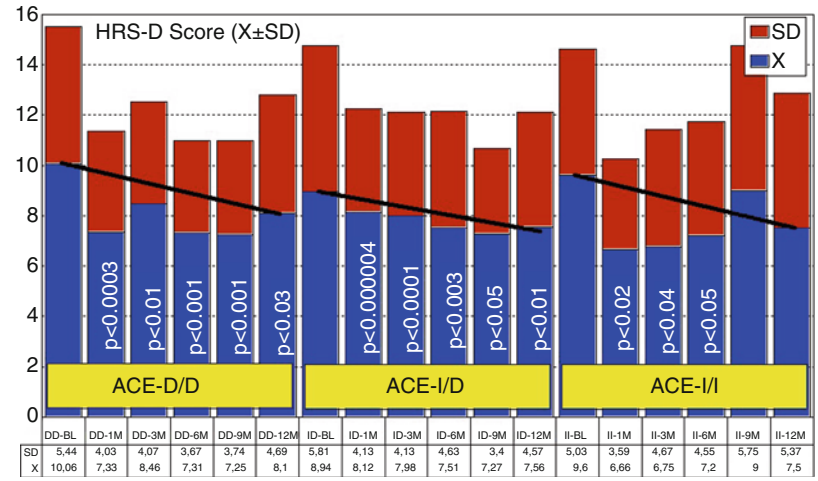


Fig. 35.8 *APOE*-related depression rate in patients with Alzheimer's disease treated with a combination therapy



	DD-BL	DD-1M	DD-3M	DD-6M	DD-9M	DD-12M	ID-BL	ID-1M	ID-3M	ID-6M	ID-9M	ID-12M	II-BL	II-1M	II-3M	II-6M	II-9M	II-12M
SD	5,28	2,88	3,41	3,95	3,42	4,66	5,92	4,45	5,09	4,92	3,1	4,48	5,98	4,46	4,14	4,94	5,68	6,13
X	11,25	8,24	8,97	8,84	7,62	8,44	10,53	9,95	9,38	8,97	7,77	8,68	11,28	8,05	7,5	8,86	9,62	9,62

Fig. 35.9 *ACE*-related anxiety rate in patients with Alzheimer's disease treated with a combination therapy



	DD-BL	DD-1M	DD-3M	DD-6M	DD-9M	DD-12M	ID-BL	ID-1M	ID-3M	ID-6M	ID-9M	ID-12M	II-BL	II-1M	II-3M	II-6M	II-9M	II-12M
SD	5,44	4,03	4,07	3,67	3,74	4,69	5,81	4,13	4,13	4,63	3,4	4,57	5,03	3,59	4,67	4,55	5,75	5,37
X	10,06	7,33	8,46	7,31	7,25	8,1	8,94	8,12	7,98	7,51	7,27	7,56	9,6	6,66	6,75	7,2	9	7,5

Fig. 35.10 *ACE*-related depression rate in patients with Alzheimer's disease treated with a combination therapy

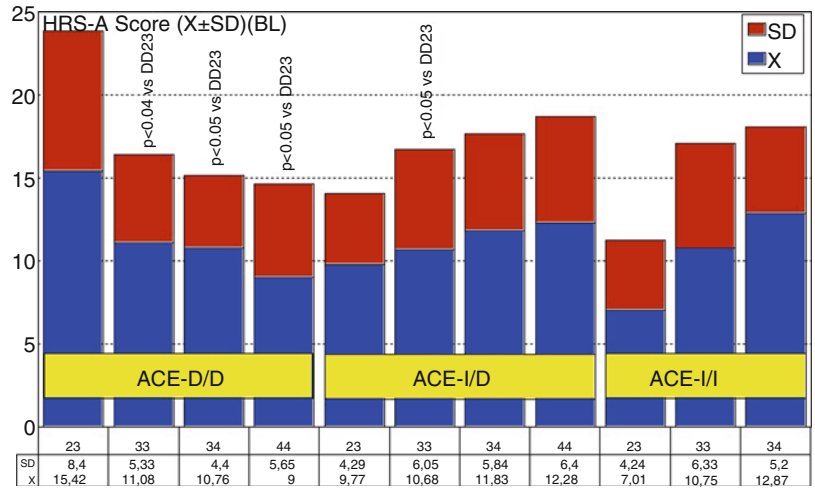


Fig. 35.11 *APOE* + *ACE*-related anxiety rate (baseline levels) in patients with Alzheimer's disease

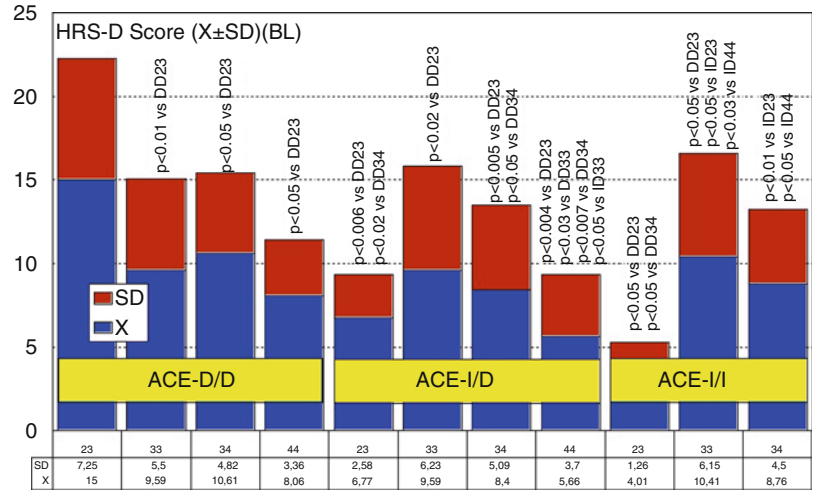


Fig. 35.12 *APOE* + *ACE*-related depression rate (baseline levels) in patients with Alzheimer's disease

faster and more sustainable during the treatment period than in the other genotypes, whereas in *ACE*-I/D the response is gradual reaching significant values after 9 months of treatment; in contrast, *ACE*-I/I patients show a very positive response during the first trimester of treatment with an apparent relapse of anxiogenic symptomatology thereafter (Fig. 35.9). This differential *ACE*-related anxiety pattern might suggest some influence of *ACE*-I/D variants on mood disorders in AD. Depressive symptoms are also similarly improved in all *ACE*-I/D variants. The best responders are the heterozygous *ACE*-I/D followed by the homozygous *ACE*-D/D and *ACE*-I/I (Fig. 35.10). Comparatively, the worst responders among *ACE*-I/D variants are carriers of the *ACE*-I/I genotype which are also the poorer responders in anxiety and cognition [23].

35.6.2 Effect of APOE–ACE Interactions on Anxiety and Depression

The combination of *APOE* and *ACE* polymorphic variants in bigenic clusters yields different anxiety and depression patterns at baseline (Figs. 35.11 and 35.12) and after 1-year treatment (Figs. 35.13 and 35.14). The most anxiogenic patients at baseline are those with the *DD23*,

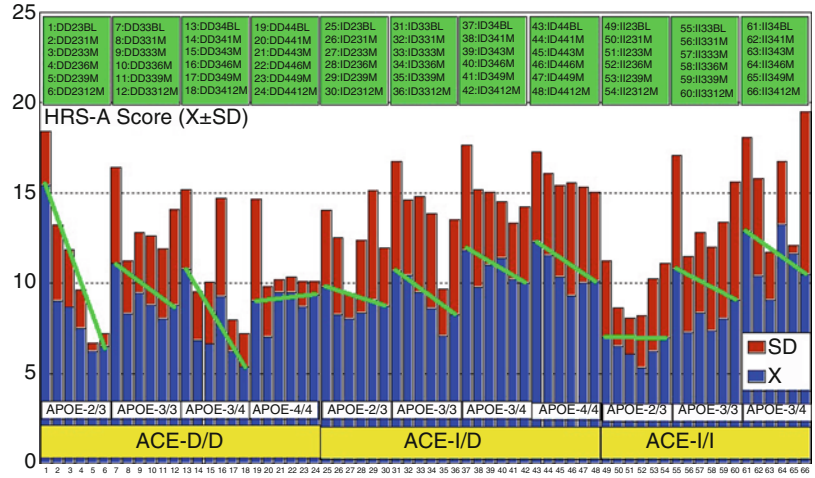


Fig. 35.13 *ACE* + *APOE*-related antianxiety effect of a multifactorial treatment in patients with Alzheimer's disease

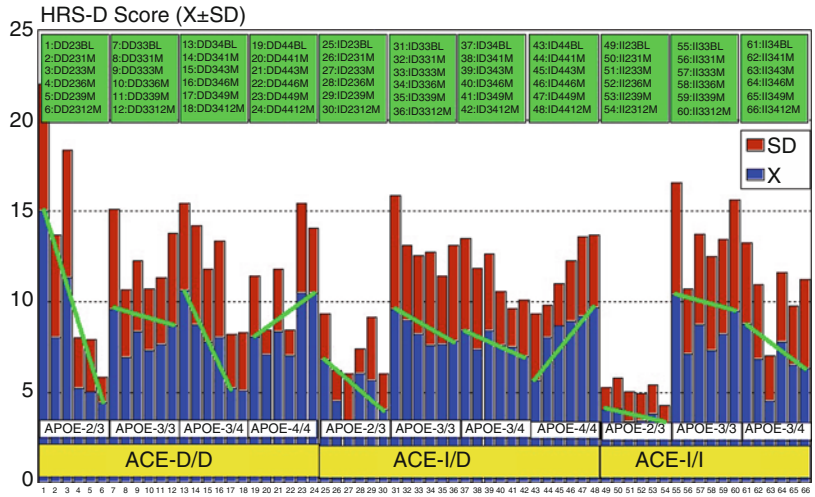


Fig. 35.14 *ACE* + *APOE*-related antidepressant effect of a multifactorial treatment in patients with Alzheimer's disease

ID44, and *II34* genotypes, and the less anxiogenic patients are those harboring the *II23*, *DD44*, and *ID23* genotypes (Fig. 35.11). The most depressive clusters at baseline are those harboring the *DD23*, *ID33*, and *II33* genotypes, with a clear accumulation of *APOE*-3/3 carriers in these groups, and the less depressive clusters are those represented by carriers of the *II23*, *ID44*, and *ID23* genotypes (Fig. 35.12). All bigenic clusters show a positive anxiolytic response to the multifactorial protocol, except *DD44* which exhibits the worst response by large (Fig. 35.13). The sequence of good anxiogenic responders from better to worse is the following: *ID33*> *ID44*> *DD34*> *DD33*> *ID34*> *II33*> *ID23*> *II23*=*II34* (Fig. 35.13). In a similar fashion, depressive symptoms gradually improved in most bigenic clusters except in *DD44* and *ID44* subjects in whom the depressive symptomatology tended to deteriorate. The best responders in depression were *DD34*> *ID33*> *DD23*> *II23*> *ID34*> *II34*> *ID23*> *DD33*> *II33*, and the worst responders were *ID44* and *DD44* (Fig. 35.14). As in the case of cognition, *DD44* patients represent the poorest responders in anxiety and depression symptoms after 1 year of treatment, clearly

indicating that the association of the *APOE*-4/4 and *ACE*-D/D genotypes plays a severe deleterious role on mental performance, at least in cognition and mood [23].

35.7 Pharmacogenomics of Antidepressants

35.7.1 Selective Serotonin Reuptake Inhibitors (SSRI) and Selective Serotonin and Norepinephrine Reuptake Inhibitors (SSNRI) (Table 35.2)

35.7.1.1 Citalopram

Citalopram is an SSRI which selectively inhibits serotonin reuptake in the presynaptic neurons and has minimal effects on norepinephrine or dopamine. Pathogenic genes potentially associated with citalopram include *ABCB1*, *BDNF*, *CREB1*, *CRHR1*, *CRHR2*, *FKBP5*, *GRIA3*, *GRIK2*, *GRIK4*, *GSK3B*, *HTR1A*, *HTR1B*, *HTR2A*, *MAOA*, *SLC6A4*, *TPH1*, and *TPH2*. Citalopram-related mechanistic genes are *ADRs*, *CHRMs*, *DRDs*, *FKBP5*, *GABRs*, *GRIK4*, *HRHs*, *HTR1A*, *HTR1B*, *HTR1D*, *HTR2A*, *SLC6A4*, and *TPH1*. Citalopram is a major substrate of ABCC1, COMT, CYP2C19, and CYP3A4/CYP3A5 and a minor substrate of CYP2D6. This antidepressant acts as a weak inhibitor of ABCB1, CYP1A2, CYP2B6, CYP2C19, CYP2D6, MAOA, and MAOB, and it is transported by ABCB1 and SLC6A4 [1] (Table 35.2).

35.7.1.2 Desvenlafaxine

Desvenlafaxine is a potent SSNRI, acting as a substrate of CYP3A4 and UGTs and a weak inhibitor of CYP2D6, SLC6A2, and SLC6A4. ABCB1, SLC6A2, and SLC6A4 proteins are current transporters of venlafaxine [1] (Table 35.2).

35.7.1.3 Duloxetine

Duloxetine is an SSNRI and a weak inhibitor of dopamine reuptake, acting as a major substrate of CYP1A2 and CYP2D6 and a moderate inhibitor of ABCB1, CYP1A2, CYP2B6, CYP2C19, CYP2D6, CYP3A4/CYP3A5, SLC6A2, and SLC6A4. Duloxetine is transported by ABCB1, SLC6A2, and SLC6A4 proteins [1] (Table 35.2).

35.7.1.4 Escitalopram

Escitalopram is an SSRI with little effect on norepinephrine or dopamine reuptake and very low affinity for 5-$HT_{1–7}$, α – and β-adrenergic, $D_{1–5}$, $H_{1–3}$, $M_{1–5}$, and benzodiazepine receptors. This drug is a major substrate of ABCB1, CYP2C19, CYP2D6, and CYP3A4 and a minor substrate of CYP2C9. Escitalopram is also a moderate inhibitor of ABCB1 and CYP2D6 and a weak inhibitor of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and SLC6A4. Its major transporters are ABCB1 and SLC6A4 proteins [1] (Table 35.2)

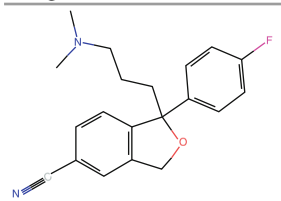
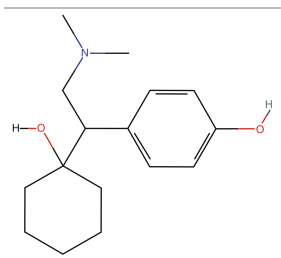
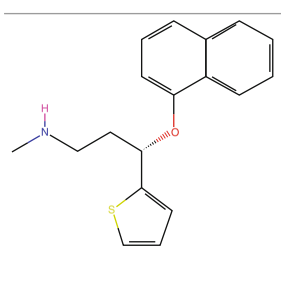
35.7.1.5 Fluoxetine

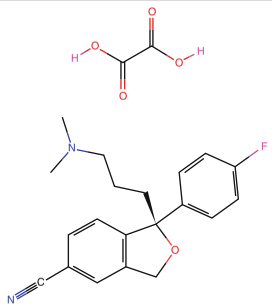
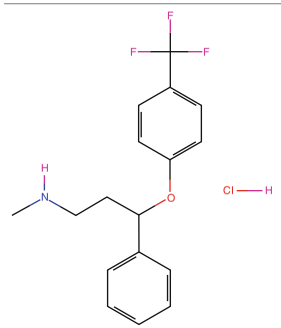
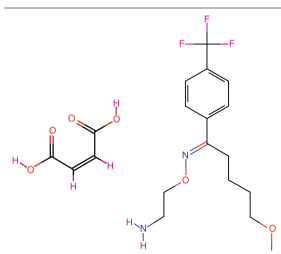
Fluoxetine is a potent SSRI. Different pathogenic (*ABCB1*, *BDNF*, *CREB1*, *FKBP5*, *GSK3B*, *HTR1A*, *HTR2A*, *MAOA*, *NR3C1*, *NTRK2*, *SLC6A4*, *TBX21*, *TPH1*, *TPH2*) and mechanistic genes (*BDNF*, *CHRMs*, *CREB1*, *DRD3*, *GSK3B*, *HTRs*, *MAOA*, *SLC6A4*, *TPH2)* and their products are involved in its therapeutic effect and mechanism of action, respectively. Fluoxetine is a major substrate of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/CYP3A5; a minor substrate of CYP2E1 and POR; a strong inhibitor of CYP2D6; a moderate inhibitor of CYP1A2 and CYP3A4/CYP3A5; and a weak inhibitor of ABCB1, CYP2B6, CYP2C9, CYP2C19, MAOA, and SLC6A4. ABCB1, KCNH2, and SLC6A4 proteins are major fluoxetine transporters [1] (Table 35.2).

35.7.1.6 Fluvoxamine

Fluvoxamine is an SSRI; a major substrate of CYP1A2, CYP2C19, CYP2D6, and CYP3A4; a strong inhibitor of CYP1A2; a moderate inhibitor of CYP2C9, CYP2C19, and CYP2D6; and a weak inhibitor of ABCB1, CYP2B6, CYP3A4, MAOA, and SLC6A4. Fluvoxamine is transported by ABCB1, KCNH2, and SCL6A4 proteins [1] (Table 35.2).

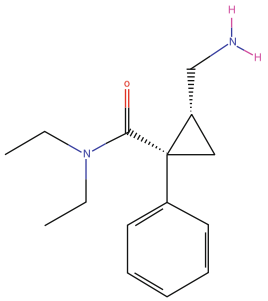
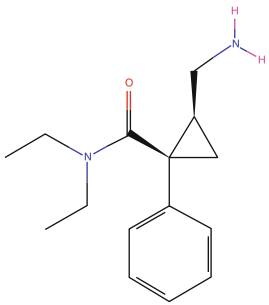
Table 35.2 Pharmacological profile and pharmacogenetics of selective serotonin reuptake inhibitors (SSRI) and selective serotonin and norepinephrine reuptake inhibitors (SSNRI)

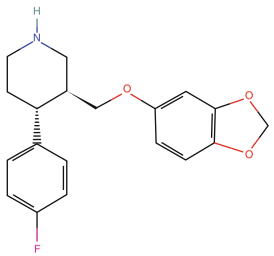
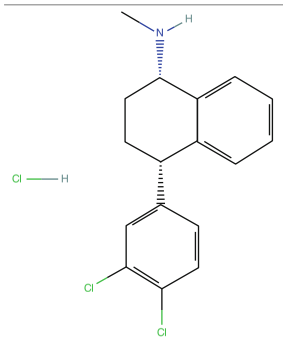
Drug	Properties	Pharmacogenetics
	*Name*: citalopram hydrobromide; Nitalapram; Cipram; Celexa; Celapram; Ciprapine *IUPAC name*: 1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile *Molecular formula*: $C_{20}H_{21}FN_2O$ *Molecular weight*: 324.391943 g/mol *Category*: selective serotonin reuptake inhibitors *Mechanism*: selectively inhibits serotonin reuptake in the presynaptic neurons and has minimal effects on norepinephrine or dopamine *Effect*: serotonin uptake inhibition; serotonergic neurotransmission enhancer; antidepressive activity, agitation reduction, and antianxiety activity	*Pathogenic genes*: *ABCB1*, *BDNF*, *CREB1*, *CRHR1*, *CRHR2*, *FKBP5*, *GRIA3*, *GRIK2*, *GRIK4*, *GSK3B*, *HTR1A*, *HTR1B*, *HTR2A*, *MAOA*, *SLC6A4*, *TPH1*, and *TPH2* *Mechanistic genes*: *ADRs*, *CHRMs*, *DRDs*, *FKBP5*, *GABRs*, *GRIK4*, *HRHs*, *HTR1A*, *HTR1B*, *HTR1D*, *HTR2A*, *SLC6A4*, and *TPH1* *Metabolic genes*: *Substrate: ABCC1*, *COMT*, *CYP2C19* (major), *CYP2D6* (minor), *CYP3A4* (major), and *CYP3A5* *Inhibitor*: *ABCB1*, *CYP1A2* (weak), *CYP2B6* (weak), *CYP2C19* (weak), *CYP2D6* (weak), *MAOA*, and *MAOB* *Transporter genes: ABCB1* and *SLC6A4* *Pleiotropic genes*: *BDNF*
	*Name*: desvenlafaxine; O-desmethylvenlafaxine; 93413-62-8; 4-(2-(dimethylamino)-1-(1-hydroxycyclohexyl)ethyl)phenol; 4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol; desvenlafaxine (INN) *IUPAC name*: 4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol *Molecular formula*: $C_{16}H_{25}NO_2$ *Molecular weight*: 263.3752 g/mol *Category*: selective serotonin and norepinephrine reuptake inhibitors *Mechanism*: it is a potent and selective serotonin and norepinephrine reuptake inhibitor *Effect*: serotonin uptake inhibition; norepinephrine uptake inhibition; antidepressant activity	*Pathogenic genes*: *ABCB1* and *SLC6A4* *Mechanistic genes*: *HTR1A*, *SLC6A2*, *SLC6A3*, and *SLC6A4* *Metabolic genes*: *Substrate: CYP3A4* (minor) and *UGTs* *Inhibitor*: *CYP2D6* (weak), *SLC6A2* and *SLC6A4* *Transporter genes: ABCB1*, *SLC6A2* and *SLC6A4*
	*Name*: duloxetine hydrochloride; 136434-34-9; duloxetine HCl; cymbalta; (S)-N-methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine hydrochloride; (S)-duloxetine HCl *IUPAC name*: methyl[(3S)-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propyl]amine *Molecular formula*: $C_{18}H_{20}ClNOS$ *Molecular weight*: 333.8755 g/mol *Category*: selective serotonin and norepinephrine reuptake inhibitors *Mechanism*: it is a selective serotonin and norepinephrine reuptake inhibitor and a weak inhibitor of dopamine reuptake *Effect*: antidepressant activity, antianxiety activity, serotonin uptake inhibition, norepinephrine uptake inhibition, anti-fibromyalgia agent, analgesic activity, urinary continence improvement	*Pathogenic genes*: *ABCB1* and *SLC6A4* *Mechanistic genes*: *COMT*, *HTR1A*, *SLC6A2*, and *SLC6A4* *Metabolic genes*: *Substrate: CYP1A2* (major) and *CYP2D6* (major) *Inhibitor*: *ABCB1*,*CYP1A2* (moderate), *CYP2B6* (moderate), *CYP2C19* (moderate), *CYP2D6* (moderate), *CYP3A4/CYP3A5* (moderate), *SLC6A2*, and *SLC6A4* *Transporter genes: ABCB1*, *SLC6A2*, and *SLC6A4*

	*Name*: escitalopram oxalate, Lexapro, Cipralex, 219861-08-2, UNII-5U85DBW7LO, Esertia *IUPAC name*: (1S)-1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile *Molecular formula*: $C_{22}H_{23}FN_2O_5$ *Molecular weight*: 414.426823 g/mol *Category*: selective serotonin reuptake inhibitors *Mechanism*: inhibits the reuptake of serotonin with little to no effect on norepinephrine or dopamine reuptake. It has very low affinity for 5-HT_{1-7}, α – , and β-adrenergic, D_{1-5}, H_{1-3}, M_{1-5}, and benzodiazepine receptors *Effect*: serotonin uptake inhibition, serotonergic neurotransmission enhancer, antidepressive activity, antianxiety activity	*Pathogenic genes*: *ABCB1*, *CREB1*, *FKBP5*, *GRIA3*, *GRIK2*, *GRIK4*, *NR3C1*, and *SLC6A4* *Mechanistic genes*: *ADRAs*, *ADRBs*, *DDC*, *DRDs*, *CHRMs*, *GABRs*, *HRHs*, *HTRs*, and *IL6* *Metabolic genes*: *Substrate: ABCB1*, *CYP2C9* (minor), *CYP2C19* (major), *CYP2D6* (major), and *CYP3A4* (major) *Inhibitor*: *ABCB1*, *CYP1A2* (weak), *CYP2C9* (weak), *CYP2C19* (weak), *CYP2D6* (moderate), *CYP2E1* (weak), *CYP3A4* (weak), and *SLC6A4* *Transporter genes*: *ABCB1* and *SLC6A4* *Pleiotropic genes*: *IL6*
	*Name*: fluoxetine hydrochloride; Prozac; fluoxetine HCl; 59333-67-4; Sarafem; Fluctin *IUPAC name*: methyl({3-phenyl-3-[4-(trifluoromethyl)phenoxy]propyl})amine *Molecular formula*: $C_{17}H_{19}ClF_3NO$ *Molecular weight*: 345.78707 g/mol *Category*: selective serotonin reuptake inhibitors *Mechanism*: potentiates serotonergic activity in CNS resulting from its inhibition of CNS neuronal reuptake of serotonin *Effect*: serotonin uptake inhibition; serotonin agent; antidepressive activity; anti-obsessive activity; antianxiety activity; anorexigenic effects	*Pathogenic genes*: *ABCB1*, *BDNF*, *CREB1*, *FKBP5*, *GSK3B*, *HTR1A*, *HTR2A*, *MAOA*, *NR3C1*, *NTRK2*, *SLC6A4*, *TBX21*, *TPH1*, and *TPH2* *Mechanistic genes*: *BDNF*, *CHRMs*, *CREB1*, *DRD3*, *GSK3B*, *HTRs*, *MAOA*, *SLC6A4*, and *TPH2* *Metabolic genes*: *Substrate: CYP1A2* (major), *CYP2B6* (major), *CYP2C8* (major), *CYP2C9* (major), *CYP2C19* (major), *CYP2D6* (major), *CYP2E1* (minor), *CYP3A4/CYP3A5* (major), and *POR* *Inhibitor*: *ABCB1*, *CYP1A2* (moderate), *CYP2B6* (weak), *CYP2C8* (moderate), *CYP2C9* (weak), *CYP2C19* (moderate), *CYP2D6* (strong), *CYP3A4* (moderate), *MAOA*, and *SLC6A4* *Transporter genes*: *ABCB1*, *KCNH2*, *SLC6A4* *Pleiotropic genes*: *DRD3*, *FABP1*, *HTR2A*, *IFNA1*, *NTRK2*, *PDE5A*, and *TPH1*
	*Name*: fluvoxamine maleate; Luvox; 61718-82-9; Fevarin; Faverin; Floxyfral *IUPAC name*: (2-aminoethoxy)({5-methoxy-1-[4-(trifluoromethyl)phenyl] pentylidene})amine *Molecular formula*: $C_{19}H_{25}F_3N_2O_6$ *Molecular weight*: 434.40681 g/mol *Category*: selective serotonin reuptake inhibitors *Mechanism*: inhibits CNS neuron serotonin uptake *Effect*: antidepressive activity; antianxiety activity; serotonin uptake inhibition	*Pathogenic genes*: *BDNF*, *HTR2A*, *SIGMAR1*, and *TPH1* *Mechanistic genes*: *BDNF*, *HTRs*, *SLC6A4*, and *SIGMAR1* *Metabolic genes*: *Substrate: CYP1A2* (major), *CYP2C19* (major), *CYP2D6* (major), and *CYP3A4* (major) *Inhibitor*: *ABCB1*, *CYP1A2* (strong), *CYP2B6* (weak), *CYP2C9* (moderate), *CYP2C19* (moderate), *CYP2D6* (moderate), *CYP3A4* (weak), *MAOA*, and *SLC6A4* *Transporter genes*: *ABCB1*, *KCNH2*, and *SCL6A4* *Pleiotropic genes*: *CREB1* and *TPH1*

(continued)

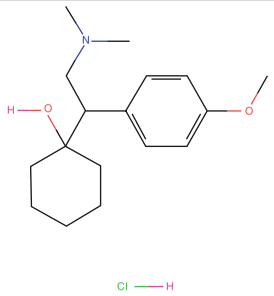
Table 35.2 (continued)

Drug	Properties	Pharmacogenetics
	*Name*: levomilnacipran; UNII-UGM0326TXX; UGM0326TXX; Fetzima; (1S,2R)-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropane-1-carboxamide; F2695 *IUPAC name*: (1S,2R)-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropane-1-carboxamide *Molecular formula*: $C_{15}H_{22}N_2O$ *Molecular weight*: 246.34798 g/mol *Category*: selective serotonin and norepinephrine reuptake inhibitors *Mechanism*: it is a potent and selective serotonin and norepinephrine reuptake inhibitor *Effect*: serotonin uptake inhibition; norepinephrine uptake inhibition; antidepressant activity	*Pathogenic genes*: *SLC6A4* *Mechanistic genes*: *HCRTR1*, *HCRTR2*, *HDC*, *HRH1*, *SLC6A2*, and *SLC6A4* *Metabolic genes*: *Substrate: ABCB1* (minor), *CYP2C19* (minor), *CYP2C8* (minor), *CYP2D6* (minor), *CYP2J2* (minor), and *CYP3A4* (major) *Transporter genes: SLC6A2* and *SLC6A4*
	*Name*: milnacipran hydrochloride; Toledomin; midalcipran; Ixel; Savella; milnacipran *IUPAC name*: (1R,2S)-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropane-1-carboxamide *Molecular formula*: $C_{15}H_{22}N_2O$ *Molecular weight*: 246.34798 g/mol *Category*: selective serotonin and norepinephrine reuptake inhibitors *Mechanism*: it is a potent inhibitor of neuronal norepinephrine and serotonin reuptake. It inhibits norepinephrine uptake with approximately threefold higher potency in vitro than serotonin without directly affecting the uptake of dopamine or other neurotransmitters *Effect*: analgesic action; anti-fibromyalgia action; serotonin uptake inhibition; adrenergic uptake inhibition; antidepressant activity	*Pathogenic genes*: *BDNF* *Mechanistic genes*: *ADRA2A*, *BDNF*, *SLC6A2*, and *SLC6A4* *Metabolic genes*: *Substrate: COMT*, *CYP1A2* (minor), *CYP2A6* (minor), *CYP2B6* (minor), *CYP2C8*, *CYP2C9* (minor), *CYP2C19* (minor), *CYP2D6* (minor), *CYP2E1* (minor), *CYP3A4/CYP3A5* (minor), and *UGTs* *Inhibitor*: *CYP3A4/CYP3A5* (moderate) *Inducer*: *CYP1A2*, *CYP2B6*, *CYP2C8*, *CYP2C9*, *CYP2C19*, and *CYP3A4/CYP3A5* *Transporter genes*: *SLC6A2* and *SLC6A4*

	*Name*: paroxetine, Paxil, Aropax, Paxil CR, Seroxat, Pexeva *IUPAC name*: (3S,4R)-3-[(2H-1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine *Molecular formula*: $C_{19}H_{20}FNO_3$ *Molecular weight*: 329.365403 g/mol *Category*: selective serotonin reuptake inhibitors *Mechanism*: it is an SSRI. Presumably acts by inhibiting serotonin reuptake from brain synapse stimulating its activity in the brain *Effect*: serotonin uptake inhibition; serotonergic neurotransmission enhancer; antidepressant activity; antianxiety activity; anti-obsessive activity	*Pathogenic genes*: *ABCB1*, *CREB1*, *HTR1B*, *HTR2A*, *HTR3B*, *MAOA*, *SLC6A3*, *SLC6A4*, *TNF*, *TPH1*, and *TPH2* *Mechanistic genes*: *CREB1*, *HTR2A*, *HTR3A*, *SLC6A4*, *STAT3*, and *TNF* *Metabolic genes*: *Substrate*: *ABCB1*, *COMT*, *CYP1A2* (minor), *CYP2C19* (minor), *CYP2D6* (major), *CYP3A4* (major), *MAOA*, and *MAOB* *Inhibitor*: *ABCB1*, *CYP1A2* (weak), *CYP2B6* (moderate), *CYP2C9* (weak), *CYP2C19* (weak), *CYP2D6* (strong), *CYP3A4* (weak), *SLC6A3*, and *SLC6A4* *Transporter genes*: *ABCB1*, *SLC6A3*, and *SLC6A4* *Pleiotropic genes*: *HTR1D*, *HTR3C*, *HTR6*, *HTT*, *TPH1*, and *TPH2*
	*Name*: sertraline hydrochloride; 79559-97-0; sertraline HCl; Zoloft; Lustral; Gladem *IUPAC name*: (1S,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine *Molecular formula*: $C_{17}H_{18}Cl_3N$ *Molecular weight*: 342.69052 g/mol *Category*: selective serotonin reuptake inhibitors *Mechanism*: it has selective inhibitory effects on presynaptic serotonin reuptake and only very weak effects on norepinephrine and dopamine neuronal uptake *Effect*: serotonin uptake inhibition; serotonergic neurotransmission enhancer; antidepressant activity; antianxiety activity; anti-obsessive activity	*Pathogenic genes*: *ABCB1*, *CREB1*, *GNB3*, *HTR1B*, *MAOA*, *SIGMAR1*, *SLC6A4*, *TNF*, *TPH1*, and *TPH2* *Mechanistic genes*: *HTR1B*, *HTR1D*, *SIGMAR1*, *SLC6A2*, *SLC6A3*, *SLC6A4*, and *TNF* *Metabolic genes*: *Substrate*: *CYP2A6*, *CYP2B6* (minor), *CYP2C9* (minor), *CYP2C19* (major), *CYP2D6* (minor), *CYP3A4* (minor), *MAOA*, *MAOB*, *UGT1A1*, and *UGT2B7* *Inhibitor*: *ABCB1*, *ACHE*, *CYP1A1*, *CYP1A2* (weak), *CYP2B6* (moderate), *CYP2C8* (weak), *CYP2C9* (weak), *CYP2C19* (moderate), *CYP2D6* (moderate), *CYP3A4* (moderate), and *SLC6A4* *Transporter genes*: *ABCB1*, *SLC6A2*, *SLC6A3*, and *SLC6A4* *Pleiotropic genes*: *FABP1*, *FOS*, *GNB3*, *TPH1*, and *TPH2*

(continued)

Table 35.2 (continued)

Drug	Properties	Pharmacogenetics
	*Name*: venlafaxine hydrochloride; 99300-78-4; venlafaxine HCl; Effexor XR; Dobupal; Trevilor *IUPAC name*: 1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexan-1-ol *Molecular formula*: $C_{17}H_{28}ClNO_2$ *Molecular weight*: 313.86272 g/mol *Category*: selective serotonin and norepinephrine reuptake inhibitors *Mechanism*: it and its active metabolite, O-desmethylvenlafaxine (ODV), are potent inhibitors of neuronal serotonin and norepinephrine reuptake and weak inhibitors of dopamine reuptake *Effect*: serotonin uptake inhibition; norepinephrine uptake inhibition; antidepressant activity; antianxiety activity; analgesic effects	*Pathogenic genes*: *ABCB1*, *BDNF*, *CREB1*, *FKBP5*, *HTR1A*, *HTR2A*, *NR3C1*, *SLC6A3*, *SLC6A4*, and *TPH2* *Mechanistic genes*: *BDNF* and *FKBP5* *Metabolic genes*: *Substrate*: *ABCB1*, *CYP2C9* (minor), *CYP2C19* (minor), *CYP2D6* (major), and *CYP3A4* (major) *Inhibitor*: *ABCB1*, *CYP1A2* (weak), *CYP2B6* (weak), *CYP2D6* (weak), *CYP3A4* (weak), *SLC6A2*, *SLC6A3*, and *SLC6A4* *Transporter genes*: *ABCB1*, *ABCC1*, *ABCG2*, *SLC6A2*, *SLC6A3*, and *SLC6A4* *Pleiotropic genes*: *DRD2*, *HTR2A*, and *TPH2*

ABCB1 ATP-binding cassette, subfamily B (MDR/TAP), member 1, *ABCC1* ATP-binding cassette, subfamily C (CFTR/MRP), member 1, *ABCG2* ATP-binding cassette, subfamily G (WHITE), member 2 (junior blood group), *ACHE* acetylcholinesterase (Yt blood group), *ADRA2A* adrenoceptor alpha 2A, *ADRAs* adrenoceptors alpha, *ADRBs* adrenoceptors beta, *ADRs* adrenoceptors, *BDNF* brain-derived neurotrophic factor, *CHRMs* cholinergic receptors; muscarinic type, *COMT* catechol-O-methyltransferase, *CREB1* cAMP-responsive element-binding protein 1, *CRHR1* corticotropin-releasing hormone receptor 1, *CRHR2* corticotropin-releasing hormone receptor 2, *CYP1A1* cytochrome P450, family 1, subfamily A, polypeptide 1, *CYP1A2* cytochrome P450, family 1, subfamily A, polypeptide 2, *CYP2A6* cytochrome P450, family 2, subfamily A, polypeptide 6, *CYP2B6* cytochrome P450, family 2, subfamily B, polypeptide 6, *CYP2C8* cytochrome P450, family 2, subfamily C, polypeptide 8, *CYP2C9* cytochrome P450, family 2, subfamily C, polypeptide 9, *CYP2C19* cytochrome P450, family 2, subfamily C, polypeptide 19, *CYP2D6* cytochrome P450, family 2, subfamily D, polypeptide 6, *CYP2E1* cytochrome P450, family 2, subfamily E, polypeptide 1, *CYP2J2* cytochrome P450, family 2, subfamily J, polypeptide 2, *CYP3A4* cytochrome P450, family 3, subfamily A, polypeptide 4, *CYP3A4/CYP3A5* cytochrome P450, family 3, subfamily A, polypeptide 4/polypeptide 5, *DDC* dopa decarboxylase (aromatic L-amino acid decarboxylase), *DRD2* dopamine receptor D2, *DRD3* dopamine receptor D3, *DRDs* dopamine receptors, *FABP1* fatty acid-binding protein 1, liver, *FKBP5* FK506-binding protein 5, *FOS* FBJ osteosarcoma oncogene, *GABRs* gamma-aminobutyric acid (GABA) receptors, *GNB3* guanine nucleotide-binding protein (G protein), beta polypeptide 3, *GRIA3* glutamate receptor, ionotropic, AMPA 3, *GRIK2* glutamate receptor, ionotropic, kainate 2, *GRIK4* glutamate receptor, ionotropic, kainate 4, *GSK3B* glycogen synthase kinase 3 beta, *HCRTR1* hypocretin (orexin) receptor 1, *HCRTR2* hypocretin (orexin) receptor 2, *HDC* histidine decarboxylase, *HRH1* histamine receptor H1, *HRHs* histamine receptors, *HTR1A* 5-hydroxytryptamine (serotonin) receptor 1A, G protein coupled, *HTR1B* 5-hydroxytryptamine (serotonin) receptor 1B, G protein coupled, *HTR1D* 5-hydroxytryptamine (serotonin) receptor 1D, G protein coupled, *HTR2A* 5-hydroxytryptamine (serotonin) receptor 2A, G protein coupled, *HTR3A* 5-hydroxytryptamine (serotonin) receptor 3A, ionotropic, *HTR3B* 5-hydroxytryptamine (serotonin) receptor 3B, ionotropic, *HTR3C* 5-hydroxytryptamine (serotonin) receptor 3C, ionotropic, *HTR6* 5-hydroxytryptamine (serotonin) receptor 6, G protein coupled, *HTRs* 5-hydroxytryptamine (serotonin) receptors, *HTT* huntingtin, *IFNA1* interferon, alpha 1, *IL6* interleukin 6, *KCNH2* potassium channel, voltage-gated age-related subfamily H, member 2, *MAOA* monoamine oxidase A, *NR3C1* nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor), *NTRK2* neurotrophic tyrosine kinase, receptor, type 2, *PDE5A* phosphodiesterase 5A, cGMP specific, *POR* P450 (cytochrome) oxidoreductase, *SCL6A4* solute carrier family 6 (neurotransmitter transporter), member 4, *SIGMAR1* sigma non-opioid intracellular receptor 1, *SLC6A2* solute carrier family 6 (neurotransmitter transporter), member 2, *SLC6A3* solute carrier family 6 (neurotransmitter transporter), member 3, *SLC6A4* solute carrier family 6 (neurotransmitter transporter), member 4, *STAT3* signal transducer and activator of transcription 3 (acute-phase response factor), *TBX21* T-box 21, *TNF* tumor necrosis factor, *TPH1* tryptophan hydroxylase 1, *UGT1A1* UDP glucuronosyltransferase 1 family, polypeptide A1, *UGT2B7* UDP glucuronosyltransferase 2 family, polypeptide B7, *UGTs* UDP glucuronosyltransferase family

35.7.1.7 Milnacipran

Milnacipran is a potent SSNRI, acting as a minor substrate of COMT, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/CYP3A5, and UGTs; a moderate inhibitor of CYP3A4/CYP3A5; and an inducer of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4/CYP3A5. Milnacipran is transported by SLC6A2 and SLC6A4 proteins [1] (Table 35.2).

35.7.1.8 Paroxetine

Paroxetine is an SSRI, a major substrate of ABCB1, COMT, CYP2D6, and CYP3A4, a minor substrate of CYP1A2, CYP2C19, MAOA, and MAOB, a strong inhibitor of CYP2D6, a moderate inhibitor of CYP2B6, and a weak inhibitor of ABCB1, CYP1A2, CYP2C9, CYP2C19, CYP3A4, SLC6A3, and SLC6A4. ABCB1, SLC6A3, and SLC6A4 are regular transporters of paroxetine. Some important pathogenic genes *(ABCB1*, *CREB1*, *HTR1B*, *HTR2A*, *HTR3B*, *MAOA*, *SLC6A3*, *SLC6A4*, *TNF*, *TPH1*, *TPH2)* might be involved in the efficacy of paroxetine [1] (Table 35.2).

35.7.1.9 Sertraline

Sertraline is an SSRI with very weak effects on norepinephrine and dopamine neuronal uptake. Pathogenic (*ABCB1*, *CREB1*, *GNB3*, *HTR1B*, *MAOA*, *SIGMAR1*, *SLC6A4*, *TNF*, *TPH1*, *TPH2*) and mechanistic genes (*HTR1B*, *HTR1D*, *SIGMAR1*, *SLC6A2*, *SLC6A3*, *SLC6A4*, *TNF*) are involved in the antidepressant effect of sertraline, which is a major substrate of CYP2C19; a minor substrate of CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP3A4, MAOA, MAOB, UGT1A1, and UGT2B7; a moderate inhibitor of CYP2B6, CYP2C19, CYP2D6, and CYP3A4; and a weak inhibitor of ABCB1, ACHE, CYP1A1, CYP1A2, CYP2C8, CYP2C9, and SLC6A4. Its principal transporters are ABCB1, SLC6A2, SLC6A3, and SLC6A4 proteins [1] (Table 35.2).

35.7.1.10 Venlafaxine

Venlafaxine is an SSNRI and a weak inhibitor of dopamine reuptake, with potential, reciprocal influence on some pathogenic genes (*ABCB1*, *BDNF*, *CREB1*, *FKBP5*, *HTR1A*, *HTR2A*, *NR3C1*, *SLC6A3*, *SLC6A4*, *TPH2)*. Venlafaxine is a major substrate of CYP2D6 and CYP3A4 enzymes; a minor substrate of ABCB1, CYP2C9, and CYP2C19; and a weak inhibitor of ABCB1, CYP1A2, CYP2B6, CYP2D6, CYP3A4, SLC6A2, SLC6A3, and SLC6A4. Several genes *(ABCB1*, *ABCC1*, *ABCG2*, *SLC6A2*, *SLC6A3*, *SLC6A4)* encode transporter proteins for the penetration of venlafaxine into the brain [1] (Table 35.2).

35.7.2 Tricyclics (TCAs) and Other Norepinephrine Reuptake Inhibitors (Table 35.3)

35.7.2.1 Amitriptyline

Amitriptyline is a tricyclic drug which increases the synaptic concentration of serotonin and/or norepinephrine in the CNS by inhibiting their reuptake at the presynaptic neuronal membrane. Pathogenic and mechanistic genes are associated with the therapeutic effects of amitriptyline. It is a major substrate of ABCB1, CYP2D6, CYP3A4/CYP3A5, GSTP1, UGT1A3, UGT1A4, and UGT2B10; a minor substrate of CYP1A2, CYP2B6, CYP2C9, and CYP2C19; a moderate inhibitor of ABCB1, ABCC2, ABCG2, CYP1A2, CYP2C9, CYP2C19, and CYP2D6; and a weak inhibitor of CYP2E1. ABCB1, ABCC2, ABCG2, KCNE2, KCNH2, KCNQ1, SCN5A, and SLC6A4 proteins are current transporters of amitriptyline [1] (Table 35.3).

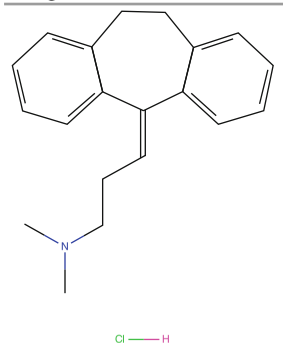
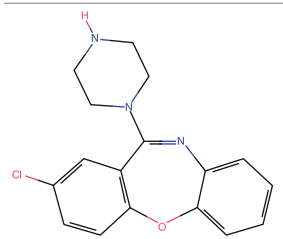
35.7.2.2 Amoxapine

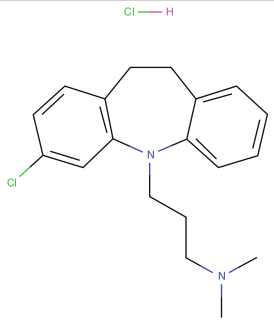
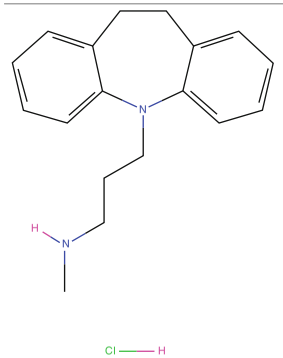
Amoxapine is a tricyclic drug which reduces the reuptake of serotonin and norepinephrine at the synaptic cleft. Its metabolite 7-OH-amoxapine has a significant dopamine receptor-blocking activity. Amoxapine is a major substrate of CYP2D6 enzymes and is transported by SLC6A2 and SLC6A4 [1] (Table 35.3).

35.7.2.3 Clomipramine

Clomipramine is a strong, nonselective serotonin reuptake inhibitor. Its main metabolite

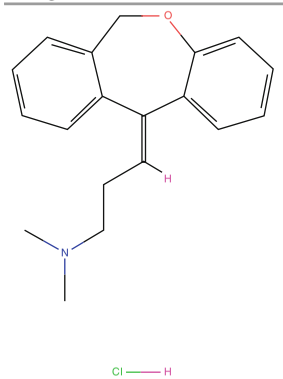
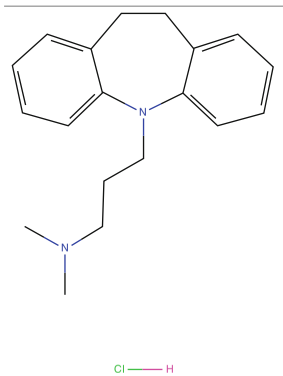
Table 35.3 Pharmacological profile and pharmacogenetics of tricyclics (TCAs) and other norepinephrine reuptake inhibitors

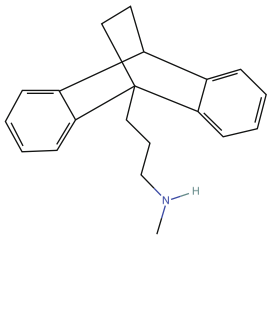
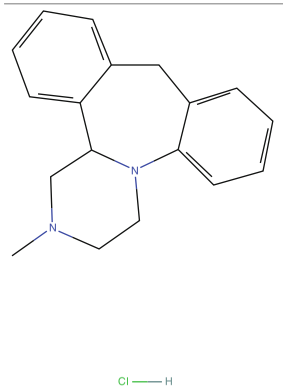
Drug	Properties	Pharmacogenetics
	*Name*: amitriptyline hydrochloride; Annoyltin; amitriptyline HCl; 549-18-8; Tryptizol; Domical *IUPAC name*: dimethyl(3-{tricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,11,13-hexaen-2-ylidene}propyl)amine *Molecular formula*: $C_{20}H_{24}ClN$ *Molecular weight*: 313.86426 g/mol Category: tricyclics *Mechanism*: increases synaptic concentration of serotonin and/or norepinephrine in the central nervous system by inhibiting their reuptake in the presynaptic neuronal membrane *Effect*: adrenergic uptake inhibition; antimigraine activity; analgesic (nonnarcotic) activity; antidepressant action	*Pathogenic genes*: *ABCB1*, *GNB3*, *HTRs*, *NTRK2*, *SLC6A4*, and *TNF* *Mechanistic genes*: *ADRA1A*, *HTRs*, *NTRK1*, and *NTRK2* *Metabolic genes*: *Substrate*: *ABCB1*, *CYP1A2* (minor), *CYP2B6* (minor), *CYP2C9* (minor), *CYP2C19* (minor), *CYP2D6* (major), *CYP3A4/CYP3A5* (major), *GSTP1*, *UGT1A3*, *UGT1A4*, and *UGT2B10* *Inhibitor*: *ABCB1*, *ABCC2*, *ABCG2*, *CYP1A2* (moderate), *CYP2C9* (moderate), *CYP2C19* (moderate), *CYP2D6* (moderate), and *CYP2E1* (weak) *Transporter genes*: *ABCB1*, *ABCC2*, *ABCG2*, *KCNE2*, *KCNH2*, *KCNQ1*, *SCN5A*, and *SLC6A4* *Pleiotropic genes*: *FABP1*, *GNAS*, *GNB3*, *NTRK1*, and *TNF*
	*Name*: amoxapine; Asendin; Demolox; 14028-44-5; Asendis; Moxadi *IUPAC name*: 13-chloro-10-(piperazin-1-yl)-2-oxa-9-azatricyclo[9.4.0.0^{3,8}]pentadeca-1(11),3,5,7,9,12,14-heptaene *Molecular formula*: $C_{17}H_{16}ClN_3O$ *Molecular weight*: 313.78144 g/mol *Category*: tricyclics *Mechanism*: reduces reuptake of serotonin and norepinephrine. The metabolite, 7-OH-amoxapine, has significant dopamine receptor-blocking activity *Effect*: serotonin uptake inhibition; adrenergic uptake inhibition; dopamine antagonism; neurotransmitter uptake inhibition; antidepressant action; antianxiety activity	*Pathogenic genes*: *GNB3* and *SLC6A4* *Mechanistic genes*: *ADRA1A*, *ADRA2A*, *CHRMs*, *DRD1*, *DRD2*, *GABRs*, *GABBRs*, and *HTRs* *Metabolic genes*: *Substrate*: *CYP2D6* (major) *Transporter genes*: *SLC6A2* and *SLC6A4* *Pleiotropic genes*: *DRD2*, *GNAS*, and *GNB3*

	*Name*: clomipramine hydrochloride; Anafranil; clomipramine HCl; 17321-77-6; Anafranil; 3-(3-chloro-10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethylpropan-1-amine hydrochloride *IUPAC name*: (3-{14-chloro-2-azatricyclo[9.4.0.0^{3,8}]pentadeca-1(11),3,5,7,12,14-hexaen-2-yl}propyl)dimethylamine *Molecular formula*: $C_{19}H_{24}Cl_2N_2$ *Molecular weight*: 351.31326 g/mol *Category*: tricyclics *Mechanism*: it is a strong, but not completely selective serotonin reuptake inhibitor; as its active main metabolite, desmethylclomipramine acts preferably as an inhibitor of noradrenaline reuptake. α1-receptor blockage and β-downregulation have been noted and most likely play a role in its short-term effects. A blockade of sodium channels and NDMA receptors *Effect*: *serotonin uptake inhibition*; *antidepressant action*; *antianxiety activity*; *anti-obsessional effects*; *analgesic effects*	*Pathogenic genes*: *HTR2A* and *SLC6A4* *Mechanistic genes*: *ADRA1s*, *CHRMs*, *CHRNs*, *HRH1*, *HTR2s*, and *HTR3* *Metabolic genes*: *Substrate*: *CYP1A2* (major), *CYP2A6*, *CYP2B6*, *CYP2C19* (major), *CYP2D6* (minor), *CYP3A4* (major), *CYP3A5* (major), and *UGT1A4* *Inhibitor*: *CYP2C9* (moderate), *CYP2C19* (strong), *CYP2D6* (moderate), *GSTP1*, and *SLC6A4* *Transporter genes*: *SLC6A4* *Pleiotropic genes*: *FABP1* and *PTGS2*
	*Name*: desipramine hydrochloride; Norpramin; Desipramine HCl; DMI hydrochloride; Pertofrane; Pertofran *IUPAC name*: (3-{2-azatricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,11,13-hexaen-2-yl}propyl)(methyl)amine *Molecular formula*: $C_{18}H_{23}ClN_2$ *Molecular weight*: 302.84162 g/mol *Category*: tricyclics *Mechanism*: increases the synaptic concentration of norepinephrine in the CNS by inhibition of its reuptake by the presynaptic neuronal membrane. Additional receptor effects including desensitization of adenylate cyclase, downregulation of β-adrenergic receptors, and downregulation of serotonin receptors *Effect*: enzyme inhibition; adrenergic uptake inhibition; antidepressant action; analgesic activity	*Pathogenic genes*: *ABCB1*, *CRHR1*, *CRHR2*, *FKBP5*, *HTR1A*, *IL1B*, *NR3C1*, *NTRK2*, *PDE5A*, *SLC6A4*, and *TBX21* *Mechanistic genes*: *ADCY1*, *ADRA1A*, *ADRBs*, *CHRMs*, *HTR1A*, *IFNA1*, *PDE1C*, *PSMD9*, *PRKCSH*, and *STAT3* *Metabolic genes*: *Substrate*: *CYP1A2* (minor), *CYP2C9*, and *CYP2D6* (major) *Inhibitor*: *ABCB1*, *CYP2A6* (moderate), *CYP2B6* (moderate), *CYP2C19* (moderate), *CYP2D6* (moderate), *CYP2E1* (weak), *CYP3A4* (moderate), *SLC6A2*, and *SLC22A3* *Transporter genes*: *ABCB1*, *SLC6A2*, *SLC6A3*, *SLC6A4*, and *SLC22A3* *Pleiotropic genes*: *NTRK2* and *FOS*

(continued)

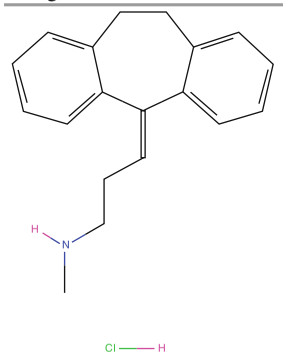
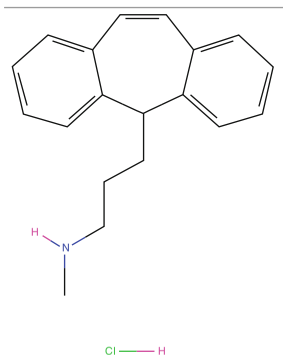
Table 35.3 (continued)

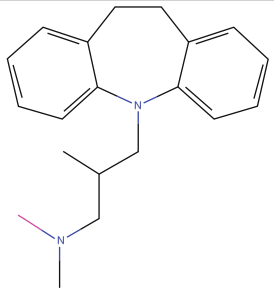
Drug	Properties	Pharmacogenetics
	*Name*: doxepin hydrochloride; Silenor; Adapin; Novoxapin; Toruan; Curatin *IUPAC name*: dimethyl(3-{9-oxatricyclo[9.4.0.0^{3},^{8}]pentadeca-1(15),3,5,7,11,13-hexaen-2-ylidene}propyl)amine *Molecular formula*: $C_{19}H_{22}ClNO$ *Molecular weight*: 315.83708 g/mol *Category*: tricyclics *Mechanism*: it increases the synaptic concentration of serotonin and norepinephrine in the CNS by inhibition of their reuptake by the presynaptic neuronal membrane *Effect*: adrenergic uptake inhibition; histamine antagonism; antidepressant action; analgesic effects; pruritus reduction	*Pathogenic genes*: *ABCB1* and *SLC6A4* *Mechanistic genes*: *ADRBs*, *CHRMs*, *HRH1*, *HRH2*, and *HTRs* *Metabolic genes*: *Substrate*: *CYP1A1* (minor), *CYP1A2* (minor), *CYP2C9* (minor), *CYP2C19* (major), *CYP2D6* (major), *CYP3A4/CYP3A5* (minor), *GSTP1*, *UGT1A3*, and *UGT1A4* *Inhibitor*: *CYP2C19* (strong) and *CYP2D6* (moderate) *Transporter genes*: *ABCB1*, *KCNH2*, *SLC6A2*, and *SLC6A4*
	*Name*: imipramine hydrochloride; Tofranil; imipramine HCl; 113-52-0; Chimoreptin; Feinalmin *IUPAC name*: (3-{2-azatricyclo[9.4.0.0^{3},^{8}]pentadeca-1(15),3,5,7,11,13-hexaen-2-yl}propyl)dimethylamine *Molecular formula*: $C_{19}H_{25}ClN_2$ *Molecular weight*: 316.8682 g/mol *Category*: tricyclics *Mechanism*: it binds the sodium-dependent serotonin transporter and sodium-dependent norepinephrine transporter preventing or reducing the reuptake of norepinephrine and serotonin by nerve cells. It causes downregulation of cerebral cortical beta-adrenergic receptors *Effect*: adrenergic uptake inhibition; antidepressant action; antienuretic effects; analgesic activity; attention enhancer	*Pathogenic genes*: *ABCB1*, *BDNF*, *HTR2A*, and *SLC6A4* *Mechanistic genes*: *ADRB2*, *DRD2*, *CHRMs*, *HTR2A*, and *SCNs* *Metabolic genes*: *Substrate*: *CYP1A2* (minor), *CYP2B6* (minor), *CYP2C19* (major), *CYP2D6* (major), *CYP3A4* (minor), *CYP3A7*, *GSTP1*, *UGT1A3*, *UGT1A4*, and *UGT2B10* *Inhibitor*: *CYP1A2* (weak), *CYP2C9* (moderate), *CYP2C19* (weak), *CYP2D6* (moderate), *CYP2E1* (weak), *CYP3A4* (moderate), *FMO1*, *SLC22A2*, and *SLC22A3* *Transporter genes*: *ABCB1*, *SLC6A2*, *SLC6A4*, *SLC22A2*, and *SLC22A3* *Pleiotropic genes*: *ADRB2*, *BDNF*, *FABP1*, *FOS*, and *ORM1*

	*Name*: maprotiline hydrochloride; Ludiomil; Psymion; Maprotiline HCl; 10347-81-6; Maprotilline HCl *IUPAC name*: methyl(3-{tetracyclo[6.6.2.0^{2},^{7}.0^{9},^{14}]hexadeca-2,4,6,9,11,13-hexaen-1-yl}propyl)amine *Molecular formula*: $C_{20}H_{24}ClN$ *Molecular weight*: 313.86426 g/mol Category: tetracyclics *Mechanism*: inhibits presynaptic uptake of catecholamines, thereby increasing their concentration at the synaptic cleft and acts as an antagonist at central presynaptic α2-adrenergic inhibitory autoreceptors and hetero-receptors. It is also a moderate peripheral α1-adrenergic antagonist, and it is a strong inhibitor of the histamine H1 receptor. It also inhibits the amine transporter, delaying the reuptake of noradrenaline and norepinephrine *Effect*: adrenergic uptake inhibition; antidepressant action; sedative action; anxiolytic effects; hypotensive effects	*Pathogenic genes*: *ABCB1* *Mechanistic genes*: *ADRA2s*, *ADRA1s*, *CHRM4*, *CHRM5*, and *HRH1* *Metabolic genes*: *Substrate*: *CYP1A2* (minor), *CYP2C19*, *CYP2D6* (major), and *CYP3A4* *Inhibitor*: *MAOB* and *SLC6A2* *Transporter genes: ABCB1* and *SLC6A2*
	*Name*: mianserin hydrochloride; 21535-47-7; Athymil; mianserin hydrochloride; mianserin HCl; Bolvidon *IUPAC name*: 5-methyl-2,5-diazatetracyclo[13.4.0.0^{2},^{7}.0^{8},^{13}] nonadeca-1(19),8,10,12,15,17-hexaene *Molecular formula*: $C_{18}H_{21}ClN_2$ *Molecular weight*: 300.82574 g/mol *Category*: tetracyclics *Mechanism*: increases central noradrenergic neurotransmission by α_2-autoreceptor blockade and noradrenaline reuptake inhibition. In addition, interactions with serotonin receptors in CNS have been found *Effect*: serotonin antagonism; histamine H1 antagonism (antihistaminic action); adrenergic alpha-antagonism; antidepressant agent; hypnosedative activity	*Pathogenic genes*: *HTR2A* *Mechanistic genes*: *ADRA2A*, *HRH1*, and *HTR2s* *Metabolic genes*: *Substrate*: *CYP1A2*, *CYP2B6*, *CYP2D6* (major), *CYP3A4* (major), and *UGTs* *Inhibitor*: *SLC6A2* *Transporter genes*: *SLC6A2*

(continued)

Table 35.3 (continued)

Drug	Properties	Pharmacogenetics
	*Name*: nortriptyline hydrochloride; Pamelor; Allegron; Altilev; Nortrilen; 894-71-3 *IUPAC name*: methyl(3-{tricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,11,13-hexaen-2-ylidene}propyl)amine *Molecular formula*: $C_{19}H_{22}ClN$ *Molecular weight*: 299.83768 g/mol *Category*: tricyclics *Mechanism*: inhibits the reuptake of the neurotransmitter serotonin at the neuronal membrane or acts at beta-adrenergic receptors. It has additional receptor effects including desensitization of adenylate cyclase, downregulation of β-adrenergic receptors, and downregulation of serotonin receptors *Effect*: adrenergic uptake inhibitor; antidepressant agent; analgesic activity; hypnosedative activity	*Pathogenic genes*: *ABCB1*, *GNB3*, *HTR1B*, *NR3C1*, and *SLC6A4* *Mechanistic genes*: *ADCY1*, *ADRA2s*, *ADRBs*, *GNB3*, *HRH1*, and *HTRs* *Metabolic genes*: *Substrate*: *CYP1A2* (minor), *CYP2C19* (minor), *CYP2D6* (major), *CYP3A4* (minor), and *UGTs* *Inhibitor*: *CYP2C8* (moderate), *CYP2C9* (moderate), *CYP2C19* (moderate), *CYP2D6* (weak), *CYP2E1* (weak), and *CYP3A4* (moderate) *Transporter genes*: *ABCB1*, *SLC6A2*, and *SLC6A4* *Pleiotropic genes*: *HTR1B*
	*Name*: protriptyline hydrochloride; protriptyline HCl; Concordin; Maximed; Triptyl; Triptil hydrochloride *IUPAC name*: methyl(3-{tricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,9,11,13-heptaen-2-yl}propyl)amine *Molecular formula*: $C_{19}H_{22}ClN$ *molecular weight*: 299.83768 g/mol *Category*: tricyclics *Mechanism*: increases synaptic concentration of serotonin and/or norepinephrine in CNS by inhibition of their reuptake by presynaptic neuronal membrane *Effect*: adrenergic uptake inhibitor; antidepressant agent; analgesic activity; anti-migraine effect	*Mechanistic genes*: *SLC6A2* and *SLC6A4* *Metabolic genes*: *Substrate*: *CYP1A2* (minor), *CYP2C19* (minor), *CYP2D6* (major), and *CYP3A4* (minor) *Inhibitor*: *CYP1A2* (moderate), *CYP2C9* (moderate), *CYP2C19* (moderate), *CYP2D6* (moderate), and *CYP3A4* (moderate) *Transporter genes: SLC6A2* and *SLC6A4* *Pleiotropic genes*: *ADRA1A*, *GNAS*, and *ITGB3*



Name: trimipramine; Sapilent; Surmontil; beta-methylimipramine; Trimeprimine
IUPAC name: (3-{2-azatricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,11,13-hexaen-2-yl}-2-methylpropyl)dimethylamine
Molecular formula: $C_{20}H_{26}N_2$
Molecular weight: 294.43384 g/mol
Category: tricyclics
Mechanism: increases synaptic concentration of serotonin and/or norepinephrine in CNS by inhibition of their reuptake by presynaptic neuronal membrane
Effect: adrenergic uptake inhibition; antidepressant action; antihistaminic activity; sedative effect

Pathogenic genes: *ABCB1* and *SLC6A4*
Mechanistic genes: *SLC6A2*, *SLC6A4*, *SLC22A1*, and *SLC22A2*
Metabolic genes:
Substrate: *CYP2C19* (major), *CYP2D6* (major), and *CYP3A4/CYP3A5* (major)
Inhibitor: *ABCB1*
Transporter genes: *SLC6A2*, *SLC6A4*, *SLC22A1*, and *SLC22A2*

ABCB1 ATP-binding cassette, subfamily B (MDR/TAP), member 1, *ABCC2* ATP-binding cassette, subfamily C (CFTR/MRP), member 2, *ABCG2* ATP-binding cassette, subfamily G (WHITE), member 2 (junior blood group), *ADCY1* adenylate cyclase 1 (brain), *ADRA1A* adrenoceptor alpha 1A, *ADRA1s* adrenoceptors alpha 1, *ADRA2A* adrenoceptor alpha 2A, *ADRA2s* adrenoceptors alpha 2, *ADRB2* adrenoceptor beta 2, surface, *ADRBs* adrenoceptors beta, *BDNF* brain-derived neurotrophic factor, *CHRM4* cholinergic receptor, muscarinic 4, *CHRM5* cholinergic receptor, muscarinic 5, *CHRMs* cholinergic receptors, muscarinic type, *CHRNs* cholinergic receptors, muscarinic type, *CRHR1* corticotropin-releasing hormone receptor 1, *CRHR2* corticotropin-releasing hormone receptor 2, *CYP1A1* cytochrome P450, family 1, subfamily A, polypeptide 1, *CYP1A2* cytochrome P450, family 1, subfamily A, polypeptide 2, *CYP2A6* cytochrome P450, family 2, subfamily A, polypeptide 6, *CYP2B6* cytochrome P450, family 2, subfamily B, polypeptide 6, *CYP2C19* cytochrome P450, family 2, subfamily C, polypeptide 19, *CYP2C8* cytochrome P450, family 2, subfamily C, polypeptide 8, *CYP2C9* cytochrome P450, family 2, subfamily C, polypeptide 9, *CYP2D6* cytochrome P450, family 2, subfamily D, polypeptide 6, *CYP2E1* cytochrome P450, family 2, subfamily E, polypeptide 1, *CYP3A4* cytochrome P450, family 3, subfamily A, polypeptide 4, *CYP3A4/CYP3A5* cytochrome P450, family 3, subfamily A, polypeptide 4/polypeptide 5, *CYP3A5* cytochrome P450, family 3, subfamily A, polypeptide 5, *CYP3A7* cytochrome P450, family 3, subfamily A, polypeptide 7, *DRD1* dopamine receptor D1, *DRD2* dopamine receptor D2, *FABP1* fatty acid-binding protein 1, liver, *FKBP5* binding protein 5, *FMO1* flavin-containing monooxygenase 1, *FOS* FBJ murine osteosarcoma viral oncogene homolog, *GABBRs* gamma-aminobutyric acid (GABA) A receptors, beta, *GABRs* gamma-aminobutyric acid (GABA) A receptors, *GNAS* GNAS complex locus, *GNB3* guanine nucleotide-binding protein (G protein), beta polypeptide 3, *GSTP1* glutathione S-transferase pi 1, *HRH1* histamine receptor H1, *HRH2* histamine receptor H2, *HTR1A* 5-hydroxytryptamine (serotonin) receptor 1A; G protein coupled, *HTR1B* 5-hydroxytryptamine (serotonin) receptor 1B, G protein coupled, *HTR2A* 5-hydroxytryptamine (serotonin) receptor 2A, G protein coupled, *HTR2s* 5-hydroxytryptamine (serotonin) receptors 2, *HTR3* histone H3, *HTRs* 5-hydroxytryptamine (serotonin) receptors, *IFNA1* interferon, alpha 1, *IL1B* interleukin 1, beta, *ITGB3* integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61), *KCNE2* potassium channel, voltage-gated subfamily E regulatory beta subunit 2, *KCNH2* potassium channel, voltage-gated age-related subfamily H, member 2, *KCNQ1* potassium channel, voltage-gated KQT-like subfamily Q, member 1, *MAOB* monoamine oxidase B, *NR3C1* nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor), *NTRK1* neurotrophic tyrosine kinase, receptor, type 1, *NTRK2* neurotrophic tyrosine kinase, receptor, type 2, *ORM1* orosomucoid 1, *PDE1C* phosphodiesterase 1C, calmodulin-dependent 70 kDa, *PDE5A* phosphodiesterase 5A, cGMP specific, *PRKCSH* protein kinase C substrate 80 K-H, *PSMD9* proteasome (prosome, macropain) 26S subunit, non-ATPase, 9, *PTGS2* prostaglandin-endoperoxide synthase 2 (prostaglandin G/prostaglandin H synthase and cyclooxygenase), *SCN5A* sodium channel, voltage-gated, type V alpha subunit, *SCNs* sodium channels, voltage gated, *SLC22A1* solute carrier family 22 (organic cation transporter), member 1, *SLC22A2* solute carrier family 22 (organic cation transporter), member 2, *SLC22A3* solute carrier family 22 (organic cation transporter), member 3, *SLC6A2* solute carrier family 6 (neurotransmitter transporter), member 2, *SLC6A3* solute carrier family 6 (neurotransmitter transporter), member 3, *SLC6A4* solute carrier family 6 (neurotransmitter transporter), member 4, *STAT3* signal transducer and activator of transcription 3 (acute-phase response factor), *TBX21* T-box 21, *TNF* tumor necrosis factor, *UGT1A3* UDP glucuronosyltransferase 1 family, polypeptide A3, *UGT1A4* UDP glucuronosyltransferase 1 family, polypeptide A4, *UGT2B10* UDP glucuronosyltransferase 2 family, polypeptide B10, *UGTs* UDP glucuronosyltransferase family

desmethylclomipramine acts as an inhibitor of noradrenaline reuptake. This tricyclic also displays α_1-receptor blocking activity, β-downregulation, and blockade of sodium channels and NMDA receptors. Clomipramine is a major substrate of CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP3A4/CYP3A5, and UGT1A4, a minor substrate of CYP2D6, a strong inhibitor of CYP2C19, and a moderate inhibitor of CYP2C9, CYP2D6, GSTP1, and SLC6A4. SLC6A4 is its main transporter [1] (Table 35.3).

35.7.2.4 Desipramine

Desipramine is a tricyclic drug which increases the synaptic concentration of norepinephrine in the CNS by inhibition of its reuptake at the presynaptic neuronal membrane. Additional receptor effects include desensitization of adenylate cyclase, downregulation of β-adrenergic receptors, and downregulation of serotonin receptors. Diverse pathogenic (*ABCB1*, *CRHR1*, *CRHR2*, *FKBP5*, *HTR1A*, *IL1B*, *NR3C1*, *NTRK2*, *PDE5A*, *SLC6A4*, *TBX21*) and mechanistic genes (*ADCY1*, *ADRA1A*, *ADRBs*, *CHRMs*, *HTR1A*, *IFNA1*, *PDE1C*, *PSMD9*, *PRKCSH*, *STAT3*) influence the effects of desipramine. This antidepressant is a major substrate of CYP2D6; an intermediate substrate of CYP2C9; a minor substrate of CYP1A2; a moderate inhibitor of ABCB1, CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP3A4, SLC6A2, and SLC22A3; and a weak inhibitor of CYP2E1. Its principal transporters are ABCB1, SLC6A2, SLC6A3, SLC6A4, and SLC22A3 [1] (Table 35.3).

35.7.2.5 Doxepin

Doxepin increases the synaptic concentration of serotonin and norepinephrine in the CNS by inhibition of their reuptake at the presynaptic neuronal membrane. This tricyclic is a major substrate of CYP2C19, CYP2D6, GSTP1, UGT1A3, and UGT1A4; a minor substrate of CYP1A1, CYP1A2, CYP2C9, and CYP3A4/CYP3A5; a moderate inhibitor of CYP2D6; and a strong inhibitor of CYP2C19 enzymes. ABCB1, KCNH2, SLC6A2, and SLC6A4 are its current transporters [1] (Table 35.3).

35.7.2.6 Imipramine

Imipramine is a tricyclic drug that binds the sodium-dependent serotonin transporter and sodium-dependent norepinephrine transporter preventing or reducing the reuptake of norepinephrine and serotonin by nerve cells. It also causes downregulation of cerebral cortical β-adrenergic receptors. Pathogenic genes (*ABCB1*, *BDNF*, *HTR2A*, and *SLC6A4*) and mechanistic genes associated with adrenergic, dopaminergic, cholinergic, serotonergic, and histaminergic neurotransmission (*ADRB2*, *DRD2*, *CHRMs*, *HTR2A*, and *SCNs*) may affect its pharmacodynamic properties. Imipramine is a major substrate of CYP2C19, CYP2D6, GSTP1, UGT1A3, UGT1A4, and UGT2B10; a minor substrate of CYP1A2, CYP2B6, CYP3A4, and CYP3A7; a weak inhibitor of CYP1A2, CYP2C19, and CYP2E1; and a moderate inhibitor of CYP2C9, CYP2D6, CYP3A4, FMO1, SLC22A2, and SLC22A3. Imipramine is transported by ABCB1, SLC6A2, SLC6A4, SLC22A2, and SLC22A3 proteins [1] (Table 35.3).

35.7.2.7 Maprotiline

Maprotiline is a pleiotropic tetracyclic that inhibits the presynaptic uptake of catecholamines, thereby increasing their concentration at the synaptic cleft. Maprotiline also acts as an antagonist at central presynaptic α_2-adrenergic inhibitory autoreceptors and hetero-receptors, is a moderate peripheral α_1-adrenergic antagonist, and is a strong inhibitor of the histamine H_1 receptor. It also inhibits the amine transporter, delaying the reuptake of noradrenaline and norepinephrine. Maprotiline is a major substrate of CYP2D6; is a minor substrate of CYP1A2, CYP2C19, and CYP3A4 enzymes; inhibits MAOB and SLC6A2; and is transported by ABCB1 and SLC6A2 [1] (Table 35.3).

35.7.2.8 Mianserin

Mianserin is a tetracyclic drug that increases central noradrenergic neurotransmission by

α_2-autoreceptor blockade and noradrenaline reuptake inhibition and also interacts with serotonin receptors. This antidepressant is a major substrate of CYP2D6 and CYP3A4/CYP3A5 enzymes; a minor substrate of CYP1A2, CYP2B6, and UGTs; and an inhibitor of SLC6A2, which is also its main transporter [1] (Table 35.3).

35.7.2.9 Nortriptyline

Nortriptyline is a tricyclic antidepressant that inhibits the reuptake of serotonin at the neuronal membrane and also interacts with β-adrenergic receptors. Other mechanistic effects of nortriptyline include desensitization of adenylate cyclase, downregulation of β-adrenergic receptors, and downregulation of serotonin receptors. It is a major substrate of CYP2D6 and UGTs; a minor substrate of CYP1A2, CYP2C19, and CYP3A4; a moderate inhibitor of CYP2C8, CYP2C9, CYP2C19, and CYP3A4; and a weak inhibitor of CYP2D6 and CYP2E1 enzymes. ABCB1, SLC6A2, and SLC6A4 are its principal transporters [1] (Table 35.3).

35.7.2.10 Protriptyline

Protriptyline is a tricyclic compound that increases the intersynaptic concentration of serotonin and/or norepinephrine by inhibition of their reuptake at the presynaptic neuronal membrane. It is a major substrate of CYP2D6; a minor substrate of CYP1A2, CYP2C19, and CYP3A4; and a moderate inhibitor of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Protriptyline is transported by SLC6A2 and SLC6A4 proteins [1] (Table 35.3).

35.7.2.11 Trimipramine

Trimipramine is a tricyclic antidepressant that enhances the concentration of serotonin and/or norepinephrine by inhibition of their reuptake at the presynaptic neuronal membrane. It is a major substrate of CYP2C19, CYP2D6, and CYP3A4/CYP3A5; inhibits ABCB1; and is transported by SLC6A2, SLC6A4, SLC22A1, and SLC22A2 proteins [1] (Table 35.3).

35.7.3 Monoamine Oxidase Inhibitors (MAOIs) (Table 35.4)

35.7.3.1 Isocarboxazid

Isocarboxazid is a nonselective MAOI that increases endogenous concentrations of epinephrine, norepinephrine, dopamine, and serotonin through inhibition of MAOA and MAOB enzymes. It is a substrate of COMT and CYP2D6, and a strong inhibitor of MAOs [1] (Table 35.4).

35.7.3.2 Moclobemide

Moclobemide is a MAOA inhibitor that inhibits deamination of serotonin, norepinephrine, and dopamine, leading to increased concentrations of these neurotransmitters in the CNS. It is a major substrate of CYP2C19 and CYP2D6; a minor substrate of CYP2E1; a weak inhibitor of CYP1A2, CYP2C19, and CYP2D6; a strong inhibitor of MAOA; and a moderate inhibitor of MAOB [1] (Table 35.4).

35.7.3.3 Phenelzine

Phenelzine is a nonselective MAOI that increases endogenous concentrations of norepinephrine, dopamine, and serotonin through inhibition of MAOs. It is a substrate of COMT, MAOA, and MAOB and a moderate inhibitor of CYP2C8, CYP2D6, CYP3A4, MAOA, and MAOB [1] (Table 35.4).

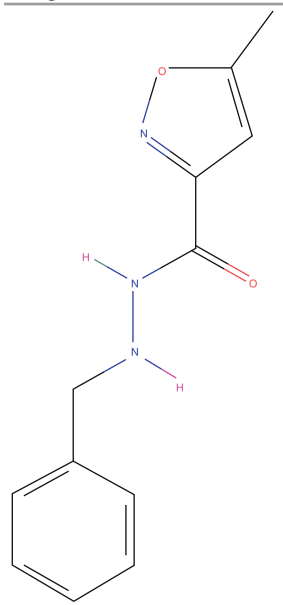
35.7.3.4 Rasagiline

Rasagiline is a potent, irreversible, selective inhibitor of brain MAOB. It is a major substrate of CYP1A2, CYP2D6, UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A9, UGT1A10, UGT2B7, and UGT2B15 and a strong inhibitor of MAOB [1] (Table 35.4).

35.7.3.5 Selegiline

Selegiline is a selective, irreversible MAOB inhibitor that binds to MAOB within the nigrostriatal pathway, thus blocking microsomal metabolism of dopamine and enhancing the dopaminergic activity in the substantia nigra. At high doses, it can also inhibit MAOA. Selegiline

Table 35.4 Pharmacological profile and pharmacogenetics of monoamine oxidase inhibitors (MAOIs)

Drug	Properties	Pharmacogenetics
	*Name*: isocarboxazid; Isocarboxazide; Marplan; Isocarbonazid; Benazide; Enerzer *IUPAC name*: N′-benzyl-5-methyl-1,2-oxazole-3-carbohydrazide *Molecular formula*: $C_{12}H_{13}N_3O_2$ *Molecular weight*: 231.25052 g/mol *Category*: monoamine oxidase inhibitors, nonselective *Mechanism*: thought to act by increasing endogenous concentrations of epinephrine, norepinephrine, dopamine, and serotonin through inhibition of enzyme (monoamine oxidase) responsible for breakdown of these neurotransmitters *Effect*: antidepressant activity; monoamine oxidase inhibition; antianxiety activity	*Pathogenic genes*: *MAOA* *Mechanistic genes*: *MAOA* and *MAOB* *Metabolic genes*: *Substrate: COMT* and *CYP2D6* *Inhibitor*: *MAOA* and *MAOB*

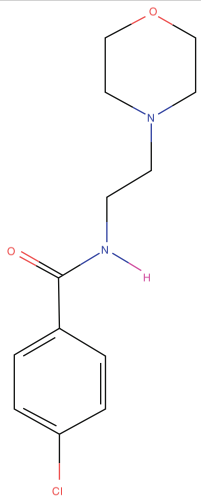
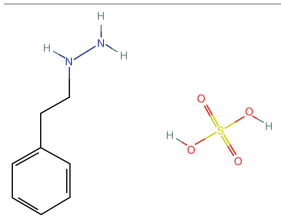
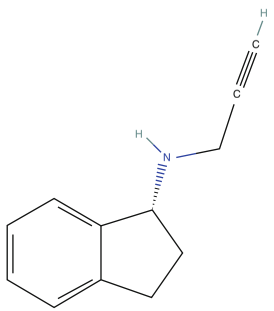
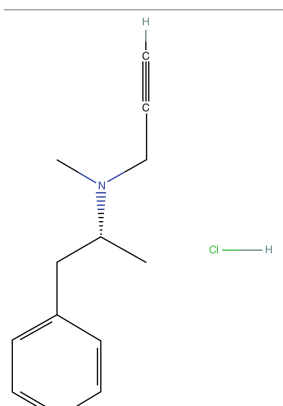
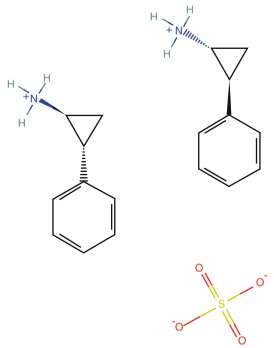
	*Name*: moclobemide; Aurorix; Moclamine; Manerix; Moclobemidum; Moclobemid *IUPAC name*: 4-chloro-N-[2-(morpholin-4-yl)ethyl]benzamide *Molecular formula*: $C_{13}H_{17}ClN_2O_2$ *Molecular weight*: 268.73928 g/mol *Category*: monoamine oxidase A inhibitors *Mechanism*: involves the selective, reversible inhibition of MAOA. This inhibition leads to a decrease in the metabolism and destruction of monoamines in the neurotransmitters. This results in an increase in the monoamines *Effect*: antidepressant agent; monoamine oxidase inhibition	*Pathogenic genes*: *MAOA* *Mechanistic genes*: *MAOA* *Metabolic genes*: *Substrate: CYP2C19* (major), *CYP2D6* (major), and CYP2E1 *Inhibitor*: *CYP1A2* (weak), *CYP2C19* (weak), *CYP2D6* (weak), *MAOA* (strong), and *MAOB* (moderate)
	*Name*: phenelzine sulfate; Estinerval; Nardelzine; Kalgan; Phenethylhydrazine; phenelzine sulfate salt *IUPAC name*: (2-phenylethyl)hydrazine *Molecular formula*: $C_8H_{14}N_2O_4S$ *Molecular weight*: 234.27276 g/mol *Category*: monoamine oxidase inhibitors, nonselective *Mechanism*: irreversible, nonselective inhibition of MAO. It causes an increase in the levels of serotonin, norepinephrine, and dopamine in the neuron *Effect*: antidepressant activity; monoamine oxidase inhibition	*Pathogenic genes*: *MAOA* *Mechanistic genes*: *MAOA* and *MAOB* *Metabolic genes*: *Substrate: COMT*, *MAOA*, and *MAOB* *Inhibitor*: *CYP2C8* (moderate), *CYP2D6*, *CYP3A4* (moderate), *MAOA*, and *MAOB*

Table 35.4 (continued)

Drug	Properties	Pharmacogenetics
	*Name*: rasagiline mesylate; 161735-79-1; Azilect; rasagiline mesilate; TVP-1012; Agilect *IUPAC name*: (1R)-N-(prop-2-yn-1-yl)-2,3-dihydro-1H-inden-1-amine *Molecular formula*: $C_{13}H_{17}NO_3S$ *Molecular weight*: 267.34398 g/mol *Category*: monoamine oxidase B inhibitors *Mechanism*: potent, irreversible, selective inhibitor of brain monoamine oxidase (MAO) type B, which plays a major role in catabolism of dopamine *Effect*: antidepressant activity; monoamine oxidase inhibition; neuroprotective agent; antiparkinsonian agent	*Pathogenic genes*: *PARK2* *Mechanistic genes*: *BCL2*, *BCL2L1*, and *MAOB* *Metabolic genes*: *Substrate: CYP1A2* (major), *CYP2D6*, *UGT1A1*, *UGT1A3*, *UGT1A4*, *UGT1A6*, *UGT1A7*, *UGT1A9*, *UGT1A10*, *UGT2B7*, and *UGT2B15* *Inhibitor*: *MAOB*
	*Name*: selegiline; Selegiline hydrochloride; L-deprenyl hydrochloride; 14611-52-0; Eldepryl; Selegiline HCl; Zelapar *IUPAC name*: methyl(1-phenylpropan-2-yl)(prop-2-yn-1-yl)amine *Molecular formula*: $C_{13}H_{18}ClN$ *Molecular weight*: 223.74172 g/mol *Category*: monoamine oxidase B inhibitors *Mechanism*: selective, irreversible inhibition of MAOB. It binds to MAOB within the nigrostriatal pathways in the central nervous system, thus blocking microsomal metabolism of dopamine and enhancing the dopaminergic activity in the substantia nigra. It may also increase dopaminergic activity through mechanisms other than inhibition of MAOB. At higher doses, it can also inhibit MAOA *Effect*: antidepressant activity; monoamine oxidase inhibition neuroprotective agent; antiparkinsonian agent	*Pathogenic genes*: *MAOA* and *PARK2* *Mechanistic genes*: *MAOA* and *MAOB* *Metabolic genes*: *Substrate: CYP1A2* (minor), *CYP2A6* (minor), *CYP2B6* (major), *CYP2C8* (minor), *CYP2C19* (major), *CYP2D6* (minor), *CYP2E1* (minor), *CYP3A4* (minor), and *MAOA* *Inhibitor*: *CYP1A2* (weak), *CYP2A6* (weak), *CYP2C9* (weak), *CYP2C19* (weak), *CYP2D6* (weak), *CYP2E1* (weak), *CYP3A4* (weak), *MAOB* (strong), and *MAOA* *Transporter genes: SCNA*

	*Name*: tranylcypromine sulfate; tylciprine; phenylcyclopromine sulfate; Dl-tranylcypromine sulfate; EINECS 236-807-1; 1-amino-2-phenylcyclopropane sulfate *IUPAC name*: (1R)-2-phenylcyclopropan-1-amine *Molecular formula*: $C_{18}H_{24}N_2O_4S$ *Molecular weight*: 364.45916 g/mol *Category*: monoamine oxidase inhibitors, nonselective *Mechanism*: it increases endogenous concentrations of epinephrine, norepinephrine, dopamine, and serotonin through inhibition of MAO responsible for breakdown of these neurotransmitters *Effect*: monoamine oxidase inhibition; antidepressant activity; antianxiety activity	*Pathogenic genes*: *MAOA*, *NTRK2*, and *SLC6A4* *Mechanistic genes*: *MAOA* and *MAOB* *Metabolic genes*: *Substrate: CYP2A6* (major) *Inhibitor*: *CYP1A2* (moderate), *CYP2A6* (strong), *CYP2C8* (weak), *CYP2C9* (weak), *CYP2C19* (moderate), *CYP2D6* (moderate), *CYP2E1* (weak), *CYP3A4* (weak), *MAOA*, and *MAOB* *Transporter genes: SLC6A4* *Pleiotropic genes*: *FOS* and *NTRK2*

BCL2 B-cell CLL/lymphoma 2, *BCL2L1* BCL2-like 1, *COMT* catechol-O-methyltransferase, *CYP1A2* cytochrome P450, family 1, subfamily A, polypeptide 2, *CYP2A6* cytochrome P450, family 2, subfamily A, polypeptide 6, *CYP2B6* cytochrome P450, family 2, subfamily B, polypeptide 6, *CYP2C19* cytochrome P450, family 2, subfamily C, polypeptide 19, *CYP2C8* cytochrome P450, family 2, subfamily C, polypeptide 8, *CYP2C9* cytochrome P450, family 2, subfamily C, polypeptide 9, *CYP2D6* cytochrome P450, family 2, subfamily D, polypeptide 6, *CYP2E1* cytochrome P450, family 2, subfamily E, polypeptide 1, *CYP3A4* cytochrome P450, family 3, subfamily A, polypeptide 4, *FOS* FBJ osteosarcoma oncogene, *MAOA* monoamine oxidase A, *MAOB* monoamine oxidase B, *NTRK2* neurotrophic tyrosine kinase, receptor, type 2, *PARK2* parkin RBR E3 ubiquitin protein ligase, *SCNA* synuclein, alpha (non-A4 component of amyloid precursor), *SLC6A4* solute carrier family 6 (neurotransmitter transporter), member 4, *UGT1A1* UDP glucuronosyltransferase 1 family, polypeptide A1, *UGT1A10* UDP glucuronosyltransferase 1 family, polypeptide A10, *UGT1A3* UDP glucuronosyltransferase 1 family, polypeptide A3, *UGT1A4* UDP glucuronosyltransferase 1 family, polypeptide A4, *UGT1A6* UDP glucuronosyltransferase 1 family, polypeptide A6, *UGT1A7* UDP glucuronosyltransferase 1 family, polypeptide A7, *UGT1A9* UDP glucuronosyltransferase 1 family, polypeptide A9, *UGT2B15* UDP glucuronosyltransferase 2 family, polypeptide B15, *UGT2B7* UDP glucuronosyltransferase 2 family, polypeptide B7

is a major substrate of CYP2B6 and CYP2C19; a minor substrate of CYP1A2, CYP2A6, CYP2C8, CYP2D6, CYP2E1, and CYP3A4; a weak inhibitor of CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and MAOA; and a strong inhibitor of MAOB. SCNA is involved in its transport into the brain [1] (Table 35.4).

35.7.3.6 Tranylcypromine

Tranylcypromine is a nonselective MAOI that increases endogenous concentrations of epinephrine, norepinephrine, dopamine, and serotonin through inhibition of MAOs. It is a major substrate of CYP2A6; a weak inhibitor of CYP2C8, CYP2E1, and CYP3A4; a moderate inhibitor of CYP1A2 and CYP2C19; and a strong inhibitor of CYP2A6 and MAOs. It is transported by SLC6A4 [1] (Table 35.4).

35.7.4 Other Categories of Antidepressants (Table 35.5)

35.7.4.1 Agomelatine

Agomelatine is a melatonergic agonist and a 5-HT2C antagonist. It is a major substrate of CYP1A1 and CYP1A2 and a minor substrate of CYP2C9 and CYP2C19 [1] (Table 35.5).

35.7.4.2 Bupropion

Bupropion is a dopamine reuptake inhibitor. It is a substrate of COMT; a major substrate of CYP2B6; a minor substrate of CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4; and a strong inhibitor of CYP2D6. Its transport is modulated by SLC6A2, SLC6A3, and SLC6A4 [1] (Table 35.5).

35.7.4.3 Mirtazapine

Mirtazapine is a α_2-adrenergic antagonist with pleiotropic effects on other neurotransmitters. It has central presynaptic α_2-adrenergic antagonist effects, which result in increased release of norepinephrine and serotonin, and is also a potent antagonist of 5-HT_2 and 5-HT_3 serotonin receptors and histamine H_1 receptors and a moderate antagonist of peripheral α_1-adrenergic and muscarinic receptors. Mirtazapine is a major substrate of CYP1A2, CYP2D6, CYP3A4, UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A9, UGT1A10, UGT2B7, and UGT2B15; a minor substrate of CYP2C9; a weak inhibitor of CYP1A2, CYP3A4, CYP2D6, MAOA, and MAOB; and an inducer of CYP1A2, CYP2D6, and CYP3A4. Several pathogenic (*ABCB1*, *FKBP5*, *HTR1A*, *HTR2A*, *MAOA*, *SLC6A3*, *SLC6A4*, *TPH2)* and mechanistic genes (*ADRA1s*, *ADRA2A*, *FKBP5*, *HRH1*, *HTRs*) are involved in its pharmacodynamic properties and are transported by ABCB1, SLC6A3, and SLC6A4 [1] (Table 35.5).

35.7.4.4 Nefazodone

Nefazodone is a serotonin modulator that potently and selectively blocks postsynaptic 5-HT_{2A} receptors and moderately inhibits serotonin and noradrenaline reuptake. It is an antagonist of α-1 adrenoceptors and 5-hydroxytryptamine receptor 2. This compound is a major substrate of CYP2D6, CYP3A4, and CYP3A5; a strong inhibitor of CYP3A4; a moderate inhibitor of ABCB1 and ABCC2; and a weak inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2D6, and SLC6A2. ABCB1, ABCC2, ABCB11, and SLC6A2 participate in its transport [1] (Table 35.5).

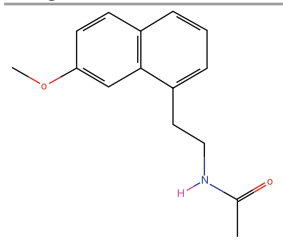
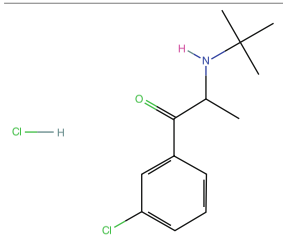
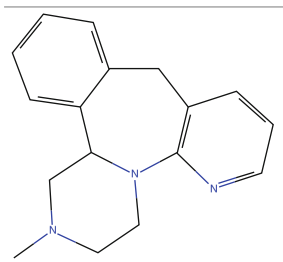
35.7.4.5 Reboxetine

Reboxetine is a highly selective and potent inhibitor of noradrenaline reuptake, with weak effects on 5-HT reuptake. It is a major substrate of CYP3A4 and a weak inhibitor of ABCB1, CYP2D6, and CYP3A4. ABCB1, SLC6A2, SLC6A3, and SLC6A4 are its preferential transporters [1] (Table 35.5).

35.7.4.6 Trazodone

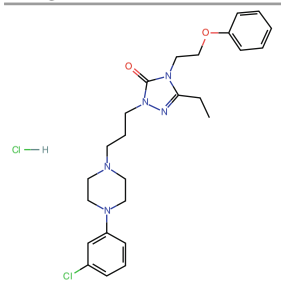
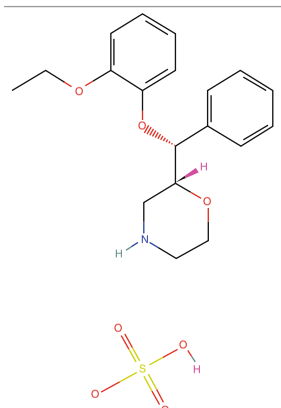
Trazodone is a pleiotropic serotonin modulator that inhibits the reuptake of serotonin, causes adrenoreceptor subsensitivity, and induces significant changes in 5-HT presynaptic receptors, also blocking histamine (H_1) and α_1-adrenergic receptors. It is a major substrate of CYP3A4; a minor substrate

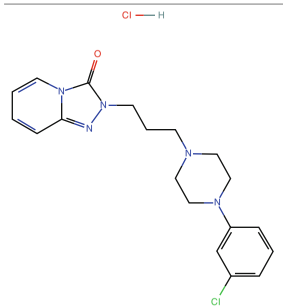
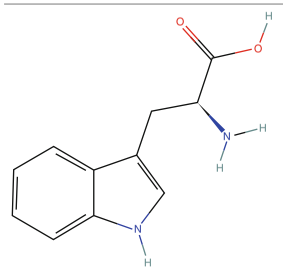
Table 35.5 Pharmacological profile and pharmacogenetics of other categories of antidepressants

Drug	Properties	Pharmacogenetics
	*Name*: agomelatine; 138112-76-2; Valdoxan; Thymanax; Melitor; N-(2-(7-methoxynaphthalen-1-yl)ethyl)acetamide *IUPAC name*: N-[2-(7-methoxynaphthalen-1-yl)ethyl]acetamide *Molecular formula*: $C_{15}H_{17}NO_2$ *Molecular weight*: 243.30098 g/mol *Category*: melatonergic agonist and 5-HT2C antagonists *Mechanism*: it behaves as an agonist at melatonin receptors and as an antagonist at serotonin (5-HT)(2C) receptors *Effect*: norepinephrine–dopamine disinhibitor; antidepressant activity; antianxiety activity; sleep induction; circadian rhythms resynchronization; psychological excitement reduction	*Mechanistic genes*: *HTR2C*, *MTNR1A*, and *MTNR1B* *Metabolic genes*: *Substrate*: *CYP1A1* (major), *CYP1A2* (major), *CYP2C9* (minor), and *CYP2C19* (minor)
	*Name*: bupropion hydrochloride; 31677-93-7; Wellbutrin; Zyban; Bupropion HCl *IUPAC name*: 2-(tert-butylamino)-1-(3-chlorophenyl)propan-1-one *Molecular formula*: $C_{13}H_{19}Cl_2NO$ *Molecular weight*: 276.20206 g/mol *Category*: dopamine reuptake inhibitor *Mechanism*: it is a relatively weak inhibitor of the neuronal uptake of norepinephrine and dopamine *Effect*: dopamine uptake inhibition; anti-addiction/substance abuse treatment agent; smoking cessation enhancer; antidepressant activity	*Pathogenic genes*: *SLC6A3* and *SLC6A4* *Mechanistic genes*: *ADRA1A*, *CHRNB2*, and *DRD2* *Metabolic genes*: *Substrate*: *COMT*, *CYP1A2* (minor), *CYP2A6* (minor), *CYP2B6* (major) *CYP2C9* (minor), *CYP2C19* (minor), *CYP2D6* (minor), *CYP2E1* (minor), and *CYP3A4* (minor) *Inhibitor*: *CYP2D6* (strong) *Transporter genes*: *SLC6A2*, *SLC6A3*, and *SLC6A4* *Pleiotropic genes*: *DRD2*
	*Name*: mirtazapine; Zispin; Remergil; Remeron; 6-azamianserin; Remergon *IUPAC name*: 5-methyl-2,5,19-triazatetracyclo[13.4.0.0^{2}, .0^{8,13}] nonadeca-1(15),8,10,12,16,18-hexaene *Molecular formula*: $C_{17}H_{19}N_3$ *Molecular weight*: 265.35286 g/mol *Category*: α2-adrenergic antagonist *Mechanism*: it has central presynaptic α_2-adrenergic antagonist effects, which result in increased release of norepinephrine and serotonin. Also a potent antagonist of 5-HT_2 and 5-HT_3 serotonin receptors and H_1 histamine receptors and a moderate peripheral α_1-adrenergic and muscarinic antagonist *Effect*: histamine H_1 antagonism; adrenergic alpha-antagonism; antidepressant activity; anxiolytic effects	*Pathogenic genes*: *ABCB1*, *FKBP5*, *HTR1A*, *HTR2A*, *MAOA*, *SLC6A3*, *SLC6A4*, and *TPH2* *Mechanistic genes*: *ADRA1s*, *ADRA2A*, *CHRMs FKBP5*, *HRH1*, *HTR2s*, and *HTR3s* *Metabolic genes*: *Substrate*: *CYP1A2* (major), *CYP2C9* (minor), *CYP2D6* (major), *CYP3A4* (major), *UGT1A1*, *UGT1A3*, *UGT1A4*, *UGT1A6*, *UGT1A7*, *UGT1A9*, *UGT1A10*, *UGT2B7*, and *UGT2B15* *Inhibitor*: *CYP1A2* (weak), *CYP3A4* (weak), *CYP2D6* (weak), *MAOA*, and *MAOB* *Inducer*: *CYP1A2*, *CYP2D6*, and *CYP3A4* *Transporter genes*: *ABCB1*, *SLC6A3*, and *SLC6A4* *Pleiotropic genes*: *TPH2*

(continued)

Table 35.5 (continued)

Drug	Properties	Pharmacogenetics
	*Name*: nefazodone hydrochloride; Serzone; Nefazodone HCl; Dutonin; 82752-99-6; Menfazon *IUPAC name*: 1-{3-[4-(3-chlorophenyl)piperazin-1-yl] propyl}-3-ethyl-4-(2-phenoxyethyl)-4,5-dihydro-1H-1,2,4-triazol-5-one *Molecular formula*: $C_{25}H_{33}Cl_2N_5O_2$ *Molecular weight*: 506.46782 g/mol *Category*: serotonin modulators *Mechanism*: blocks potently and selectively postsynaptic 5-HT_{2A} receptors and moderately inhibits serotonin and noradrenaline reuptake. Also blocks α1 receptors. It is an antagonist of adrenoceptors alpha 1 and 5-hydroxytryptamine receptors 2 *Effect*: serotonin uptake inhibition; noradrenaline uptake inhibition; alpha-adrenergic antagonism; antidepressant activity; muscle relaxation; sedation	*Pathogenic genes*: *ABCB1*, *HTR1A*, and *HTR2A* *Mechanistic genes*: *ADRA1A*, *HTR1s*, and *HTR2s* *Metabolic genes*: *Substrate*: *CYP2D6* (major), *CYP3A4* (major), and *CYP3A5* (major) *Inhibitor*: *ABCB1*, *ABCC2*, *CYP1A2* (weak), *CYP2B6* (weak), *CYP2C8* (weak), *CYP2D6* (weak), *CYP3A4* (strong), and *SLC6A2* *Transporter genes*: *ABCB1*, *ABCC2*, *ABCB11*, and *SLC6A2*
	*Name*: reboxetine mesylate; vestra mesylate; davedax mesylate; reboxetine mesilate; Edronax; 98769-84-7 *IUPAC name*: (2S)-2-[(S)-2-ethoxyphenoxy(phenyl)methyl]morpholine *Molecular formula*: $C_{20}H_{27}NO_6S$ *Molecular weight*: 409.49648 g/mol *Category*: norepinephrine inhibitor *Mechanism*: it is a highly selective and potent inhibitor of noradrenaline reuptake. Only it has weak effect on 5-HT reuptake Effect: adrenergic uptake inhibitor; antidepressant activity; antianxiety activity; attention enhancer	*Pathogenic genes*: *ABCB1*, *SLC6A3*, and *SLC6A4* *Mechanistic genes*: *ADRs*, *CHRMs*, *CHRNs*, *HRHs*, *DRDs*, *GH1*, *HTRs*, *POMC*, and *PRL* *Metabolic genes*: *Substrate*: *CYP3A4* (major) *Inhibitor*: *ABCB1*, *CYP2D6* (weak), and *CYP3A4* (weak) *Transporter genes*: *ABCB1*, *SLC6A2*, *SLC6A3*, and *SLC6A4* *Pleiotropic genes*: *ADRB2* and *POMC*

	*Name*: trazodone hydrochloride; Desyrel; 25332-39-2; trazodone HCl; Molipaxin; Trittico *IUPAC name*: 2-{3-[4-(3-chlorophenyl)piperazin-1-yl]propyl}-2H,3H-[1,2,4]triazolo[4,3-a]pyridin-3-one *Molecular formula*: $C_{19}H_{23}Cl_2N_5O$ *Molecular weight*: 408.32482 g/mol *Category*: serotonin modulators *Mechanism*: inhibits reuptake of serotonin, causes adrenoreceptor subsensitivity, and induces significant changes in 5-HT presynaptic receptor adrenoreceptors. It also significantly blocks histamine (H_1) and α_1-adrenergic receptors *Effect*: serotonin uptake inhibitor; antianxiety activity; antidepressant agent; hypnotic effects	*Pathogenic genes*: *ABCB1*, *GNB3*, *HTR1A*, *HTR2A*, and *SLC6A4* *Mechanistic genes*: *ADRA1s*, *ADRA2s*, *HRH1*, *HTR2A*, and *HTR2C* *Metabolic genes*: *Substrate*: *CYP1A2* (minor), *CYP2D6* (minor), *CYP3A4* (major), *GSTs*, and *SOD2* *Inhibitor*: *CYP2D6* (moderate), *CYP3A4* (weak), and *SLC6A4* *Inducer*: *ABCB1* *Transporter genes*: *ABCB1* and *SLC6A4* *Pleiotropic genes*: *GNAS*, *GNB3*, and *HTR2A*
	*Name*: tryptophan; L-tryptophan; (S)-tryptophan; L-tryptophane; tryptophane; 73-22-3 *IUPAC name*: (2S)-2-amino-3-(1H-indol-3-yl)propanoic acid *Molecular formula*: $C_{11}H_{12}N_2O_2$ *Molecular weight*: 204.22518 g/mol *Category*: amino acid *Mechanism*: reestablishes inhibitory action of 5-HT on amygdaloid nuclei *Effect*: antidepressant activity; antianxiety activity; sleep latency reduction and healthy sleep promoter; child normal growth; nitrogen balance; pain tolerance; mental and emotional well-being enhancer	*Pathogenic genes*: *IDO1*, *TPH1*, and *TPH2* *Mechanistic genes*: *GPR35* *Metabolic genes*: *Substrate*: *CYP2D6*, *DDC*, *IDO1*, *TDO2*, *TPH1*, *TPH2*, *WARS*, and *WARS2* *Pleiotropic genes*: *DIO2*

ABCB1 ATP-binding cassette, subfamily B (MDR/TAP), member 1, *ABCB11* ATP-binding cassette, subfamily B (MDR/TAP), member 11, *ABCC2* ATP-binding cassette, subfamily C (CFTR/MRP), member 2, *ADRA1A* adrenoceptor alpha 1A, *ADRA1s* adrenoceptors alpha 1, *ADRA2A* adrenoceptor alpha 2A, *ADRA2s* adrenoceptors alpha 2, *ADRB2* adrenoceptor beta 2, surface, *ADRs* adrenoceptors, *CHRMs* cholinergic receptors, muscarinic type, *CHRNB2* cholinergic receptor, nicotinic, beta 2 (neuronal), *CHRNs* cholinergic receptors, nicotinic type, *COMT* catechol-O-methyltransferase, *CYP1A1* cytochrome P450, family 1, subfamily A, polypeptide 1, *CYP1A2* cytochrome P450, family 1, subfamily A, polypeptide 2, *CYP2A6* cytochrome P450, family 2, subfamily A, polypeptide 6, *CYP2B6* cytochrome P450, family 2, subfamily B, polypeptide 6, *CYP2C19* cytochrome P450, family 2, subfamily C, polypeptide 19, *CYP2C8* cytochrome P450, family 2, subfamily C, polypeptide 8, *CYP2C9* cytochrome P450, family 2, subfamily C, polypeptide 9, *CYP2D6* cytochrome P450, family 2, subfamily D, polypeptide 6, *CYP2E1* cytochrome P450, family 2, subfamily E, polypeptide 1, *CYP3A4* cytochrome P450, family 3, subfamily A, polypeptide 4, *CYP3A5* cytochrome P450, family 3, subfamily A, polypeptide 5, *DDC* dopa decarboxylase (aromatic L-amino acid decarboxylase), *DIO2* deiodinase, iodothyronine, type II, *DRD2* dopamine receptor D2, *DRDs* dopamine receptors, *FKBP5* FK506-binding protein 5, *GH1* growth hormone 1, *GNAS* GNAS complex locus, *GNB3* guanine nucleotide-binding protein (G protein), beta polypeptide 3, *GPR35* G protein-coupled receptor 35, *GSTs* glutathione S-transferases, *HRH1* histamine receptor H1, *HRHs* histamine receptors, *HTR1A* 5-hydroxytryptamine (serotonin) receptor 1A, G protein coupled, *HTR1s* 5-hydroxytryptamine (serotonin) receptors 1, *HTR2A* 5-hydroxytryptamine (serotonin) receptor 2A, G protein coupled, *HTR2C* 5-hydroxytryptamine (serotonin) receptor 2C, G protein coupled, *HTR2s* 5-hydroxytryptamine (serotonin) receptors 2, *HTR3s* 5-hydroxytryptamine (serotonin) receptors 3, *IDO1* indoleamine 2,3-dioxygenase 1, *MAOA* monoamine oxidase A, *MAOB* monoamine oxidase B, *MTNR1A* melatonin receptor 1A, *MTNR1B* melatonin receptor 1B, *POMC* proopiomelanocortin, *PRL* prolactin, *SLC6A2* solute carrier family 6 (neurotransmitter transporter), member 2; *SLC6A3* solute carrier family 6 (neurotransmitter transporter), member 3, *SLC6A4* solute carrier family 6 (neurotransmitter transporter), member 4, *SOD2* superoxide dismutase 2, mitochondrial, *TDO2* tryptophan 2,3-dioxygenase, *TPH1* tryptophan hydroxylase 1, *TPH2* tryptophan hydroxylase 2, *UGT1A* UDP glucuronosyltransferase 1 family, polypeptide A complex locus, *UGT1A3* UDP glucuronosyltransferase 1 family, polypeptide A3, *UGT1A4* UDP glucuronosyltransferase 1 family, polypeptide A4, *UGT1A6* UDP glucuronosyltransferase 1 family, polypeptide A6, *UGT1A7* UDP glucuronosyltransferase 1 family, polypeptide A7, *UGT1A9* UDP glucuronosyltransferase 1 family, polypeptide A9, *UGT1A10* UDP glucuronosyltransferase 1 family, polypeptide A10, *UGT2B7* UDP glucuronosyltransferase 2 family, polypeptide B7, *UGT2B15* UDP glucuronosyltransferase 2 family, polypeptide B15, *WARS* tryptophanyl-tRNA synthetase, *WARS2* tryptophanyl-tRNA synthetase 2, mitochondrial

of CYP1A2, CYP2D6, GSTs, and SOD2; a moderate inhibitor of CYP2D6; a weak inhibitor of CYP3A4 and SLC6A4; and an inducer of ABCB1. Its transport is mediated by ABCB1 and SLC6A4 transporters [1] (Table 35.5).

Conclusions

The optimization of CNS therapeutics requires the establishment of new postulates regarding (i) the costs of medicines, (ii) the assessment of protocols for multifactorial treatment in chronic disorders, (iii) the implementation of novel therapeutics addressing causative factors, and (iv) the setting up of pharmacogenomic strategies for drug development [80]. Pharmacogenomics accounts for 30–90 % variability in pharmacokinetics and pharmacodynamics. Personalized therapeutics based on individual genomic profiles implies the characterization of five types of gene clusters: (i) genes associated with disease pathogenesis, (ii) genes associated with the mechanism of action of drugs, (iii) genes associated with drug metabolism (phase I and II reactions), (iv) genes associated with drug transporters, and (v) pleiotropic genes involved in multifaceted cascades and metabolic reactions [4].

Priority areas for pharmacogenetic research are the prediction of serious adverse reactions and the establishment of variation in efficacy [81]. Both requirements are necessary in depression, to cope with efficacy and safety issues associated with current antidepressant drugs and new CNS drugs as well.

With regard to the future of pharmacogenomics as a practical discipline to efficiently optimize therapeutics, several issues should be addressed:

(i) The education of physicians in medical genomics and pharmacogenomics is fundamental (less than 2 % of the members of the medical community are familiar with genomic science).
(ii) Genomic screening of gene clusters involved in pharmacogenomic outcomes must become a clinical routine (without genetic testing there is no pharmacogenetics).
(iii) Each patient must be a carrier of a pharmacogenetic card [82] indicating what kind of drugs he/she can take and which medications he/she should avoid.
(iv) Regulatory agencies should request pharmacogenetic data from the pharmaceutical industry when applying for drug approval.
(v) Pharmacogenetic data must be incorporated into the patient information leaflet and the pharmaceutical vademecum.
(vi) New guidelines for daily praxis, such as that of the first World Guide for Drug Use and Pharmacogenomics [1], will facilitate the understanding of the relationship between drugs and genes (and vice versa) to make drug prescription a real personalized procedure [16].

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36 Antidepressants Modulate Microglia Beyond the Neurotransmitters Doctrine of Mood Disorders

Masahiro Ohgidani, Takahiro A. Kato, Yoshito Mizoguchi, Hideki Horikawa, Akira Monji, and Shigenobu Kanba

36.1 Introduction

Mood disorders have long and dominantly been regarded to be induced by disturbances of neuronal networks including synapses and neurotransmitters. Thus, the effects of antidepressants have long been understood to modulate synaptic regulation via receptors and transporters of neurotransmitters such as serotonin and dopamine [5, 71]. Recently, microglia, immune cells in the brain, have been indicated to have positive links not only to neurodegenerative disorders [25, 26] but also to psychiatric disorders [58, 59]. Recent human imaging studies have shown microglial activation in the brain of patients with psychiatric disorders such autism, depression, and schizophrenia, especially suicide victims [19, 60, 83, 84, 87, 88, 92]. Animal models of psychiatric disorders including depression models have revealed the underlying microglial pathologies [15, 34, 45, 48]. In addition, various psychotropic drugs have been suggested to have direct effects on microglia [38]. We herein introduce up-to-date knowledge of the effects of antidepressants on microglial modulation. In addition, we summarize recent findings of the interaction between microglia and neurotransmitters such as serotonin and melatonin. Finally, we propose the possibility that modulating microglia may be a key target in the treatment of various psychiatric disorders.

36.2 Antidepressants and Microglia

36.2.1 Antidepressants

Several studies have indicated the importance of immunological dysfunction in the pathophysiology of depression [3, 20, 52, 61, 74, 76]. Also, several studies have explored the association between inflammatory cytokines and major depressive disorder [20]. Interestingly, Steiner et al. reported that an elevated microglial density had been observed in patients with depression who committed suicide [84], and a recent animal study has revealed that microglial activation is a key pathological factor in stress-induced

M. Ohgidani, PhD • H. Horikawa, MD, PhD
S. Kanba, MD, PhD
Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi Higashi-ku, Fukuoka 812-8582, Japan

T.A. Kato, MD, PhD (✉)
Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi Higashi-ku, Fukuoka 812-8582, Japan

Brain Research Unit, Innovation Center for Medical Redox Navigation, Kyushu University, 3-1-1 Maidashi Higashi-ku, Fukuoka 812-8582, Japan
e-mail: takahiro@npsych.med.kyushu-u.ac.jp

Y. Mizoguchi, MD, PhD • A. Monji, MD, PhD
Department of Psychiatry, Faculty of Medicine, Saga University, Saga, Japan

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depressive-like behavior in rodents [45]. These reports have suggested a positive linkage between depression and microglial maladaptive activation.

Recent in vitro studies have shown direct anti-inflammatory effects of antidepressants on microglia. A variety of antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants suppressed the production of NO and/or proinflammatory cytokines from LPS-activated microglia in vitro [27, 32, 49, 50, 67, 91, 93]. LPS is known as a famous activator of microglia, and it is reported that peripheral administration of LPS activates indoleamine 2,3-dioxygenase and culminates in a distinct depressive-like behavioral syndrome [66]. On the other hand, IFN-γ is a typical Th1 cytokine, and some reports have suggested a relationship between depression and IFN-γ [31, 77, 78]. We previously revealed that various types of antidepressants including SSRIs inhibited the production of NO and proinflammatory cytokines from IFN-γ-activated microglia [27, 30]. Other researchers have shown similar microglial inhibitory effects of various antidepressants including fluoxetine, imipramine, paroxetine, citalopram, and escitalopram [16, 51, 86]. In addition, lithium chloride also has similar anti-inflammatory effects [27]. On the other hand, we also revealed that various antipsychotics including aripiprazole have anti-inflammatory effects via suppressing microglial activation [6, 35, 36, 39, 79]. These antidepressants' and antipsychotics' intracellular mechanisms have not been well clarified, while we suggest the possible mechanisms in Fig. 36.1. To our knowledge, the target of both antidepressants and antipsychotics in microglia seems to be beyond the classical monoamine pathways. Further studies should be conducted to understand the deeper molecular pathways.

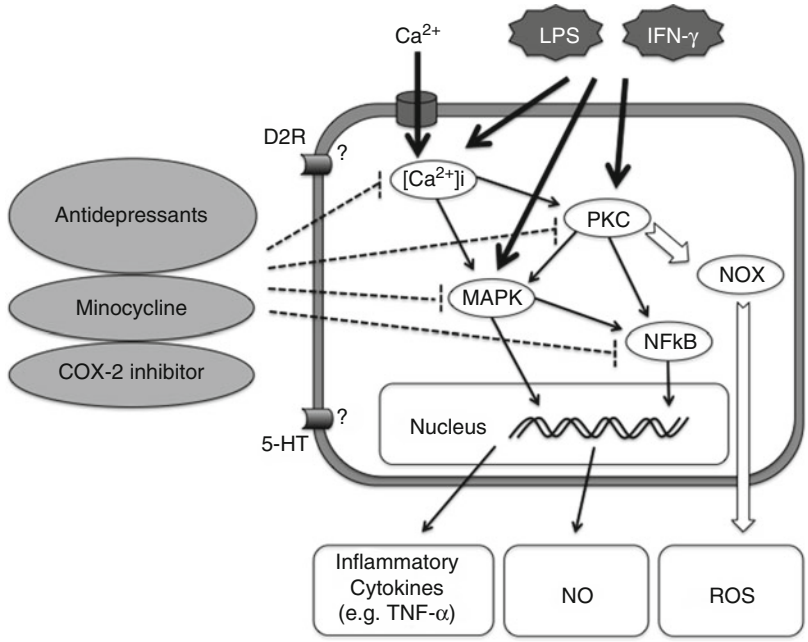

Fig. 36.1 Molecular pathways of microglial modulation by antidepressants. The anti-inflammatory actions of antidepressants (and also minocycline and COX-2 inhibitor) on microglia seem to be modulated beyond neurotransmitters such as serotonin receptors (5-HT). IFN-γ and/or LPS evokes inflammatory transactivations by upregulation of MAPK-PKC pathway and elevation of [Ca2+] in microglia. These activations translocate NFκB from the cytosol to the nucleus, which produce inflammatory cytokines and NO via nucleus response. Antidepressants seem to have potential inhibitory effects of microglial activation by modulating the intracellular cascades such as MAPK-PKC pathway, calcium signaling, NFκB cascade, and NADPH oxidase pathway

36.2.2 Other Candidate Psychotropic Drugs

Another pharmacological strategy to suppress neuroinflammation via microglial modulation is being explored for clinical therapies.

36.2.2.1 Minocycline

Tetracyclines are bacteriostatic agents that bind to the 30S ribosomal subunit of bacteria and inhibit protein synthesis. Semisynthetic second-generation tetracyclines are used as typical antibiotics in humans [18]. The minocycline, a semisynthetic tetracycline antibiotic, not only has anti-inflammatory properties but also has been shown to promote dendritic spine maturation and encourage the remyelination in oligodendrocytes [7, 13]. In addition to these evidences, minocycline readily crosses the blood-brain barrier and attenuates inflammation associated with microglial activation [28].

Minocycline has been capable to activate various molecules as follows: (1) suppresses microglial proliferation/activation as well as subsequent release of cytokines such as IL-1β, IL- 6, and TNF-α [43, 85]; [(2) inhibition of T-cell migration into the central nervous system (CNS) [14]; (3) p38 MAPK inhibition, which both preserves neurons and inhibits microglial activation [72]; and (4) possessing antioxidant properties [4]. It has been reported that minocycline can be beneficial for the treatment via suppressing microglial activation in animal models of psychiatric disorders [23, 57, 99]. A recent clinical study has shown that the minocycline add-on therapy group significantly improved both psychotic and depressive symptoms in unipolar psychotic depression [56]. In addition, therapeutic improvement in psychotic symptoms has been demonstrated by minocycline in patients with schizophrenia [2, 8, 9, 41, 47, 54, 55]. However, the primary target of minocycline for the treatment of depression and schizophrenia has not yet to be clarified. Interestingly, we have recently reported that minocycline modulates human social decision-making in healthy volunteers [37, 94, 95]. Therefore, minocycline may effect not only on psychiatric patients but also on healthy persons.

36.2.2.2 COX-2 Inhibitor

Cyclooxygenase (COX) catalyzed arachidonic acid synthesis of prostaglandins and thromboxane [82]. There are two known COX isoforms, COX-1 and COX-2, which are 90 % similar in amino acid sequence and 60 % homologous [81]. COX-1 is constitutively synthesized in many tissues, whereas COX-2, which is normally undetectable in most tissues, can be rapidly induced by proinflammatory cytokines (e.g., IL-2 and IL-6) [29]. COX-2 interacts with neurotransmitters such as acetylcholine, 5-hydroxytryptamine, and glutamate and is also involved in the regulation of the brain immune system and in inflammation via the effects of prostaglandins, in particular prostaglandin E2 (PGE2) [64]. COX-2 is known to be constitutively expressed not only in the neuron but also in microglia. In addition, COX-2 expression has been detected in activated microglia preceding neuronal cell death [1, 11], where inhibition of COX-2 was shown to be protective [11]. Therefore, COX-2 inhibitor has been speculated in the protection of neuronal cells via suppressing the activated microglia [62, 63]. The regulation of COX-2 including microglial activation was considered to be important for establishing the new therapy of psychiatric disorders. Further clinical trials are warranted to validate the effectiveness of these drugs in depression therapy [22]. Müller et al. reported the therapeutic effect of the COX-2 inhibitor, celecoxib, on symptoms of schizophrenia; the celecoxib add-on therapy group showed a significant improving effect in the positive and negative syndrome scale (PANSS) total score than solely the risperidone treatment group [65].

To verify the effects of minocycline and COX-2 inhibitors for psychiatric disorders and related mental functions, in vitro/in vivo animal studies and a variety of clinical trials should be conducted.

36.3 Neurotransmitters and Microglia

Microglia are known to have various receptors of neurotransmitters [73]. We herein introduce the microglial interactions with the following two neurotransmitters: serotonin and melatonin.

36.3.1 Serotonin

Traditionally, serotonin is known to have effects dominantly on neurons and synapses, while recent studies have suggested the direct effects of serotonin on microglia. Murine microglia express mRNA encoding serotonin receptor 5-HT1 (1a and 1f), 5-HT2 (2a, 2b and 2c), 5-HT5a, and 5-HT7 [44]. In the presence of serotonin, murine microglia migrated more rapidly toward the laser lesion in the brain slices, which is considered to be a chemotactic response to ATP. In that case, the phagocytic activity of ameboid microglia was suppressed, but that of ramified microglia was not observed [44]. This report has suggested the presence of serotonin receptors that regulate the function of microglia. Another report has suggested that serotonin did not inhibit the production of TNF-α from LPS-stimulated microglia and did not show anti-inflammatory effects in vitro [91]. On the other hand, exosomes, small vesicles that derive from fusion of multivesicular bodies with the plasma membrane, are known to be involved in secretion of certain cytokines. A latest study has shown the expression of serotonin receptors, 5-HT2a, 2b, and 5-HT4 in mice primary and BV2 microglial cells, and their functional involvement in the modulation of exosome release by serotonin [24]. This study has proposed that serotonergic neurons may stimulate exosome release from microglia in the brain. Further study should be conducted in the interaction.

36.3.2 Melatonin

Melatonin is a hormone secreted by pineal gland, which has representative function as maintenance of circadian rhythm, and recently melatonin is regarded to have a variety of neurotransmitter functions. In mammal, two types of melatonin receptors (MEL1a and MEL1b) are known to exist [21], and microglia have both of the melatonin receptors [70]. In fact, melatonin acts on microglia, and melatonin increases the microglial number and upregulates the immune genes such as complement type 3 receptor (CR3), major histocompatibility complex (MHC) class 1, MHC class 2, and CD4 antigen in rat [40].

Almost all previous studies related to the effects of melatonin on microglia have shown the function as a microglial suppressant against external stimuli. These studies have been carried out both in in vitro and in vivo studies as follows.

36.3.2.1 In Vitro Studies

Murine-derived BV2 microglial cell line and rat-derived HAPI microglial cell line are frequently used for in vitro experiments focusing on melatonin. Melatonin inhibits the apoptosis of BV2 cells caused by stimulation of amyloid beta (Aβ). Melatonin inhibits the activity of NF-κB pathway and caspase-3 due to the suppression of reactive oxygen species (ROS) production [33]. Melatonin also inhibits the upregulation of CC chemokines such as CCL2, CCL3, and CCL9 caused by stimulation of lipopolysaccharide (LPS) in BV2 cells, due to the inhibition of Akt phosphorylation [53]. Further, the production of ROS, tumor necrosis factor (TNF)-α, and interleukin (IL)-1β induced by fluoride is also suppressed by melatonin via the inhibition of Jun-N-terminal kinase (JNK) phosphorylation [98]. However, lower doses (no more than 1 μM) of melatonin do not affect the production of TNF-α and IL-1β caused by LPS stimulation in BV2 microglia [80], suggesting that suppressive effects of melatonin on microglia require the higher doses (1 μM and more). On the other hand, these suppressive effects are also confirmed by the in vitro experiments using HAPI cells. Melatonin suppresses the production of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) caused by amphetamine [89]. Furthermore, melatonin also suppresses the production of reactive nitrogen species (RNS), TNF-α, IL-1β, and IL-6 in HAPI cells [90].

Thus, suppressive effects of melatonin on microglia are demonstrated by numerous in vitro studies, while detailed molecular mechanisms of melatonin's suppressive effects remain unclear. Microglia has two types of melatonin receptors [70]; thus melatonin is supposed to affect microglia directly. However, a previous study reported that both an antagonist of melatonin receptor

(luzindole) and an inhibitor of G-protein (pertussis toxin) did not inhibit the microglial-suppressive effects of melatonin on BV2 microglia [53]. These data have indicated the existence of signaling pathways without melatonin receptors. Furthermore, it has been thought that most of these actions depend on high antioxidant ability of melatonin [33, 75, 89, 90, 98]. Contrarily, it is showed that ROS pathway does not relate to the actions of melatonin by inhibition experiment using antioxidant [53]. Taken together, suppressive effects of melatonin on microglia certainly exist in vitro; however, further studies are needed in order to clarify the molecular mechanisms of melatonin's actions.

36.3.2.2 In Vivo Studies

The effects of melatonin on microglia have also been reported using in vivo models. Almost all previous reports have shown neuroprotective effects of melatonin via suppression of microglia. In the models of encephalopathy induced by agent, melatonin inhibits the hippocampal neurodegeneration caused by kainic acid [12]. Further, melatonin inhibits the upregulation of nitrosation and oxidative stress induced by LPS, due to suppression of microglial activation. Thereby, melatonin suppresses the decline in the sensorimotor behavior [96]. In ischemia model, melatonin protects the brain against ischemic effects due to the inhibition of microglial invasion [46]. Melatonin inhibits the neuronal death and production of inflammatory cytokines via suppression of microglial activity in bacterial infection model [97]. Interestingly, melatonin inhibits the production of inflammatory cytokines via suppression of mitogen-activated protein kinase (MAPK) pathway of microglia and thereby reduces median nerve injury-induced mechanical hypersensitivity [10]. Furthermore, melatonin reduces the microglial activation and production of inflammatory cytokines by dephosphorylation of mammalian target of rapamycin (mTOR) pathway, which protects neuron from apoptosis at the site of damage [17]. There are many in vivo microglial studies related to the melatonin, and almost all of the studies demonstrate the neuroprotective action via microglial suppression. As well as in vitro studies, the mechanisms of these actions are considered as the antioxidant ability of melatonin; however detailed molecular mechanisms are still not cleared. Moreover, contradictory results were also reported in the same model of traumatic brain injury [42], which could be contributed to various factors such as melatonin dosage and administration plans.

Altogether, it is probable that melatonin affects microglia in vitro and in vivo. Additionally, melatonin suppresses the microglial activity and protects neuron against external stimuli. Further work is needed to understand these actions, which opened up new possibilities as therapeutic targets for neuropsychiatric disorders.

Conclusion

In this review, we have shown up-to-date knowledge of the effects of psychotropic drugs including antidepressants on microglial modulation and summarized recent findings of the interaction between microglia and neurotransmitters such serotonin and melatonin. Antidepressants are thought to affect mainly the neurons or neural networks over a long period of time. However, as shown above, recent studies have demonstrated that some antidepressants have a strong anti-inflammatory effect via the inhibition of microglial activation in in vitro, which may indicate that these drugs may have a deleterious effect on the brains of patients with depression. Interestingly, a recent randomized controlled trial of infliximab, a TNF-α antagonist, has suggested that infliximab improves depressive symptoms in patients with high baseline inflammatory biomarkers (assessed by high-sensitivity C-reactive protein (hs-CRP)). Thus, immunosuppressive agents themselves may thus be useful for the treatment of depression. Herein, we propose the possibility that modulating microglia may be a key target in the treatment of depression and other psychiatric disorders (Fig. 36.2). Further translational studies should be conducted to clarify this perspective, using animal in vivo studies and clinical studies with human subjects. We have recently developed a novel

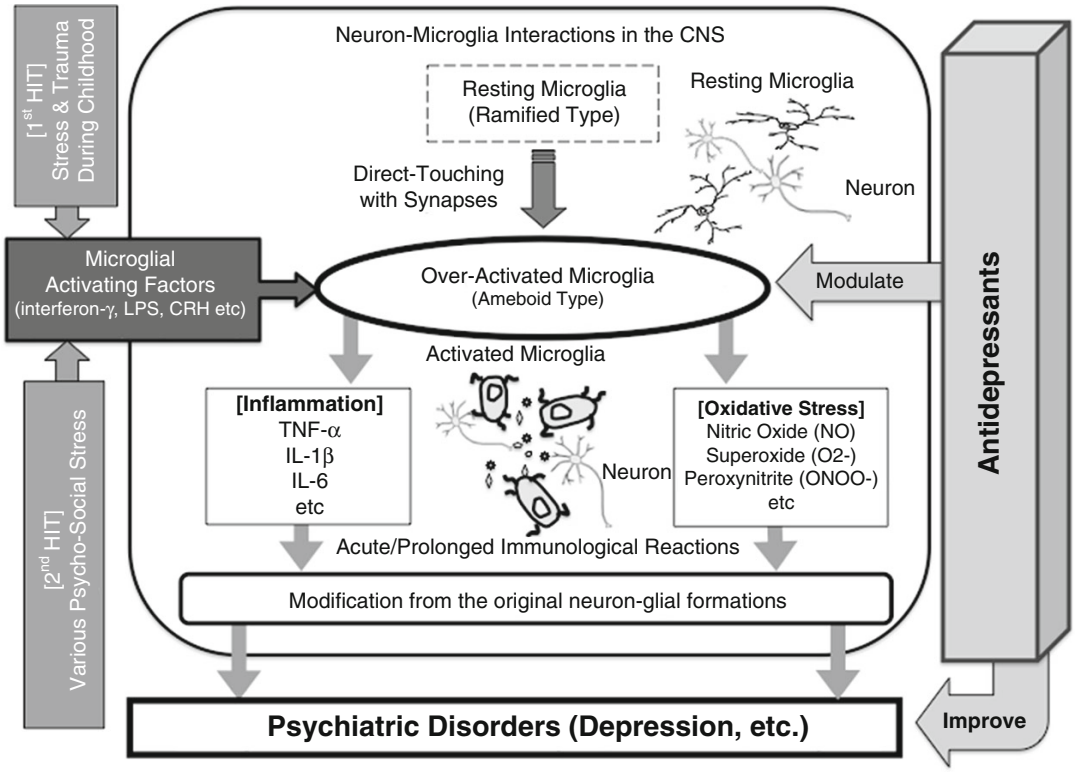

Fig. 36.2 Microglial therapeutic hypothesis of antidepressants on psychiatric disorders. Microglia are activated by various immunological/inflammatory activators during stressful life episodes (e.g., infections and physical/psychological traumas) both in pre- and postnatal states, developmental states, and other life events. Activated microglia release proinflammatory cytokines and free radicals. These mediators are known to cause brain dysfunctions such as neuronal degeneration, decreased neurogenesis, and white matter abnormalities. These neuron-microglia interactions may thus be one of the important factors in the pathophysiology of psychiatric disorders including depression. Antidepressants may have therapeutic effects on psychiatric patients, by reducing microglial inflammatory/oxidative reactions and following neuronal protective effects, which puts forward a novel therapeutic hypothesis beyond the neuron-neurotransmitter-synapse doctrine in the field of psychiatric research

translational research tool to create directly induced microglia-like (iMG) cells from human peripheral blood [68, 69]. We suppose that the iMG cells may express different activation patterns based on each patient's diagnosis, psychopathologies, and severity and may show different reactions toward psychotropic drugs including antidepressants, according to our preliminary data (unpublished). The iMG technique may help to predict drug responses before treating patients. Drug efficacy screening using the iMG cells can predict which drug will respond best to each respective patient, and the technique may be applied as a companion diagnostic tool, which has raised expectations for the application of "order-made"medicine with a reduction in side effects and a shortening of treatment period [68]. These novel research tools will open the door to explore various unknown dynamic aspects of human microglia in psychiatric disorders.

Conflict of Interest All authors declare that they have no financial conflict of interest.

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37 Brain-Derived Neurotrophic Factor (BDNF): TrkB Signaling in Depression – Biomarker and Novel Therapeutic Target

Kenji Hashimoto

Abbreviations

BD	Bipolar disorder
BDNF	Brain-derived neurotrophic factor
DG	Dentate gyrus
MDD	Major depressive disorder
NAc	Nucleus accumbens
PFC	Prefrontal cortex
proBDNF	Precursor of BDNF
TrkB	Tropomyosin receptor kinase B
VTA	Ventral tegmental area

37.1 Introduction

Mood disorders are among the most prevalent, recurrent, and disabling of mental disorders. Major depressive disorder (MDD) is a serious illness that affects approximately 17 % of the population at some point in life, resulting in major social and economic consequences [1, 2]. Very little is known about the neurobiological alterations that underlie the pathophysiology and treatment of MDD. Bipolar disorder (BD), also known as manic-depressive illness, is a brain disorder that causes unusual shifts in mood, energy, and ability to function. More than two million American adults, or approximately 1 % of the population aged 18 and older, in any given year, suffer from BD [3, 4]. BD typically develops in late adolescence or early adulthood. However, some patients suffer their first symptoms during childhood, while others develop the disease later in life. BD is often not recognized as an illness, and people may suffer for years before being properly diagnosed and treated. BD is also a long-term illness requiring careful management throughout the lifetime of a patient [3–6].

Since the precise neurobiology behind these mood disorders is unknown, concerted efforts have focused on the development of novel biomarkers which could potentially revolutionize disease recognition and management [7, 8]. Identification of biomarkers would also aid both in the diagnosis of these disorders and in the development of new therapeutic drugs. In addition, biomarkers could provide the basis for early intervention and prevention efforts, targeting at-risk individuals. To date, there are no diagnostic biomarkers available for mood disorders, although easily accessible body fluids, including blood, urine, and cerebral spinal fluid, could be potential sources for the identification of biomarkers [7, 8].

In this chapter, the author provides a review of recent findings on the role of brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB) in the

K. Hashimoto, PhD
Division of Clinical Neuroscience,
Chiba University Center for Forensic Mental Health,
1-8-1 Inohana, Chiba 260-8670, Japan
e-mail: hashimoto@faculty.chiba-u.jp

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pathophysiology of mood disorders such as MDD and BD. Secondly, the author discusses BDNF-TrkB signaling as a novel therapeutic target for mood disorders. Finally, the author will discuss the use of BDNF and its precursor, proBDNF as diagnostic biomarkers for MDD and BD.

37.2 BDNF-TrkB Signaling

BDNF, one of neurotrophic factors, is a 27-kDa polypeptide which is important in the survival, differentiation, and outgrowth of select peripheral and central neurons during development and in adulthood [8–11] (Fig. 37.1). It is well known that BDNF participates in use-dependent plasticity mechanisms such as long-term potentiation, learning, and memory [8–14].

The *BDNF* gene encodes a precursor peptide, proBDNF. Mature BDNF is initially synthesized as a precursor protein (preproBDNF) in the endoplasmic reticulum. Following cleavage of the signal peptide, proBDNF is transported to the Golgi for sorting into either constitutive or regulated secretory vesicles. ProBDNF is converted intracellularly into mature BDNF in the trans-Golgi, by members of the subtilisin-kexin family of endoproteases, or in immature secretory granules, by proprotein convertases [12]. ProBDNF is converted to mature BDNF by extracellular proteases, and this mature BDNF binds to TrkB. It was long thought that only secreted, mature BDNF was biologically active, and that proBDNF was localized exclusively in intracellular vesicles, serving as an inactive precursor. However, recent evidence suggests that proBDNF may also

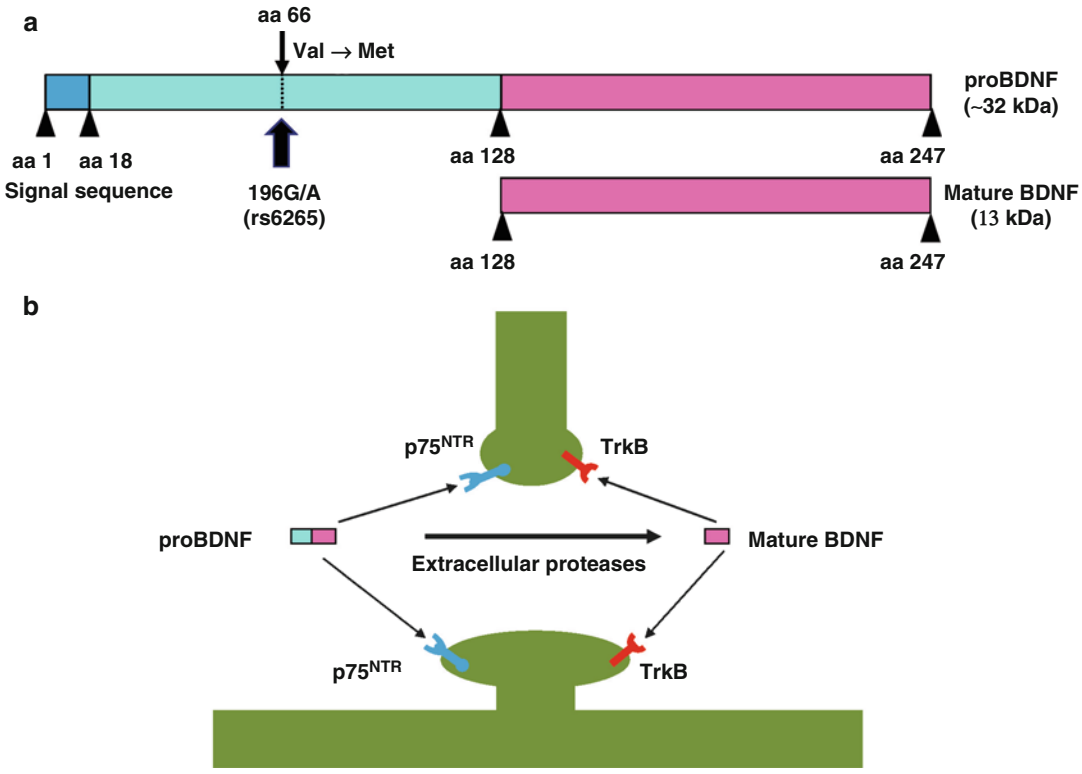

Fig. 37.1 (**a**) Structure of proBDNF and mature BDNF. *Arrowheads* indicate known protease cleavage sites involved in the processing of mature BDNF. The position of the single nucleotide polymorphism (rs6265, Val66Met) in the human *BDNF* gene is indicated by *arrows*. (**b**) Extrasynaptic cleavage of proBDNF to mature BDNF. ProBDNF preferentially binds $p75^{NTR}$. ProBDNF is cleaved by extracellular proteases at the synapses, and converted to mature BDNF. Mature BDNF preferentially binds the TrkB receptor (This figure is a modified version of previously published figures [18, 67])

be biologically active. It has been reported that proBDNF induced neuronal apoptosis, via activation of the $p75^{NTR}$ receptor. Taken together, these findings suggest that proBDNF and BDNF elicit opposite effects via the $p75^{NTR}$ and TrkB receptors, respectively, and that both proBDNF and BDNF are instrumental in several physiological functions [8, 15–19] (Fig. 37.1).

37.3 Role of BDNF in Prefrontal Cortex and Hippocampus

BDNF is implicated in the pathophysiology and treatment mechanisms of depression [18, 20–25]. A number of reports have documented antidepressant-like effects for BDNF in animal models of depression. First, infusion of BDNF into the midbrain induces antidepressant-like activity in two animal models of depression, namely, learned helplessness following exposure to inescapable shock, and learned helplessness following exposure to inescapable shock, as well as in response to the forced-swim test [26]. Another report noted that a single bilateral infusion of BDNF into the dentate gyrus (DG) and CA3 pyramidal cell layers of the hippocampus produced an antidepressant effect in the learned helplessness model of rats [27]. These effects were observed as early as 3 days after a single infusion of BDNF, and lasted for at least 10 days [27]. In addition, infusions of a broad-spectrum Trk inhibitor, K252a, blocked the antidepressant effects of BDNF, suggesting that BDNF-TrkB signaling was integral to the therapeutic action of antidepressants [27].

Loss of BDNF in the forebrain attenuated the actions of antidepressants [28], while responses typically elicited by antidepressants were lost in mice with either reduced brain BDNF levels, or inhibited TrkB signaling [29, 30]. A viral-mediated gene transfer approach found that BDNF in the DG was probably essential for mediating the therapeutic effect of antidepressants [31]. Studies using postmortem brain samples found reduced BDNF protein in the hippocampus and prefrontal cortex (PFC) of psychiatric disorder patients who had committed suicide, compared with non-psychiatric controls [32, 33]. Recently, we reported that lipopolysaccharide (LPS)-induced inflammation caused a reduction of BDNF in CA3 and DG of the hippocampus and PFC, resulting in depression-like behavior in mice [34]. Therefore, it would appear that decreased levels of BDNF in forebrain regions, such as hippocampus and PFC play a role in the pathophysiology of depression.

37.4 Role of BDNF in Ventral Tegmental Area (VTA)-Nucleus Accumbens (NAc)

Nestler's group demonstrated that increased BDNF in the ventral tegmental area (VTA)-nucleus accumbens (NAc) pathway is required for depression onset [35, 36]. It was demonstrated that intra-VTA infusions of BDNF resulted in a 57 % shorter latency to immobility, relative to control animals (a depression-like effect), and that rats given intra-NAc injections of a virus expressing a truncated version of the BDNF receptor had an almost fivefold longer latency to immobility, relative to rats that had received a vehicle injection, or a virus expressing a full-length version of the BDNF receptor (an antidepressant-like effect). Using a viral-mediated, mesolimbic dopamine pathway-specific knockdown of BDNF, Berton et al. [37] identified an essential role for BDNF in this pathway, in the depression-like behavior seen after social defeat stress. These findings suggest that BDNF activity in the VTA-NAc pathway might contribute to the development of a depressive-like phenotype [36, 37]. A study using postmortem brain samples showed 40 % higher levels of BDNF protein in the NAc of patients with depression, relative to controls [38]. Recently, we also reported that LPS-induced inflammation increased BDNF levels in the NAc, resulting in depression-like behavior in mice [34].

This data implies that BDNF acts within the VTA-NAc pathway inducing a depression-like phenotype [34, 35, 40], whereas it produces antidepressant-like effects in the hippocampus and PFC [22, 34, 39, 40].

37.5 BDNF-TrkB Signaling as a Novel Therapeutic Target for Depression

The aforementioned findings imply that decreased levels of BDNF in DG and CA3 of the hippocampus and PFC, as well as increased levels of BDNF in the NAc, may promote depression-like behavior in rodents. Recently, we reported that the TrkB agonist, 7,8-dihydroxyflavone (7,8-DHF) [41–43], and TrkB antagonist, ANA-12 [42–44], show antidepressant effects on depression-like behavior and induce morphological changes in mice after LPS administration [34]. Dosing with 7,8-DHF promoted antidepressant effects in LPS-induced depression-like behavior, and pretreatment with ANA-12 blocked this activity. Surprisingly, we found that ANA-12 alone showed antidepressant-like effects on LPS-induced depression-like behavior and that bilateral infusion of ANA-12 into the NAc produced antidepressant activity in this model. These results point to the possibility that LPS-induced inflammation causes depression-like behavior by altering BDNF levels and spine density in CA3, DG, PFC, and NAc, areas which may be targeted in the antidepressant effects of both 7,8-DHF and ANA-12 [34]. Most recently, we found that direct infusion of 7,8-DHF (but not ANA-12) into the hippocampus (CA3 and DG) and PFC and direct infusions of ANA-12 (but not 7,8-DHF) into the NAc promoted antidepressant activity in the rat learned helplessness model [45], implying that stimulation at TrkB in CA3 and DG of the hippocampus and PFC and blockade of TrkB in the NAc confer antidepressant effects. In summary, it is reasonable to infer that decreased levels of BDNF in DG and CA3 of the hippocampus and in PFC and, conversely, increased levels of BDNF in the NAc may promote depression-like behavior (Fig. 37.2).

From this point of view, we propose the use of TrkB ligands as potential therapeutic drugs for depression (Fig. 37.2). Postmortem data from depressed patients identified an association between depression and decreases in BDNF levels in the hippocampus and PFC and increases in BDNF in the NAc [32, 33, 38, 40]. From our results, TrkB agonists could represent effective therapeutic drugs for patients with decreased

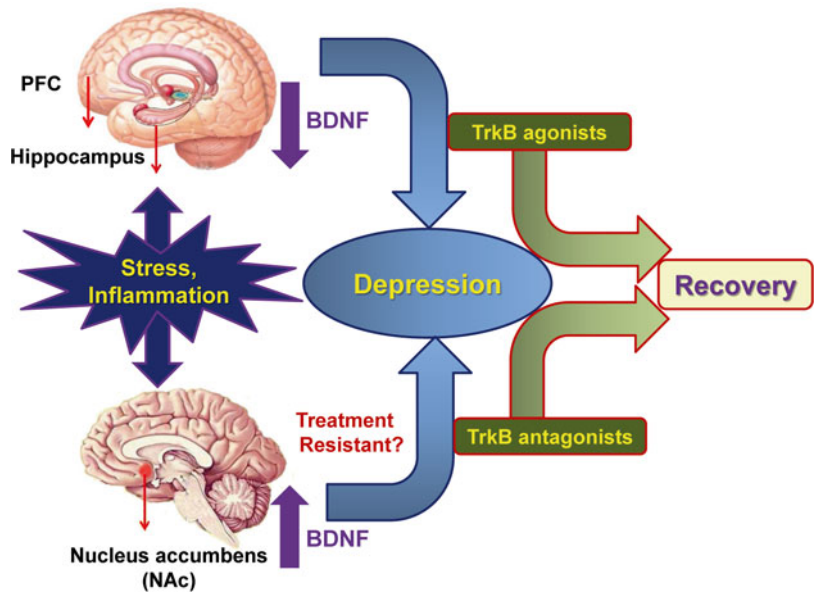

Fig. 37.2 Schematic outlining the proposed use of TrkB ligands as novel therapeutic drugs for depression. TrkB agonists could be effective therapeutic drugs for depressed patients with decreased levels of BDNF in the hippocampus and PFC. In contrast, TrkB antagonists may be potential therapeutic drugs for patients with treatment-resistant depression who may show increased BDNF levels in the NAc [34]

BDNF levels in the hippocampus and PFC. In addition, these findings add weight to the theory that TrkB antagonists can act as therapeutic agents for treatment-resistant depression in patients who show increased BDNF-TrkB signaling in VTA-NAc pathways (Fig. 37.2).

37.6 BDNF as a Biomarker for Mood Disorders

Human blood samples from unaffected individuals typically show high concentrations of BDNF [46, 47]. In 2003, we reported that serum levels of BDNF in antidepressant-naive patients with MDD were significantly lower than those of patients medicated with antidepressants or normal controls and that serum BDNF levels correlated negatively with the severity of depression [48]. Interestingly, we also reported a preliminary finding that decreased serum BDNF levels in antidepressant-naive patients recovered to normal levels and exhibited lower Hamilton Depression Rating Scale scores, after treatment with antidepressant medication [48]. Several subsequent meta-analyses have supported our findings [49–52]. A recent meta-analysis noted low serum BDNF levels in 2,384 antidepressant-free depressed patients, relative to 2,982 healthy controls and 1,249 antidepressant-treated depressed patients ($p<0.0000001$) [53]. These data indicate that serum BDNF levels could constitute a biomarker for MDD [8, 18].

Accumulating evidence also suggests that BDNF is integral to the pathogenesis of BD. Blood levels of BDNF were decreased in BD patients during manic [54–57], depressed [54, 57], and even euthymic states [58]. However, these findings have not been consistently replicated in other reports [59–61]. A meta-analysis demonstrated that patients with BD had lower levels of BDNF than healthy controls ($p=1\times10^{-4}$) [62]. A subsequent meta-analysis of 13 studies showed decreased BDNF levels in both mania and depression, when compared with controls (mania: $p<0.0001$ and depression: $p=0.02$) [63]. There were no differences in BDNF levels in euthymia, when compared to controls ($p=0.33$). Meta-regression analyses in euthymia showed that only age ($p<0.0001$) and length of illness ($p=0.04$) influenced the variation in effect size. Also highlighted was an increase in BDNF levels following treatment for acute mania ($p=0.01$). It would appear that blood BDNF levels are abnormally reduced in the manic and depressed states of BD patients and that this reduction in the manic state can be corrected by pharmacological treatment. This strengthens the case of a potential role for blood BDNF levels as a state-dependent biomarker of BD.

37.7 BDNF and Its Precursor proBDNF as Differential Biomarkers for Mood Disorders

Considering the important physiological roles of both proBDNF and mature BDNF in normal functions, potentially, invaluable clinical information could be derived from measuring the individual levels of proBDNF and mature BDNF in the body fluids of human subjects [8, 64–66]. Although BDNF levels in human blood can be measured using commercially available human BDNF ELISA kits, due to the limited specificity of the BDNF antibody, early versions of these kits were unable to distinguish between pro and mature forms of BDNF [67]. Very recently, we reported on serum levels of proBDNF and mature BDNF in healthy subjects using newly available human proBDNF and BDNF ELISA kits capable of distinguishing between the two forms [67].

Using this high specificity kit, we found that in medicated patients with MDD, serum levels of mature BDNF, but not proBDNF, were significantly lower than those of healthy controls [68]. Recently, Yoshimura et al. [69] reported on serum levels of mature BDNF, in first-episode drug-naive patients with MDD, and detected significantly lower levels in patients, compared with healthy control subjects, consistent with our report [68]. In contrast, we recently found that serum levels of mature

BDNF and the ratio of mature BDNF to proBDNF in mood-stabilized BD patients were significantly higher than in healthy controls [69]. Interestingly, serum levels of proBDNF in mood-stabilized patients with BD were significantly lower than those of healthy controls [70]. These findings in BD were confirmed in two independent cohorts (Sahlgrenska set and Karolinska set in Sweden) [70].

It is therefore possible that measuring the blood levels of mature BDNF and proBDNF could assist in the clinical problem of distinguishing between MDD and BD, thus reducing the high rates of misdiagnosis between these two diseases [71].

Conclusion

As described above, BDNF-TrkB signaling is crucial to the pathophysiology of mood disorders such as MDD and BD, and it is probable that serum BDNF levels could act as a biomarker for these disorders. Given the opposing biological effects of proBDNF and BDNF, it would be of great interest to study the precise mechanisms controlling cleavage of proBDNF to BDNF in these mood disorders. Since high specificity human proBDNF and mature BDNF ELISA kits are now available commercially, it would also be of great interest to quantify concentrations of proBDNF proteins and mature BDNF in patients and utilize these measurements as novel biological markers for mood disorders. Furthermore, TrkB agonists could represent rapid-acting antidepressants for depressive patients showing decreased BDNF levels in the hippocampus and PFC. In contrast, we propose that TrkB antagonists are capable of acting as rapid antidepressants for treatment-resistant depressive patients who show increased BDNF-TrkB signaling in VTA-NAc pathways.

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38 Targeting Opioid Receptors for Innovative Antidepressant Therapies: Rediscovering the Opioid Cure

Emmanuel Darcq, Paul Chu-Sin-Chung, Brigitte L. Kieffer, and Pierre-Eric Lutz

38.1 Introduction

Opiates and the endogenous opioid system were discovered from the early use of opium in both medicinal and recreational practices. Opiate drugs act by hijacking a complex neuromodulatory system composed of three receptors, mu, delta, and kappa, which are activated by endogenous opioid peptides known as β-endorphin, enkephalins, and dynorphins. Opioid receptors form a family of G protein-coupled receptors (GPCRs), which also includes the homologous but non-opioid nociceptin/orphanin FQ receptor. Both receptors and peptides are expressed throughout peripheral and central nervous systems [1] and have been intensively investigated for several decades. Opioids play a central role in pain processing and regulate many other aspects of physiology, including stress responses, respiration, gastrointestinal transit, as well as endocrine and immune functions [2]. Regulation of mood states by the endogenous opioid system represents another important facet of opioid physiology. The potent euphoric effects of known opiate drugs, and the high density of peptides and receptors in limbic brain areas, set the opioid system as a central player in both reward processing and mood control (see Glossary) and a feasible target to treat emotional dysfunction [3].

Mood disorders are defined as a group of diagnoses where mood disturbance and reward dysfunction are core underlying features. Among these, major depressive disorder is a chronic relapsing disease characterized by the repetition of major depressive episodes. The natural course of a depressive episode is remission (50 % at 1 year), even in the absence of medical intervention. Unfortunately, 80 % of patients experience relapse within 15 years [4]. Depression is a worldwide leading cause of disability, expected to even worsen in the next decades [5]. In the early 1900s, an opioid cure was proposed for the treatment of depressed patients, through progressive exposure to low doses of an opiate mixture. Although seemingly effective [6], this approach was hampered by the inherent addictive properties of available opiates, as is the case for pain treatment. Since the advent of monoamine targeting drugs in the 1950s, the antidepressant utility of opiates was less considered, and first-line treatments in modern medicine currently rely on

E. Darcq • B.L. Kieffer
Douglas Mental Health University Institute, McGill University, 6875 LaSalle Boulevard, Verdun, QC, Canada, H4H 1R3

P. Chu-Sin-Chung
Centre National de la Recherche Scientifique (CNRS)-UPR 3212, Institute of Cellular and Integrative Neurosciences, Strasbourg, France

P.-E. Lutz (✉)
McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, 6875 LaSalle Boulevard, Verdun, QC, Canada, H4H 1R3
e-mail: pierreeric.lutz@gmail.com

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selective serotonin reuptake inhibitors (SSRIs). SSRIs however are effective in only 40–50 % of patients [7], and identifying new targets for therapeutic intervention stands as a major challenge.

Today, opioids are reentering the therapeutic arsenal for MDD treatment. Agonists such as buprenorphine are used in the specific contexts of refractory depression [8], and depression-addiction comorbidity [9], and may have broader indications. Recent clinical and animal research data further strengthen the notion that endogenous opioids contribute to the etiology of mood disorders. Here we will summarize both genetic and pharmacological approaches that reveal mu, delta, and kappa opioid receptors as highly distinct players in reward processes and emotional responses and very different targets for clinical intervention in MDD [10–13]. The present chapter synthesizes previous reviews [11–13] and updates this rapidly evolving field of investigation.

38.2 The MOR as an Antidepressant Target

Genetic studies and behavioral pharmacology reveal an implication of MORs in psychiatric and neurological disorders. Specifically, pharmacological data demonstrate effects of MOR drugs in rodent models of depression. Historically, antidepressant-like effects of enkephalins and endorphins were first reported in the FS in rats [14]. Inhibitors of enkephalinase [15], and the general opioid antagonist naloxone [16], were later found to have antidepressant- and prodepressant-like effects, respectively, in the LH model. These results indicated that endogenous opioid peptides regulate despair-like behaviors. Later studies strengthened these findings using genetic approaches. Data from MOR KO mice concurred to establish that this receptor represents a key molecular player for reward processing of both natural stimuli and drugs of abuse. This robust activity contributes to recreational drug use and facilitates the onset of addictive behaviors [17]. The strong rewarding effects of MOR agonists likely contribute to the reported success of the "opioid depression cure" [6]. Besides, two groups reported decreased anxiety- and depressive-like behaviors in MOR KO mice, indicating the possibility of a paradoxical prodepressant role of MOR in regulating emotional responses [18, 19]. MOR-mediated mechanisms of mood control may therefore be more complex than previously anticipated, a notion that is supported by the pharmacology. Acute pharmacological activation of the MOR reduces depressive-like behaviors in some (e.g., LH in rat, TS and FS test in mice; see [16, 20–24] and Glossary), but not all [25], studies. Pharmacological antidepressant-like effects of MOR agonists are in apparent contradiction with the decreased depressive-like behaviors observed in MOR KO mice (Fig. 38.1). There are several possibilities to explain this discrepancy. First, constitutive MOR KO mice may have developed a compensatory high mood state. Second, MOR expression may be dynamically regulated during brain development, and MOR-dependent signaling may show both negative and positive effects on mood during development and adulthood, respectively. Third, acute (i.e., pharmacological treatment) versus chronic (i.e., constitutive KO) MOR activation may have antidepressant versus depressant-like effects, respectively.

The MOR is essential to mediate rewarding properties of opiates (morphine, heroin), as well as non-opioid drugs of abuse and natural stimuli [1, 17] (Fig. 38.1). Among these, social behaviors, which are recognized as intrinsically rewarding [26] and represent a major determinant of emotional well-being in humans [27], involve this receptor. Recent animal research supports a key role for MOR in social attachment and anhedonia in mice [28], as well as affiliative behaviors in prairie voles [29]. Evidence is accumulating that reward processes are highly dynamic during ontogeny, particularly in the area of social behaviors, and MOR likely contributes to these processes all along life. Maternal attachment was reduced in MOR gene knockout (KO) pups [30], stressing a very early role of MOR in parent-infant affiliative behavior. Furthermore, a recent study demonstrate that the lack of MOR signaling in MOR KO animals produces behavioral

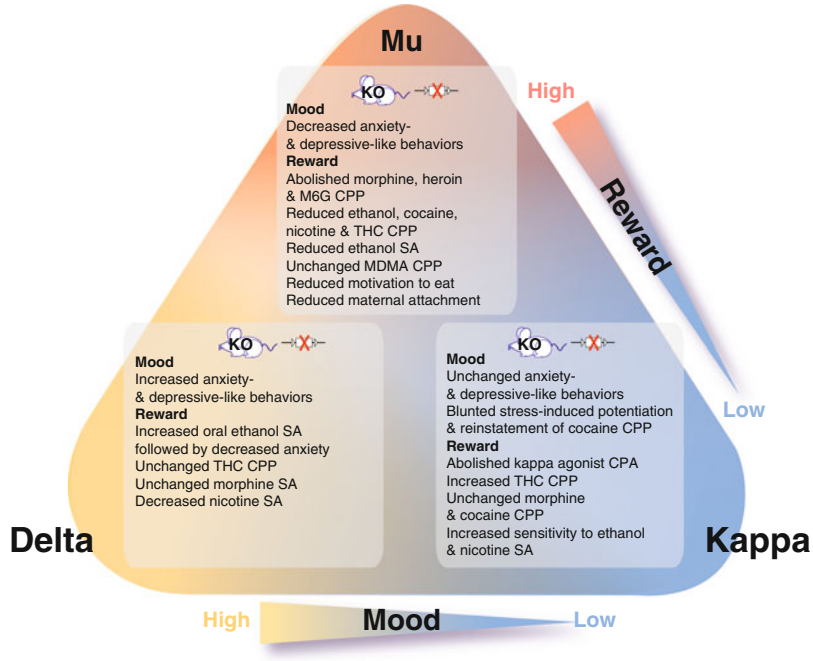

Fig. 38.1 Opioid receptor knockout (KO) mice phenotypes in models of addiction and mood disorders. Behavioral modifications are summarized for each receptor constitutive KO mouse line. Important conclusions from these data are the following: mu opioid receptor (MOR) is a key mediator of both natural and artificial rewards [17, 189, 190] and may show prodepressant activity [18, 19] although this is not supported by the pharmacology. Kappa opioid receptor (KOR) mediates dysphoria particularly under stressful conditions where the dynorphin/KOR activity is higher [158, 191, 192]. Delta opioid receptor (DOR) decreases levels of anxiety and reduces depressive-like behaviors [18], while its role in reward remains debated [78–80, 97, 98, 120]. Exhaustive reviews are available on the role of MOR in reward processes [1], the emerging roles of DOR in brain disorders [120], the potential of MOR and DOR receptors as targets to treat addiction [193], and the role of KOR in stress-induced and pro-addictive behaviors or more general psychiatric disorders [194]. Abbreviations: *CPA* conditioned place aversion, *CPP* conditioned place preference, *MDMA* 3,4-methylenedioxy-*N*-methylamphetamine, *M6G* morphine-6-glucoronide, the active metabolite of morphine, *SA* self-administration, *THC* delta-9-tetrahydrocannabinol (Adapted from Lutz and Kieffer [11])

symptoms reminiscent of autism spectrum disorders, including deficient social abilities, aggressiveness, stereotyped behaviors, and high anxiety [31]. Consistently, MOR gene variants correlate with the quality of parental attachment in infant primates (C77G [32]) and humans (A118G [33]). Recent studies also suggest that MOR-mediated responses vary with age, particularly during adolescence as social behaviors become particularly important [34]. Social play, acting as a natural reinforcer in adolescent rats and mice, induced a conditioned place preference (CPP) and was potentiated by activation of MORs in the nucleus accumbens (NAc) [35]. Further, MOR stimulation by morphine substituted for social peer exploration (with different sensitivity across inbred strains) in adolescent but not adult mice [36]. Also, heroin self-administration and seeking [37] as well as emotional responses to opioid withdrawal [38, 39] differed between adolescent and adult rodents. Together, the latter studies show increased reinforcing effects of mu opiates and decreased aversive effects of opioid withdrawal in adolescent animals, which is consistent with the notion that susceptibility to initiate addictive behaviors is higher during this life period [40].

The dopaminergic (DA) mesolimbic system regulates hedonic homeostasis and, as such, contributes to mood disorders [41]. In addiction research, a large body of work has documented the activation of DA neurons by MOR activation

in the ventral tegmental area (VTA) [17, 42]. Data indicate that activation of VTA MOR receptors regulate DA release in the NAc, with distinct effects across various subnuclei of the NAc [43]. Although much is known about the complex DA/opioid interaction in addictive behaviors, additional studies are required to understand functional interaction of MOR and DA systems in animal models of mood disorders.

Interaction of the MOR with the serotonergic (5-HT) and noradrenergic (NA) systems has been also investigated. The monoamine theory of depression posits that decreased activity of either serotonergic or noradrenergic neurons underlies depressive disorders [44]. Pharmacological studies show that the combination of subeffective doses of codeine, a weak MOR agonist, with a SSRI is behaviorally effective in the TS test [21] and that effects of two tricyclic antidepressants in the FS are antagonized by the general opioid antagonist naloxone [45], establishing interactions between 5-HT, NA, and opioid systems. The MOR tightly controls the activity of 5-HT neurons. Systemic acute morphine injection increases 5-HT release in various limbic regions in rats [46] and mice [47], an effect antagonized or mimicked by infusion into the dorsal raphe nucleus (DRN) of a MOR antagonist [48] or agonist [46], respectively. MOR in the median raphe nucleus (MRN) does not control 5-HT neurons, as morphine infusion in the MRN had no effect on local or forebrain 5-HT release [46]. Similar to DA neurons, morphine is proposed to activate 5-HT neurons through a disinhibition mechanism, with local GABAergic interneurons in the DRN expressing the MOR [48]. Chronic morphine had opposing effects and led to a compensatory upregulation of the GABAergic tone on 5-HT neurons, resulting in decreased 5-HT activity upon cessation of chronic treatment and during withdrawal [49].

Long-term effects of chronic morphine on mood have only recently been investigated in rodents. Depressive-like behaviors were detectable in acute withdrawal from chronic morphine treatment in rat [50, 51] and after more prolonged withdrawal, or abstinence, in rat [52] and mouse [38, 53]. The latter mouse studies showed that behavioral deficits progressively strengthen during 4–7 weeks of opiate abstinence [53, 54]. Importantly, these emotional deficits were prevented by chronic SSRI treatment during the withdrawal period. In this model, chronic morphine induced sequential signaling adaptations at the level of the 5-HT_{1A} receptor [55], the main autoreceptor controlling 5-HT neuron activity which transiently desensitized during morphine abstinence. Interestingly, this molecular adaptation was previously identified in several rodent models of depression [56]. Finally, mice lacking most central 5-HT neurons [57] showed normal morphine conditioned place preference (CPP), suggesting an interesting anatomical dissociation between MOR-mediated mechanisms underlying the regulation of reward processes and affective states.

NA neurons are also sensitive to endogenous opioidergic modulation. In contrast with 5-HT and DA neurons, NA neurons express the MOR. Thus, acute morphine injection increased 5-HT and DA release, but decreased NA release in the forebrain [58]. Following chronic opiate treatment, rebound from this inhibitory effect produced hyperactivity of the NA system. In particular, hyperactivity of NA neurons targeting the bed nucleus of the stria terminalis (BNST) was shown to mediate place aversion induced by acute precipitated opiate withdrawal [59] and may also be implicated in altered rewarding properties of natural stimuli (e.g., food) during abstinence from opiates [60]. To our knowledge, there is no report on the long-term consequences of MOR-mediated adaptations in the NA system on emotional responses.

Social anhedonia is raising increasing interest in drug abuse research [61]. As addiction develops, hedonic homeostasis is compromised, and reward processes gradually shift, leading to increased motivation for drugs of abuse at the expense of naturally rewarding stimuli [62]. Social dysfunction and isolation are frequently encountered in addicted individuals and used as diagnostic criteria. A growing body of evidence suggest a possible overlap in the neural circuitry underlying "social pain" and physical pain, which are both modulated by opiates, and MOR

activity may well be at the center of the intriguing relationship between these two unpleasant experiences [63]. In humans, neural sensitivity to social rejection [64] and social hedonic capacity [65] show significant association with a common variant of the MOR gene (A118G), although the functional significance of this polymorphism remains debated [66] and may implicate genetic-epigenetic interactions [67]. Specifically, a recent study shows that the A118G polymorphism in the *MOR* gene (OPRM1, rs1799971) increases susceptibility to develop major depressive disorder following major rejection life events [68]. In contrast, genetically engineered mice carrying the human A118G polymorphism present with increased social interactions and appear resilient to social defeat [69]. Positron emission tomography scanning in patients with current major depressive disorder demonstrated a reduced endogenous opioid release in brain structures involved in stress, mood, and motivation including the amygdala, thalamus, anterior insula, and nucleus accumbens [70]. This altered endogenous opioid activity seems to be the mechanism underlying the impaired emotion regulation during social rejection and acceptance in patients with major depressive disorder [70, 71]. Altogether, these data emphasize a crucial role for MOR in the mechanisms leading to depression and highlight the importance of taking the patients genetic profile into consideration when developing strategies for the treatment and prevention of major depressive disorder. Due to this positive role on mood, reward, and motivation, MOR represents an ideal target for novel antidepressants drugs.

38.3 The DOR as an Antidepressant Target

38.3.1 The DOR and the Reward System

Drugs of abuse activate brain reward systems and initially produce pleasurable effects. Repeated drug exposure may lead to loss of control over drug intake and drug dependence. A well-accepted view describes drug abuse as a three-stage vicious circle involving intoxication/withdrawal/craving episodes [72]. Animal studies have demonstrated the development of altered reward processes and enhanced stress responses [73], the setting of aberrant learning mechanisms [74] and habitual behaviors [75], the disruption of self-control [76], and the engagement of cue-induced relapse mechanisms [77], which all contribute to maintaining drug use. All three opioid receptors are largely expressed in reward and associated neural circuits [1, 72], which adapt to chronic drug exposure, and are involved in both recreational drug use (reward) and the many aspects of addictive behaviors.

Animal and human studies have clearly established that mu opioid receptors are essential to mediate rewarding properties of both natural stimuli and drugs of abuse and that kappa receptors mediate dysphoria, particularly under stressful conditions (see below) [11]. The implication of DOR in drug reward is more complex and differs across drugs of abuse. Data from conditioned place preference (CPP) and self-administration (SA) experiments for four distinct classes of drugs of abuse have been recently compiled [12] and are summarized here. Beyond drug reward, delta receptors also contribute to the development of adaptations upon chronic drug exposure, mainly examined for morphine. Overall, the large body of evidence documenting the regulation of reward processes by the DOR has immediate implications for the understanding of depression, which largely stem from altered perception of reinforcing properties of various stimuli, beyond the sole context of drugs of abuse.

38.3.1.1 Morphine

DOR knockout mice showed decreased morphine-induced CPP in two studies [78, 79]. However, this effect was independent from rewarding properties of the drug, since mutant mice also exhibited decreased conditioned place aversion to lithium, as well as normal motivation to obtain morphine in a SA paradigm [78, 80]. The association of stimuli that predict morphine administration was able to restore full expression of morphine CPP in these KO animals [81]. This

set of experiments strongly suggests that DOR does not mediate morphine reward per se, but rather modulates learning processes in a place conditioning setting. Pharmacological studies using CPP experiments in rodents also support a role for DOR involvement in place conditioning paradigms [82–84]. A potential implication from all these data is that DOR may facilitate opiate-context association, which may be critical clinically in situations of context-induced relapse. A recent study, combining gene knockout and pharmacology, suggests that DOR is required to assign hedonic value to a reward-associated stimulus, a process that might influence motivation to get a reward [85]. The latter study, involving sucrose reward, provides another indication for DOR-mediated associative processes.

Regarding chronic morphine effects, DOR knockout mice showed enhanced sensitization to locomotor effects of morphine [79], and pharmacological blockade of DOR by NTI [79] or naltriben [83] increased morphine-induced locomotor sensitization. Notably, morphine acts at mu opioid receptors in vivo [17] and does not directly activate DORs, as suggested by intact morphine analgesia [86, 87] and reward in DOR knockout mice. Therefore, the exact nature of delta-mu opioid receptor interactions in vivo and mechanisms underlying DOR-regulated chronic morphine effects remain to be clarified. Recently, refined knockin mouse models allowed the identification of subcortical networks co-expressing MOR and DOR, therefore highlighting potential functional interactions between these receptors [88].

38.3.1.2 Ethanol

Pharmacological blockade of DOR systemically by NTI, naltriben, or SORI-9409 decreased voluntary ethanol consumption [89, 90] and also cue-mediated drug seeking [91]. Those studies suggested that DORs are likely involved in both rewarding properties of alcohol and learning processes responsible for the association between drug consumption and contextual cues. Local administration of DOR antagonists into the ventral tegmental area (VTA) [92], the dorsal striatum [93], or the central nucleus of the amygdala [94] also disrupted ethanol self-administration or ethanol-induced CPP. In accordance, systemic or local administration (dorsal striatum and paraventricular nucleus of the hypothalamus) of DOR agonists stimulated ethanol SA [93, 95, 96]. Therefore, pharmacology approaches concur to indicate that DOR activation facilitates ethanol drinking in rodents at several brain sites.

Paradoxically, DOR knockout mice showed increased ethanol consumption in a two-bottle choice test (SA paradigm) [97]. Because these mutant mice exhibit high levels of anxiety [18] and ethanol SA reduced their innate high anxiety levels [97], high voluntary ethanol intake in mutant mice may reflect a self-medication approach.

38.3.1.3 Psychostimulants

DOR knockout mice showed decreased nicotine-induced CPP and SA [98]. Systemic DOR blockade by NTI produced a similar effect in rats and mice [98, 99] and also abolished amphetamine-induced CPP [100]. Endogenous DOR activity therefore seems to contribute to reinforcing properties of these two drugs, as for alcohol. NTI infused locally in the nucleus accumbens, VTA, and amygdala had contrasting effects on cocaine SA [101, 102], suggesting differing roles of DORs at distinct brain sites of reward processing. Finally, a recent SNP study showed association between an *Oprd1* variant and cocaine addiction in the African American population [103], providing support for a role of DOR in psychostimulant dependence in humans.

In sum, both genetic and pharmacologic approaches suggest a regulatory role for DOR in drug intake, seeking, and dependence, which vary depending on the drug and testing paradigm. DOR activity seems to facilitate alcohol and psychostimulant reward, but does not contribute to rewarding properties of morphine. Recently, the new delta agonist ADZ2327 (AstraZeneca) was assessed for its potential for abuse, and results only suggested a subtle behavioral effect in classical operant paradigms [104]. Examination of reinforcing effects of cannabinoids showed no difference between DOR knockout and their control mice [105], and a contribution of DOR to cannabinoid reward has not been established. DORs are also involved in other aspects

contributing to the development of drug abuse, including context learning and the development of physical dependence (morphine) or the regulation of emotional responses (alcohol). The latter aspects may be critical in the development of therapeutic strategies. Indeed, targeting aspects of DOR function other than reward (which contribute to maintain drug dependence, to the negative mood of protracted abstinence or to context-induced relapse) might be of particular interest. Finally, DOR was shown to regulate inhibitory controls in mice [106] and rats [107], revealing yet another facet of DOR function in cognitive processes with potential implication for substance abuse disorders.

38.3.2 The DOR in Emotional Regulation

Both genetic approaches and behavioral pharmacology concur to support an implication of DORs in psychiatric and neurological disorders. Furthermore, DORs agonists have recently entered clinical trials for mood disorders. The AstraZeneca compound ADZ2327 went successfully through phase II trials in patients with anxiety-associated major depressive disorder (NCT00759395) [108]. Here we will focus on the accumulating preclinical data supporting the key role of DORs in emotional processes.

Genetic studies have revealed a prominent role for DORs in emotional processing more than a decade ago. Knockout of the *Oprd1* gene, encoding DOR, led to higher anxiety-related responses and depressive-like behaviors [18]. This activity was clearly DOR selective, since neither mu receptor knockout mice nor kappa receptor knockout mice showed a similar phenotype [18]. Mice deficient for the *Penk* gene, encoding the pre-proenkephalin precursor, also showed increased levels of anxiety using a large number of experimental testing conditions [109, 110], suggesting that DOR/enkephalinergic systems critically control anxiety-related behaviors. This was later supported by experiments performed in wild-type and mu receptor mutant mice, which both showed similar decreased levels of anxiety upon systemic administration of RB101, an enkephalinase inhibitor [111]. Interestingly, overexpression of enkephalin by a virus approach in the amygdala potentiates the anxiolytic effect of benzodiazepines, and this effect is abolished by systemic naltrindole (NTI) administration [112]. Altogether, therefore, genetic approaches have opened the way to explore DOR function in the areas of anxiety (Table 38.1) and depression (Table 38.2).

Pharmacological studies using both delta agonists and antagonists in rodents confirmed anxiolytic activity of the opioid tone mediated by the DOR. As observed for knockout mice, receptor blockade by NTI, a selective DOR antagonist, increased anxiety-related behaviors in mice [113] and rats [114–116]. DOR activation by selective agonists such as SNC80 [114, 116, 117], UFP-512 [118], and ARM390 [119] decreased anxiety-related behaviors in most classical experimental paradigms (Table 38.1). Regarding depressive states, and as predicted from knockout mice data, most currently existing DOR agonists [120] consistently decreased despair-like behaviors in a large number of tests (summarized in Table 38.2) in both mice [114, 118, 121] and rats [122–125]. Although no depression-related phenotype could be detected in animals lacking *Penk* [126], systemic administration of enkephalinase inhibitors had an antidepressant effect [127, 128]. Those studies suggested that DOR/enkephalinergic signaling plays an important role in mood control.

The circuitry of emotional processing has been extensively studied [129, 130]. Sensory information reaches cortical regions mostly through the thalamus and is integrated in limbic structures such as prefrontal cortex, insula, hippocampus, and amygdala. These brain areas, which attribute emotional value to internal and external stimuli, show high DOR densities (see Fig. 38.2). Stereotaxic microinjection of several DOR agonists in the hippocampus [131], amygdala [132, 133], and cingulate cortex [113] reduced anxiety, and conversely, NTI administration at these brain sites increased levels of anxiety (Table 38.1). These data together suggest that DOR acting at the level of amygdala-cortico-hippocampal circuitry regulates emotional

Table 38.1 Delta opioid receptor function in anxiety-related behavior control

Approach	Model/compound	Test	Delta compound administration (route/dose)	Anxiety level (vs. control)	References
Genetic					
	DOR KO mice	Elevated Plus Maze		↑	[18]
		Light-Dark Box		↑	[18]
		Open Field		↔	[18]
	Enk KO mice	Open Field		↑	[109, 110]
		Elevated O-Maze		↑	[109]
		Resident-Intruder Test		↑	[109]
		Light-Dark Box		↑	[110]
		Fear Conditioning		↑	[110]
Pharmacologic					
	Rats/NTI	Elevated Plus Maze	s.c. (1, 3 or 5 mg/kg)	↑	[114–116]
		Elevated Plus Maze	Local into Hipp (0.5, 1 or 2 µg/rat)	↑	[131]
		Light-Dark Box	Local into BLA (10 pmol/rat)	↑	[113]
	Mice/NTI	Light-Dark Box	i.c.v. (1 nmol/mouse)	↑	[113]
		Light-Dark Box	s.c. (1 mg/kg)	↑	[132]
		Light-Dark Box	Local into Cingulate Cx (1 pmol/mouse)	↑	[132]
		Elevated Plus Maze	s.c. (1 mg/kg)	↑	[132]
		Elevated Plus Maze	Local into Cingulate Cx (1 pmol/mouse)	↑	[132]
	Rats/SNC80	Fear Conditioning	s.c. (1 or 3 mg/kg)	↓	[114]
		Elevated Plus Maze	s.c. (1–20 mg/kg)	↓	[114, 116]
		Open Field	s.c. (1 or 3 mg/kg)	↔	[114]
		Defensive Burying Paradigm	s.c. (5 mg/kg)	↓	[116]
		Elevated O-Maze	s.c. (5 mg/kg)	↓	[198]
	Rats/DPDPE	Elevated Plus Maze	Local into CeA (0.5 or 1.5 µg/µl; 1 µl/CeA)	↓	[133]
	Mice/UFP-512	Light-Dark Box	i.p. (1 mg/kg)	↓	[118]
		Elevated Plus Maze	i.p. (0.1 or 1 mg/kg)	↓	[118]
		Open Field	i.p. (0.1 or 1 mg/kg)	↔	[118]
	Rat/Enkephalin	Elevated Plus Maze	Local into Hipp (1, 2 or 5 µg/rat)	↓	[131]
	Mice/RB101	Elevated O-Maze	i.p. (80 mg/kg)	↓	[111]
	Rats/Opiorphin	Defensive Burying Paradigm	i.v. (1 mg/kg)	↔	[128]
	Rats/AZD2327	Modified Geller-Seifter Conflict Test	p.o. (0.5, 1 or 5 mg/kg)	↓	[108]

responses. Gene conditional approaches may be instrumental in the future to elucidate neural processes underlying DOR-controlled emotional responses at the cellular level. More recently, refined conditional knockout mice revealed that a subpopulation of DOR expressed in GABAergic neurons of the forebrain could act in an opposite manner by increasing anxiety levels, therefore

Table 38.2 Delta opioid receptor function in depressive-like behavior control

Approach	Model/compound	Test	Deltacompound administration (dose/route)	Despair level (vs. control)	References
Genetic					
	DOR KO mice	Forced Swim Test		↑	[18]
		Motility Conditioned Suppression Test		↔	[18]
	Enk KO mice	Forced Swim Test		↔	[126]
		Tail Suspension Test		↔	[126]
Pharmacologic					
	Mice/NTI	Forced Swim Test	s.c. (1 or 3 mg/kg)	↔	[114]
	Rats/SNC80	Forced Swim Test	s.c. (3.2, 10 or 32 mg/kg)	↓	[122, 123]
	Mice/SNC80	Forced Swim Test	s.c. (1 or 3 mg/kg)	↓	[114]
	Rats/DPDPE	Forced Swim Test	i.c.v. (155 nmol/rat)	↓	[124]
	Rats/Deltorphin II	Forced Swim Test	i.c.v. (0.03 or 0.1 nmol/rat)	↓	[124]
	Rats/JOM-13	Forced Swim Test	i.v. (32 mg/kg)	↓	[124]
	Mice/NIH 11082	Tail Suspension Test	i.p. (16 or 32 mg/kg)	↓	[121]
	Mice/UFP-512	Forced Swim Test	i.p. (0.1 or 0.3 mg/kg)	↓	[118]
	Rats/RB101	Forced Swim Test	i.v. (32 mg/kg)	↓	[127]
	Mice/RB101	Forced Swim Test	i.p. (80 mg/kg)	↓	[111]
		Motility Conditioned Suppression Test	i.p. (80 mg/kg)	↓	[111]
	Rats/Opiorphin	Forced Swim Test	i.v. (1 mg/kg)	↓	[128]
	Rats/AZD2327	Learned Helplessness	p.o. (1 or 10 mg/kg)	↓	[108]
	Mice/KNT-127	Forced Swim Test	s.c. (0.1, 0.3 or 1 mg/kg)	↓	[199]

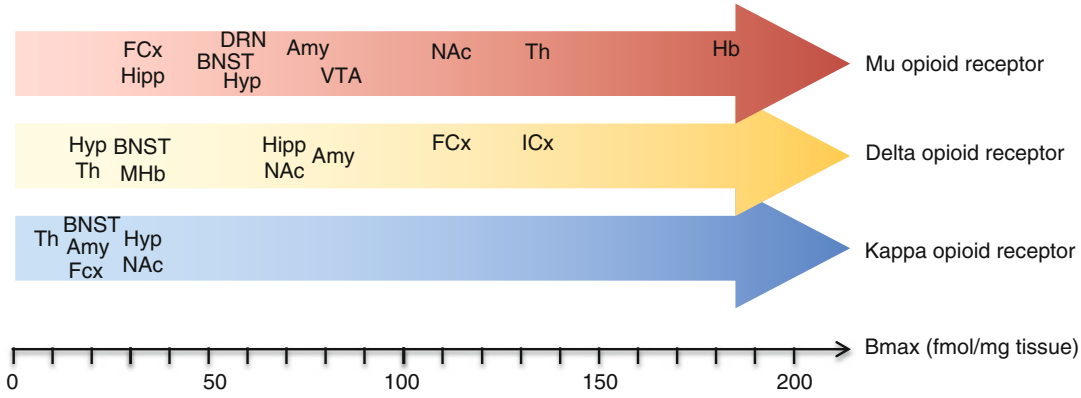

Fig. 38.2 Anatomical distribution of opioid receptors throughout neural circuits of mood in the rodent brain. Absolute protein expression levels of MOR, DOR, and KOR across relevant brain structures are shown (from [1]). Of note is that anatomical studies in humans have mainly addressed cortical networks, and comparing human with rodent data is currently difficult. For example, MOR in the anterior insular and dorsal anterior cingulate cortex has been implicated in social rejection in humans [64] yet remains unexplored in animal models of social separation or stress. Also, human imaging studies reveal a role for MORs in the thalamus (Th) in MDD [195], a receptor population ignored so far in animal models. Abbreviations: *Hb* habenula, *Hyp* hypothalamus, *ICx* insular cortex, *MHb* medial habenula, *Th* thalamus (Adapted from Lutz and Kieffer [11])

suggesting that the involvement of DOR in emotional regulation might be more complex than expected [134].

38.4 The KOR as an Antidepressant Target

38.4.1 The KOR and the Reward System

Interest in KOR pharmacology historically stemmed from the hope of developing analgesic compounds devoid of the classical abuse potential of MOR agonists (such as morphine). Unfortunately, early human studies exploring properties of KOR agonists reported potent dysphoric and psychotomimetic effects [135, 136]. While these results clearly downsized the potential of KOR agonists as painkillers, they also urged researchers to explore their intriguing dysphoric effects.

Activation of the MOR is known to induce euphoria in human and to produce reinforcement in animal models. Therefore, researchers hypothesized that MOR and KOR may have opposite effects in the regulation of reward processes, potentially through the modulation of common neuronal pathways. This framework was initially explored using CPP (see Glossary) or CPA. The seminal rat study by Shippenberg and Hertz [137] reported that, as hypothesized, systemic administration of the KOR agonist U69593 or morphine yielded opposite effects, respectively, producing CPA and CPP. While morphine-induced CPP reflects its reinforcing properties, KOR-induced CPA suggested that this receptor might be an anti-reward mechanism that contributes to a bidirectional regulation of motivation and hedonic tone.

The next step was to investigate underlying neurochemical substrates, with early studies exploring how the KOR may regulate the mesolimbic pathway (Fig. 38.3). As already mentioned, this pathway is composed of DA neurons that are located in the midbrain VTA and project to forebrain limbic structures, including the ventral striatum (or NAc) and prefrontal cortex (PFC). Animal and human data have clearly demonstrated that mood disorders and addiction both associate with major disruptions of the brain's DA reward circuitry [41], which normally acts to predict and encode the salience of environmental stimuli and natural rewards. The now classical "unitary" theory of addiction postulates that essentially all drugs of abuse enhance DA transmission in the NAc [138]. Within this line, many studies have consistently shown that acute reinforcing effects of morphine rely on disinhibition, i.e., the activation of DA neurons. This disinhibition occurs through the activation of MOR expressed by GABAergic interneurons located mainly in the tail of the VTA (tVTA or RMTg; see [42, 139]), but also in the VTA and NAc [140]. In contrast, decreased DA signaling was hypothesized to be responsible for KOR-mediated aversion. Using microdialysis, Spanagel and colleagues [141] showed that DA release in the NAc was indeed decreased following infusion of a KOR agonist into the NAc, but not into the VTA. In addition, infusion of a KOR antagonist (nor-BNI) into the NAc increased DA release, suggesting that dynorphins tonically reduce DA neurotransmission in this region. The most compelling evidence implicating DA neurons in KOR-induced aversion came recently [142] from the use of genetically modified mice using the Cre-lox recombination system [143]. Bals-Kubik et al. took advantage of a knockin mouse expressing the Cre-recombinase under transcriptional control of the endogenous promoter of the DA transporter (DAT, a specific marker of DA neurons [144]). These mice were bred with another knockin mouse harboring a conditional "floxed" KOR allele, thereby achieving the specific deletion of KOR in DA neurons (DAT KOR-cKO). At the behavioral level, KOR-induced CPA was abolished in DAT KOR-cKO mice and restored upon virally mediated KOR re-expression in the VTA [142].

In parallel, investigators undertook a brain-wide analysis of regions where recruitment of the KOR may potentially encode aversion. The effect of local KOR activation was assessed in several areas using the CPA paradigm [145]. Infusion of the KOR agonist U50,488H in the NAc was

sufficient to induce a robust CPA, consistent with the notion that KOR activation in this region decreases DA release. Surprisingly, infusions in the PFC, lateral hypothalamus, and VTA (but not in the substantia nigra and dorsal striatum) had similar effects, suggesting that multiple KOR pools may regulate hedonic tone and mood. These results also indicate that activation of VTA KOR induces CPA in the absence of any change in NAc DA release (see aforementioned neurochemical data [141]), implying the involvement of another brain region receiving DA innervation (i.e., the PFC; see below). In addition to the regulation of DA transmission, KOR expression and function are now under investigation in many other brain regions using rodent assays relevant to reward and mood (e.g., bed nucleus of the stria terminalis, BNST, amygdala, locus coeruleus; see below).

An important next goal was to identify which neuronal cell types are controlled by the KOR. Electron microscopy approaches [146, 147] found that in the NAc, half of the axons that were KOR-immunoreactive also expressed DAT. Interestingly, this study found that almost one third (29 %) of these KOR-immunoreactive axons were DAT negative but contacted presynaptic terminals of DAT-positive neurons, suggesting that the mesolimbic pathway is regulated at the level of the NAc by afferent neurons expressing the KOR. Based on recent evidence [148], it is likely that non-DAergic KOR-positive neurons are, at least in part, serotonergic (5-HT). At the level of the NAc [149], there is also evidence for KOR-dependent modulation of glutamate release, suggesting that this receptor may be expressed presynaptically by glutamatergic cortical neurons that densely innervate the NAc. To our knowledge, the behavioral relevance of the latter KOR pool has not been addressed. Finally, in the PFC [150], the KOR was mainly located on presynaptic terminals, likely corresponding to DAergic inputs, although the neurochemical identity of these neurons was not assessed.

Electrophysiology and immunohistochemistry approaches have also been used to identify KOR-expressing neurons. Application of a KOR-selective agonist in the VTA decreased spontaneous firing of a subgroup of neurons [151]. This KOR-mediated inhibition occurred in DA cells only, as indicated by tyrosine hydroxylase immunoreactivity (the rate-limiting enzyme for DA synthesis and another marker of DA neurons). Electrophysiology, retrograde tracing and microdialysis were then combined to assess whether DA neurons projecting either to the NAc or to the PFC are differentially regulated by the KOR [152]. These elegant experiments revealed that local KOR activation in the VTA hyperpolarized PFC-targeting DA neurons, but had no effect on NAc-targeting DA neurons. Accordingly, DA release was reduced in the PFC, but not in the NAc, upon VTA KOR activation.

Overall these results are consistent with previous CPA and microdialysis studies and suggest a model whereby VTA KORs do not control NAc DA tone but rather modulate DA release in the PFC to produce CPA. The previously described DAT KOR-cKO mice recently provided strong evidence for the latter hypothesis. Tejeda et al. found that infusion of a KOR agonist in the PFC decreased DA overflow in wild type (WT) but not in DAT KOR-cKO mice [153], confirming KOR-mediated control of DA transmission in the PFC. Importantly, the authors then directly tested the behavioral relevance of PFC KORs for dysphoria in rats. Infusion of a KOR antagonist into the PFC was sufficient to prevent KOR agonist-induced CPA, clearly identifying the limbic cortex as a necessary substrate for this behavioral effect.

Collectively, these results clearly indicate that NAc-projecting DA neurons express KOR in presynaptic terminals, but not in soma and dendrites [141], while PFC-projecting DA neurons express KOR in both compartments [151–153] (Fig. 38.3).

Finally, recent elegant data using electron microscopy, electrophysiology, and ICSS (see Glossary) [154, 155] have shown that the mesolimbic pathway is controlled by the antagonistic interplay between orexin and dynorphin peptidergic systems. The hypocretin/orexin system is composed of neurons originating in the lateral hypothalamus and projecting to several mesolimbic structures [156]. Importantly, orexin and

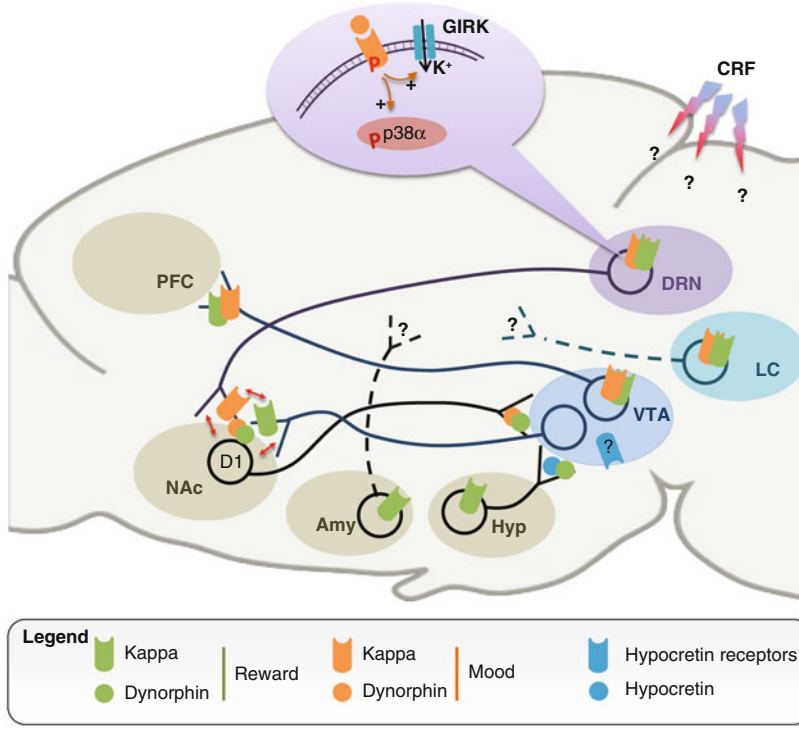

Fig. 38.3 The figure depicts a simplified scheme of neuronal circuits implicated in the regulation of reward (*green*), as well as mood and stress (*orange*), which are both modulated by dynorphins and the kappa opioid receptor (KOR). KOR-mediated inhibition of ventral tegmental area (VTA) dopaminergic neurons projecting to the prefrontal cortex (PFC) is responsible for dysphoria and conditioned place aversion [137, 141, 153]. Dynorphinergic medium spiny neurons, located in the nucleus accumbens (NAc) and expressing D1 dopamine receptors, send axonal projections back to the VTA (156), further supporting the importance of KOR in dopamine modulation and as an anti-reward agent. In addition, stressful experiences trigger widespread corticotropin-releasing factor (CRF) release in the central nervous system [172], leading to dynorphin release and KOR phosphorylation, notably in the dorsal raphe nucleus (DRN [180]) and locus coeruleus (LC [196]). Stress-induced signaling events have been extensively characterized in the DRN, where activation of KOR stimulates G protein-coupled inwardly rectifying potassium channels (GIRK; see [182]) and phosphorylation of the p38α kinase, in turn leading to translocation of the serotonin reuptake transporter to the plasma membrane and increased 5-HT reuptake [181]. Similar stress-induced activation of KOR has also been documented at the level of the NAc, which appears to be the site where SERT translocation occurs [148]. Available evidence also suggests that KOR regulation of 5-HT and DA neurotransmissions converge at the level of the NAc (*red arrows*), with important implications for comorbidity (see text for details). Further, recruitment of KOR signaling during stressful experiences has been shown: (i) in the amygdala, to potentiate conditioned place preference for drugs of abuse [197], and (ii) in the DRN [181] and LC [196], to mediate reinstatement of drug seeking. KOR-dependent modulation of monoaminergic pathways has important implications for mood regulation. Systemic treatments with KOR agonist and antagonist have pro- and antidepressant-like effects, respectively. KOR activation locally in the NAc is sufficient to achieve a prodepressant-like effect [162–166], while knockdown of dynorphins in the NAc has opposite effect [169]. Recently, hypocretin (*blue*) and dynorphin/KOR systems in the hypothalamus (Hyp) have been shown to stimulate and inhibit VTA DA neurons, respectively [154, 155]. Avenues for future investigations include the identification of (i) the signaling events following KOR activation in the LC and VTA; (ii) the brain regions receiving innervation from amygdala (Amy) and LC KOR-positive neurons; (iii) the brain sites where CRF acts to stimulate dynorphinergic neurons; and (iv) the neurochemical identity and projection targets of VTA neurons expressing hypocretin receptors. Altogether, data indicate that the KOR inhibits the activity of all three monoaminergic centers at multiple sites, thereby critically controlling their interactions in rodent models of addiction, depression, and dual diagnosis (Adapted from Lalanne et al. [13])

dynorphin were found to act as co-transmitters in neurons of the hypothalamus [154]: the two peptides co-localize in synaptic vesicles and are co-released upon electrical hypothalamic stimulation. The authors further showed that orexin and dynorphin act within the VTA to stimulate and inhibit, respectively, the excitability of DA neurons, thereby bidirectionally modulating reward (in ICSS experiments). Whether this new dual signaling pathway is relevant for mood regulation and depression will require future studies.

Activity of the dynorphin/KOR pathway on DA neurotransmission and reward function has obvious implications for addiction-related behaviors, as observed for a variety of drugs of abuse (see for recent reviews [157, 158]). More relevant to mood disorders, findings also suggest that natural rewards, such as social interactions, are also affected. In prairie voles, a monogamous rodent species, maintenance of mating pair bonds relies on the expression of aggressive behaviors toward novel conspecifics. Interestingly, this form of "social aversion" has been shown to be mediated by KOR signaling within the NAc [159]. In rodents, social play represents a highly studied, naturally occurring behavior that recruits DA neurons and triggers potent reinforcement. Systemic activation of KOR decreased social play in both rats [26, 160] and mice [161]. Altogether, these findings are relevant to our understanding of depression in human as anhedonia is a hallmark of this condition.

38.4.2 The KOR in Emotional Regulation and During Stressful Experiences

In parallel to these studies on reward, recent data have demonstrated that the KOR also controls emotional responses, in particular during stressful experiences. Pharmacological studies in rodents indicate that the dynorphin/KOR system regulates mood-related behaviors. In rats, systemic administration of KOR agonists and antagonists showed pro- and antidepressant-like effects, respectively, in the forced swim (FS) and learned helplessness (LH) tests (see [162–166] and [11] for a review). Interestingly, KOR-dependent prodepressant-like effects may be modulated by gender [167], an important aspect considering that the prevalence of depression is higher in women. Using ICSS, the authors found that the KOR agonist-induced increase in ICSS stimulation threshold was higher in male than female rats. This effect was independent from circulating levels of gonadal hormones, and was not accounted for sex differences in pharmacokinetics of the agonist. Rather, sex differences in KOR agonist-induced neuronal activation, as revealed by c-fos staining, were found in the BNST and PVN, but not in the NAc or amygdala. Therefore, in addition to the mesolimbic pathway, sex-specific KOR-dependent regulation of hedonic tone may also occur at the level of the BNST and PVN, two structures controlling stress responses and emotions.

There is evidence that dynorphins and the KOR regulate mood-related traits in part by acting at the level of the mesolimbic pathway [164, 168]. In the NAc, medium spiny neurons expressing the DA D1-receptor are known to synthesize and release dynorphins under the control of the cAMP response element-binding protein (CREB). Accordingly, prodynorphin levels were decreased in the NAc of transgenic mice overexpressing a dominant negative form of CREB. This effect was associated with decreased behavioral despair in the LH paradigm. Consistently, recent studies reported that (i) *Pdyn* knockdown (by viral expression in the NAc of an anti-*Pdyn* short hairpin RNA) decreased depressive-like behavior in the forced swim (FS) test [169] and (ii) KOR antagonism prevented depressive-like behavior that emerge during long-term withdrawal from chronic morphine exposure [170].

Beyond baseline emotional responses, data indicate that activity of the dynorphin/KOR system is potentiated by stress. Repeated exposure to FS stress produced a prodepressant-like effect that was blocked by the KOR antagonist nor-BNI and was absent in *Pdyn* KO mice [171]. Dynorphins further modulate repeated stress-dependent aversive conditioning [172]. Mice trained to associate a given odor with FS stress robustly avoided that odor. This avoidance

behavior was not observed in *Pdyn* KO mice and was blocked in WT mice by pretreatment with a KOR antagonist. Similarly, a context repeatedly paired with footshocks was aversive in WT mice; but again, this effect was absent in *Pdyn* KO mice and prevented by KOR antagonist pretreatment. Importantly, the authors showed that CRF release in the central nervous system is likely the primary event responsible for stress-induced recruitment of the dynorphin/KOR system. Results indicated that systemic injection of CRF triggered KOR phosphorylation, as revealed using a phospho-KOR antibody. Further, stress-induced CPA (mimicked by systemic or intracerebroventricular injection of CRF or the CRF_2-receptor agonist urocortin III) was absent in *Pdyn* KO mice and blocked by nor-BNI pretreatment. Following stress exposure, KOR activation and phosphorylation were identified in several brain structures, including the basolateral amygdala, hippocampus, dorsal raphe, VTA, and NAc. Altogether, these data show that dysphoric aspects of stress behaviorally manifest when CRF stimulates dynorphin release, yielding KOR activation [172].

Stress is a complex physiological process that has a primarily adaptive value but that can trigger pathological events during prolonged and excessive stressful experiences. Recently, interactions between stress and the KOR have been investigated using more sophisticated and ethologically relevant models of depression. In nature, confrontation among conspecific animals potentially generates significant consequences in terms of control over resources, access to mates, and social positions. For example, the resident-intruder social defeat paradigm [173] is a naturalistic model characterized by aggressive interactions that are unpredictable and inescapable, thereby inducing anhedonia-like symptoms such as decreased sucrose preference [149]. McLaughlin and colleagues were the first to reveal the role of dynorphins and KOR in transducing the effects of social stress [174]. Mice exposed to repeated social defeats over 3 days showed a characteristic defeated postural response, as well as an increased nociceptive threshold, or stress-induced analgesia (SIA, observed in a tail withdrawal latency assay). Both aspects were prevented in mice pretreated with a KOR antagonist or lacking the *Pdyn* gene. Another important feature of the social defeat model is that its effects show high interindividual variability, both in rats and among inbred mice, such that animals can be typically separated into susceptible and resilient groups [175]. Along this line, according to Bérubé et al. [176], expression levels of dynorphins in the NAc differ among susceptible and resilient Sprague-Dawley rats. Increased dynorphin mRNA levels (measured by qPCR) were found in the ventral striatum of susceptible rats (NAc shell, coherent with previous mice data), while surprisingly increased levels were observed in the dorsal striatum of resilient individuals, suggesting that the regulation of DA and mood by dynorphin and KOR may be more complex than anticipated. Additional studies will be necessary to further substantiate this hypothesis. In contrast, another study reported no change in dynorphin levels in VTA or NAc of socially defeated Long-Evans rats [177]. Discordance between these two studies might be explained by the different strains used or the absence of a distinction between resilient and susceptible Long-Evans rats in the latter study. Of note, Nocjar et al. found decreased dynorphin A as well as decreased orexins A and B, in the hypothalamus of defeated rats. Therefore, combined regulation of VTA DA neurons activity by these two antagonistic peptides might mediate defeat-induced KOR-dependent social aversion and be impaired following social defeat.

In addition to DAergic signaling, new findings suggest that 5-HT transmission may also be modulated by KOR in stress- and social defeat-based models of depression. Electrophysiology experiments [178, 179] initially demonstrated that the KOR regulates 5-HT neurons at the level of the dorsal raphe (DRN), a main 5-HT brain nucleus. Importantly, rescue experiments showed that the selective re-expression of KOR in the DRN of KOR KO mice is sufficient to restore the CPA induced by infusion of a KOR agonist in the NAc [180]. Together with previous findings, these results indicate that KOR in the PFC and KOR expressed by neurons present in the DRN which target the NAc (that are likely to be 5-HT neurons) are necessary and sufficient, respectively, for the

expression of KOR agonist-induced aversion. At the molecular level, acute social defeat was shown to trigger phosphorylation of KOR and the p38α kinase in the DRN [181]. Recruitment of p38α in 5-HT neurons is essential, as defeat-induced social avoidance was abolished in cKO mice in which p38α is specifically deleted from serotonin transporter (SERT)-expressing neurons (p38α-cKO^{SERT}). Phosphorylated p38α in turn promotes SERT translocation to the plasma membrane, thereby increasing 5-HT reuptake and likely mediating social avoidance. Electrophysiological recordings in brain slices [182] also showed that KOR activation dampens excitability of DRN 5-HT neurons through two mechanisms: the presynaptic inhibition of glutamatergic inputs, and the postsynaptic stimulation of G protein-gated inwardly rectifying potassium channels (GIRKs). Repeated exposure to FS stress impairs postsynaptic, but not presynaptic, effects of KOR activation. Importantly, stress-induced inhibition of KOR-mediated GIRK currents was abolished in p38α-cKO^{SERT} mice. Finally, recent evidence suggests that KOR regulation of DA and 5-HT neurons may converge at the level of the NAc to produce dysphoric and depressive-like effects. Repeated FS stress selectively increased cell-surface expression of SERT in the ventral striatum, but not in other regions examined (dorsal striatum, hippocampus, PFC, amygdala, or DRN). This effect of stress on SERT was prevented by pharmacological blockade of KOR signaling in the NAc, but not in the DRN [148]. Altogether, stressful experiences appear to recruit a CRF-dynorhin-KOR-p38α-GIRK signaling cascade within DRN 5-HT neurons, as well as KOR activation in the NAc. These molecular adaptations in turn lead to upregulation of SERT function in the NAc and ultimately affect DA function to produce behavioral symptoms. Whether similar DRN signaling is also involved in more prolonged mood-related deficits has yet to be determined.

In addition to 5-HT and DA circuits, other possible sites of KOR-dependent mood regulation notably include hippocampal neurogenesis and noradrenergic transmission. One report found that in rats, the antidepressant-like effect of the KOR antagonist nor-BNI [183] associated in the hippocampus, as well as in other structures (e.g., frontal cortex, amygdala, hippocampus, endopiriform cortex), with increased mRNA levels of BDNF, a neurotrophic factor controlling synaptic plasticity and neurogenesis. Further studies are required to better understand the relevance of this KOR/BDNF interaction.

This accumulating body of literature in the KOR field has recently prompted clinicians to undertake brain imaging studies and clinical trials [184]. Very recently, the first PET scan study using a radioactive KOR antagonist was able to demonstrate significant and widespread disruption of KOR in vivo availability in subjects suffering from fear and dysphoric symptoms following severe trauma exposure [185]. While results are nicely consistent with animal data on KOR and the mesolimbic pathway, they also suggest that other brain regions, currently poorly explored in preclinical settings, may be equally important (e.g., thalamus, insular cortex). Additional studies will be required to further assess KOR availability in well-characterized cohorts of depressed subjects. From pharmacological point of view, very recent studies have started evaluating the potential of opiate therapies combining buprenorphine (a partial MOR agonist and a KOR antagonist) with preferential (naltrexone [186]) or selective (samidorphan [187]) MOR antagonists. These efforts mainly aim at taking advantage of the reported antidepressant potential of buprenorphine (a partial MOR agonist and a KOR antagonist; see above), while dealing with the necessity to block its MOR-dependent abuse potential. In mouse models (FS and the novelty-induced hypophagia test), buprenorphine combined with naltrexone achieved antidepressant-like effects [186]. Interestingly, this dual treatment had no rewarding or aversive effect in place conditioning paradigms and did not affect anxiety-like behaviors (in the elevated plus maze and light-dark box), suggesting selective mood-enhancing properties. In human [187], a 1-week placebo-controlled study found that buprenorphine and samidorphan significantly improved depressive symptoms, while yielding no subjective opioid effects (euphoria or drug liking). Therefore, balanced agonist-antagonist opioid modulation

may represent a novel and potentially important strategy for the treatment of depression. Ideally, however, these approaches should be compared with simpler strategies using available pure KOR antagonists (e.g., nor-BNI), in order to demonstrate better efficacy on depressive symptoms and ideally better tolerance and safety profiles.

Finally, the rapidly evolving field of biased agonism (or ligand-directed signaling) raises great hopes for KOR-targeting therapeutics [188]. A major goal in the field of G protein-coupled receptors is the identification of distinct signaling pathways that may operate to control specific behavioral responses. In the near future, such approaches will likely aid in the development of antidepressants acting as KOR antagonists and devoid of potentially associated adverse effects (e.g., hyperalgesia).

38.5 Concluding Remarks

As behavioral models in rodents get more sophisticated and neurobiological mechanisms of mood disorders better understood, MOR, DOR, and KOR appear as important and highly distinct players in the regulation of emotional states. In brief, animal studies show that DOR improves mood states acutely, KOR decreases mood after stress, and MOR exerts contrasting effects on mood. Clinical trials are needed to evaluate potential benefits of DOR agonist and KOR antagonist strategies to treat depressive disorders. The risk-benefit balance of currently available MOR as antidepressants remains otherwise difficult to evaluate, partly due to their inherent abuse liability. Ongoing studies investigating biased agonism in animal models may reveal antidepressant-like efficacy independent from abuse liability at the MOR opioid receptor and open the way for novel clinical applications (Box1). Finally, research on causal factors underlying mood disorders has mainly implicated MOR receptors in the etiology of depressive states. Future studies will address the yet unknown roles of DOR and KOR in neurobiological aspects of MDD related to neurogenesis, neurodevelopment, and social behaviors.

Glossary

Anhedonia A reward-related deficit characterized by markedly diminished interest or pleasure in all, or almost all, activities.

Biased agonism A now-well recognized phenomenon, whereby GPCRs exist under distinct agonist-dependent active conformations, which form specific agonist/receptor/effector complexes that, in turn, engage distinct and agonist-specific downstream signaling and regulatory responses.

Chronic mild stress (CMS) Animals are subjected to a variety of unpredictable physical and social stressors, leading to anhedonia and despair-like behaviors.

Chronic social defeat (CSD) When exposed to resident aggressive congeners, rodents adopt a submissive characteristic posture. Repeated social defeat episodes produce long-lasting social avoidance and anhedonia.

Conditioned place preference/aversion (CPP/CPA) A Pavlovian conditioning in which a drug is repeatedly paired with a set of environmental stimuli that progressively acquire rewarding properties. The animal subsequently exhibits either preference or avoidance upon re-exposure to the environmental stimuli, a behavior dependent on learning, motivational, and hedonic mechanisms.

Conditioned suppression of motility (CSM) A close variant of learned helplessness, except the animal cannot avoid footshocks.

Forced swim (FS) Rodents, placed in an inescapable container of water, adopt a floating, immobile behavior interpreted as despair.

Intracranial self-stimulation (ICSS) An operant conditioning paradigm, in which animals learn to self-administer brief electrical pulses into specific brain regions (usually the medial forebrain bundle)

Learned helplessness (LH) Animals exposed to inescapable shocks subsequently fail to escape when able to.

Maternal separation Animals separated from their mother during early postnatal life develop depressive-like behaviors.

Mood A pervasive and sustained subjective emotional state that influences one's whole perception of the world, with generally either

a positive (mania) or negative (depression) valence; while an emotion tends to have a clear focus, mood tends to be diffuse.

Mood disorders A group of four diagnoses where mood disturbance is the main feature: (i) major depressive disorder (MDD), the repetition of major depressive episodes (MDE); (ii) dysthymic disorder, a long-lasting (2 years or more), less severe form of depression; (iii) bipolar disorder, alternation of manic and depressive episodes; and (iv) cyclothymia, a milder form of bipolar disorder.

Reinforcement A process that acutely reinforces behavior, corresponding to the positive value ascribed to natural (eating, mating, social behaviors) or artificial (drugs of abuse) stimuli.

Reward A stimulus that the brain interprets as intrinsically positive or as something to be approached.

Self-administration (SA) An instrumental conditioning in which the animals learn to self-administer (by nose poking or pressing a lever) drug of abuse into the jugular vein.

Social interactions A pair of animals is observed and scored for naturally occurring social behaviors.

Sucrose preference Rodents prefer drinking a sucrose solution over tap water. Decreased sucrose preference is widely considered a measure of anhedonia in rodents.

Stress The organism's unspecific response to internal or external challenges, with an adaptive value. When excessive or prolonged, it strongly contributes to mood disorders.

Tail suspension (TS) Rodents, when hung from the tail, adopt an immobile posture interpreted as behavioral despair.

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39 Prolactin and Somatostatin Responses to Antidepressant Therapy

Agata Faron-Górecka and Kinga Szafran-Pilch

39.1 Depression

Depression is one of the most frequently occurring psychiatric diseases and a major cause of retraction from social life, as well as premature death. The exact pathophysiology of MDD remains unknown, but the etiology has always been presumed to be heterogeneous as the diagnosis of MDD is only descriptive and likely consists of a number of syndromes with revealed symptoms. These symptoms are low mood, anhedonia, alterations in body weight not connected with diet, insomnia or hypersomnia, excitement or low motor activity, fatigue, feelings of guilt and/or worthlessness, lower intellectual abilities, lack of concentration as well as an inability to make decisions, and recurrent suicidal thoughts. Notably, major depression episodes occur twice as frequently in women (20–25 %) as in men (7–12 %) [41]. Women suffering from major depression are more frequently hospitalized, and their disorder is more likely to be chronic. Until now, there has not been a satisfactory explanation of this phenomenon, although depression more frequently occurs in women in the reproductive phase of life. Sex hormones may partly account for the higher risk of women developing depression, and transitions in the sex hormone levels in females (e.g., menarche, postpartum period, and menopause) are associated with increased vulnerability to depression [15, 56].

The main environmental factor inducing depression is stress, which – at the neurochemical level – manifests as hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis. The biological effects of stress are mediated via secretion of corticotrophin-releasing factor/hormone (CRF/CRH) leading to increased secretion of adrenocorticotropic hormone (ACTH) and release of glucocorticoids. Chronic stress results in hypersensitivity of the HPA axis, and MDD is associated with increased concentrations of CRF in the cerebrospinal fluid, increased CRF immunoreactivity and gene expression of CRF in the hypothalamic paraventricular nucleus, and down-regulation of CRFR1 receptors in frontal cortex. More than half of patients suffering from depression show hyperactivity of the HPA axis, which is resistant to feedback regulation (lack of dexamethasone inhibition). Despite the availability of many pharmacological and non-pharmacological therapies, ca. 35 % patients remain treatment resistant [67]. In addition, effective therapy leads to the normalization of HPA hyperactivity; antidepressant drugs (ADs) have been shown to inhibit HPA activity in human and animal studies [8].

One of the key neurotransmitter systems involved in the response to stress and in the development of neuropsychiatric disorders is the

A. Faron-Górecka, PhD (✉) • K. Szafran-Pilch
Department of Pharmacology, Institute of Pharmacology Polish Academy of Sciences, Smętna Street 12, Kraków 31-343, Poland
e-mail: gorecka@if-pan.krakow.pl; agatafaron-gorecka@wp.pl

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5-hydroxytryptamine (5-HT) system [9]. The majority of 5-HT neurons are located in the dorsal and median raphe nucleus. Projections from these neurons innervate several structures of the limbic system, including the amygdala and hippocampus, and it has been described that through these projections the 5-HT system regulates the fight or flight reaction to stress [34, 51], by a region-specific release of 5-HT [34, 42, 44]. Alterations in function of one of the serotonin receptors – 5-HT1A receptor – have been related to mood disorders, as imaging analyses show that depressive patients have reduced 5-HT1A receptor binding [20, 68] as well as blunted responses to 5-HT1A receptor agonists [48]. Another component of the 5-HT system is the serotonin transporter (SERT), a presynaptic protein involved in the termination of the serotonergic signal through the reuptake of 5-HT from the synapse. In the pharmacological treatment of depression, selective serotonin reuptake inhibitors (SSRIs) have been widely used. SSRIs can readily inhibit SERT activity and elevate the serotonergic tone in the brain. It has been reported that tryptophan and phenylalanine/tyrosine depletion decreases mood in patients suffering major depression [66]. Also, the role of dopamine (DA) in human-incentive motivational behavior has a considerable clinical importance. Impaired DA functioning is thought to mediate anhedonia and motor symptoms in depression [71].

The disturbances in the sleep-wake cycle are cardinal symptoms of MDD. There is clinical and epidemiological evidence that sleep disturbance in depression constitutes a risk factor for poor clinical outcomes. The risk of developing major depression is significantly increased in individuals complaining of insomnia [33]. Moreover, the persistence of REM sleep anomalies and of poor sleep quality post-psychotherapy treatment for depression is associated with nonresponse [11].

In the last decades several neuropeptide families have been discovered, and it has been shown that they have modulatory roles on neurotransmission [43, 86]. Some neuropeptides, besides CRF, have been shown to be affected by stress or to be involved in stress response in various animal models. The hyperactivity of some neuropeptides (such as substance P, corticotropin-releasing hormone, and thyrotropin-releasing hormone) and hypoactivity of other neuropeptides (such as neuropeptide Y and galanin) have been reported in major depression [86].

In this chapter we focused on two other neuropeptides: prolactin and somatostatin – two "opposite" peptides, whose involvement has been shown recently in depression and action of antidepressant drugs [25, 26, 73] (Fig. 39.1).

39.2 Prolactin

Prolactin (PRL) is a 23 kDa polypeptide hormone that is synthesized in and secreted from cells of the anterior pituitary gland, the lactotrophs. The main role of this peptide, which gave the name of this hormone (Lat. pro = for; lac = gen; lactis = milk), is to stimulate lactation, but PRL plays multiple homeostatic roles in the organism. Furthermore, other organs and tissues in the body also produce PRL, including the hypothalamus, brain stem, spinal cord, choroid plexus, mammary gland, or some immune cells, what indicates the multiple roles of this neuropeptide [6].

PRL homeostasis is the result of a complex balance between positive and negative stimuli deriving from both the external and endogenous environments. PRL regulates a wide range of biological effect including maternal, sexual, and feeding behavior [18, 32, 35], the sleep/wake cycle [63, 64], the immune system [81], the metabolism of neurotransmitters and neuropeptides [80], and stress responses [25, 29, 76, 78].

All of these functions of PRL are mediated by PRL receptors (PRLR). PRLR is a member of the class 1 cytokine receptor and it is expressed in various brain regions (e.g., the hypothalamus, cerebral cortex, hippocampus, amygdala, septum, or caudate putamen), with the highest level in the choroid plexus [25, 29, 74]. The human PRLR gene is located in chromosome 5 and contains 11 exons. These include the five alternative first exons $E1_1$–$E1_5$. Each of them has its own tissue-specific promoter. Exons 4–11 are coding

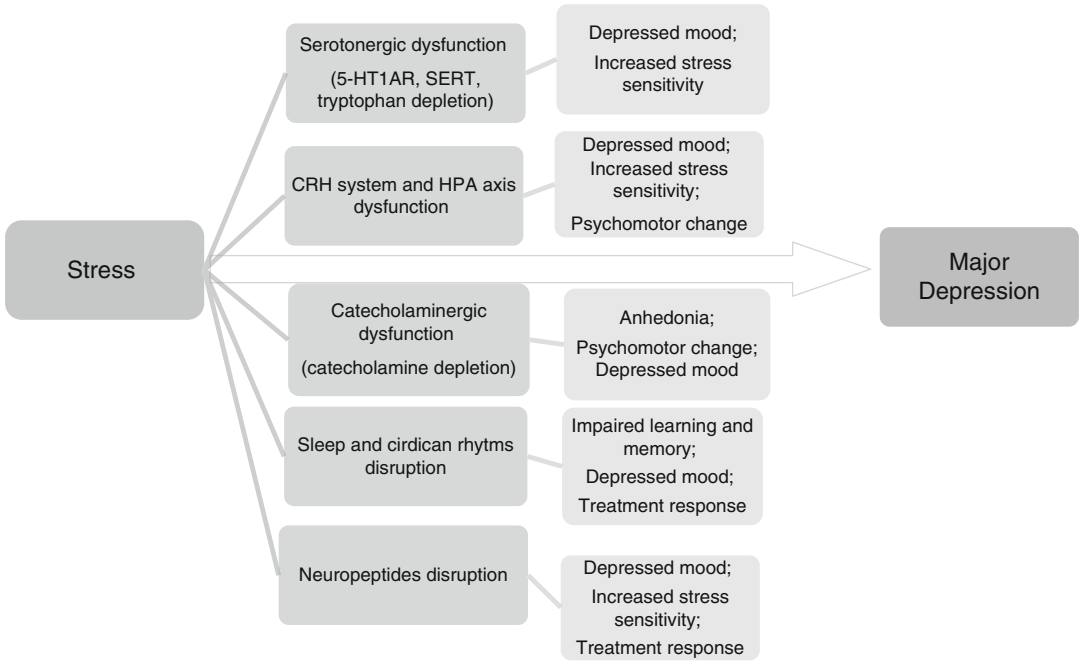

Fig. 39.1 Episodes of depression most frequently occur with various stressogenic factors

exons, whereas exon 2 is noncoding one. Sequences from exon 11 onward are present solely in the short PRLR forms. Several isoforms of PRLR have been described that result from alternative splicing and from proteolytic cleavage. The most common isoforms of PRLR are the full length activating receptors – long form and short form of PRLR. Signal transduction consists of the binding of the ligand, the PRL, to the receptor, forming a ligand-receptor complex and subsequent receptor dimerization. The JAK-2 tyrosine kinase, constitutively associated with the PRLR cytoplasmic domain, is autophoshorylated. Activated PRLR by the tyrosine residue phosphorylation binds to the STAT protein. They undergo phosphorylation and can form dimers: homo- or heterodimers. The STAT dimers translocate to the nucleus, where they regulate the expression of numerous different genes by binding to gamma-interferon-activated sequence. PRLR can involve other signal transduction pathways, e.g., MAPK cascade or activation of c-Src and Fyn kinase cascade [for more review about PRLR structure and activation mechanism: 5, 7].

39.2.1 Prolactin and Stress

PRL is often referred to as "stress hormone" because a number of physical and emotional forms of stressors stimulate its secretion from pituitary into the circulation. The increase in circulating PRL concentration is considered to be an adaptation to ensure competence of the immune system [17] and proper physiological and behavioral responses to stress [53, 69, 76]. Increase of PRL has been reported in response to different types of psychological stressors in humans. However, experimental laboratory stress studies investigating the stress response of PRL to acute psychological stress show inconsistent results [47]. The physiological importance of increased PRL in reaction to stress is not fully known. Results from animal models are also unclear. There are data indicating that PRL has a regulative role in stress response [31]. Even though ACTH is considered to play a major role in the regulation of adrenocortical secretion, it has been reported that PRL could act on the adrenal gland to drive corticosterone secretion [40]. On the other hand, it has been found that intrace-

rebral infusion of PRL into rat brain inhibits HPA axis reactivity [78]. Another postulated function of PRL is a protective role against the damage caused by stress [30]. In adult animals, PRL reduced anxiety behavior [78] and depressive-like behavior [19]. PRL administration prevents the stress-induced decrease of neurogenesis and protects the hippocampus against kainate-induced excitotoxicity [77]. PRL stimulates neurogenesis in the subventricular zone (SVZ) and activates a pool of latent precursor cells in the hippocampus [84]. Peripheral and central administration of PRL has been shown to increase PRL receptor expression in the choroid plexus [30]. The role of PRLR in the choroid plexus is to transport PRL molecules from the blood to the cerebrospinal fluid (CSF) during exposure to stress [85]. PRL is released into the blood from pituitary lactotroph cells in response to different stressors. It has been shown – using different stressful conditions – that stress has a biphasic effect on PRL secretion. When the animals were repeatedly exposed to the same stressor, some behavioral and physiological consequences of stress were reduced, suggesting that the animals become adapted to the stimulus. Among all the pituitary hormones, only ACTH and PRL levels were reduced as a consequence of repeated exposure to the same stressor. Habituation of ACTH and PRL, whenever observed, appears to be specific for the chronically applied stressor so that the potentiality of the pituitary-adrenal axis and PRL to respond to a novel stressor can be preserved [50]. Herzog et al. have shown that the stressed rats had higher PRL levels compared to control, what could have indicated either that animals were not habituated to the procedures or that the chronically stressed animals had potentiated hormonal response to a novel stressor which they were experiencing shortly before the decapitation [37]. In our experiments, using chronic mild stress model of depression (CMS), we observed similar relationship between stress and habituation to the stimulus [25]. After 2 weeks of stress, the concentration of PRL in rat plasma slightly increased in both stressed groups (stress reactive and stress nonreactive). It remains in agreement with data observed by Herzog et al. who also observed higher PRL levels after 2 weeks of chronic social instability stress. However, probably due to the use of unpredictable stress stimuli in the CMS experimental paradigm, the animals after additional 5 weeks of stress seemed not habituated to the stressed conditions, as shown by statistically significant decrease in plasma PRL level in all groups subjected to prolonged CMS. Since PRL response to acute stress appears to be sensitive to the intensity of stress experienced by the animal and also in humans [47] and repeated exposure to the same stressor results in a lesser emotional activation, it might be expected that PRL levels decrease progressively in rats having experienced particular stressor.

39.2.2 Prolactin, Depression, and Antidepressant Treatment

Given that the PRL is involved in many processes associated with mood disorders (Fig. 39.2.), its connection with depression therefore appears clear. The main symptom of disorders associated with PRL secretion is hyperprolactinemia. Hyperprolactinemia, usually defined as fasting levels of PRL at least 2 h after waking, manifests

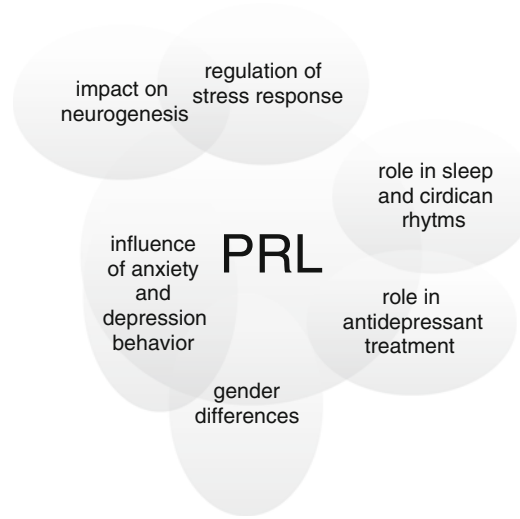

Fig. 39.2 The involvement of PRL in factors associated with mood disorders. Imposition of diagrams shows the factors linked with each other

as PRL levels greater than 20 ng/ml in men and 25 ng/ml in women and is one of the most common endocrine dysfunctions of the HPA axis. Some of the clinical manifestations of this dysfunction are anxiety and depression [27]. Stressful life events may increase PRL levels, which in turn may lead to psychiatric effects.

On the other hand, disruption of PRL release may be a consequence of antidepressant therapy. A frequent side effect of antidepressant therapy is hyperprolactinemia, but little data concerning these effects are available. Above all, drug-induced hyperprolactinemia after antipsychotic treatment is well documented, but the occurrence of this phenomenon after antidepressant treatment is less well known, although it has occasionally been reported with several classes of drugs. ADs with serotonergic activity, including SSRI, like sertraline, fluoxetine, paroxetine, citalopram, escitalopram, can cause hyperprolactinemia via the enhancement of serotonin activity by inhibiting neuronal serotonin reuptake. Monoamine oxidase inhibitors (MAOI; e.g., pargyline, clorgyline) and some tricyclics (e.g., amitriptyline, imipramine, desipramine, clomipramine, amoxapine) also can raise PRL levels by reducing catecholamines in the hypothalamus [14]. Also there has been described the relationship between the response to ADs and PRL levels [49]. In all examined groups, i.e., patients with major depression after electroconvulsive therapy, pharmacotherapy, and psychotherapy, a high-indicator PRF (prolactin response to fenfluramine) predicted a good response to ADs treatment. These data suggest that the PRF may predict the response to different forms of treatment. Similarly, it has been shown that baseline cortisol, prolactin, and L-tryptophan (TRP) availability may affect PRF and also influence the response to ADs treatment [60]. Moreover, there has been reported a decrease in PRL levels in seasonal affective disorder [16]. It is possible that low PRL levels may represent increased dopaminergic turnover, which sensitizes patients to low TRP levels and makes it less likely that they will respond to treatment. However, it has been showed that PRL responses in the fenfluramine test were increased by ADs treatment, but this effect was independent of whether depressed patients responded to treatment [13]. On the other hand, response to ADs treatment was associated with an increase in PRL response, while lack of response was not [46]. Results obtained in our laboratory – using CMS model – indicated the potential role of PRLR in the mechanism of antidepressant action [25]. After full CMS procedure combined with imipramine treatment, we observed upregulation of PRL receptor in the choroid plexus. However, the mechanism of the potential link between ADs treatment and PRL remains to be fully elucidated. Many studies have been carried out to elucidate the mechanism by which serotonin affects PRL release. One of the proposed mechanisms is through the stimulation of GABAergic neurons situated near the tuberoinfundibular dopamine cells. These dopamine cells express the 5-HT1A receptors; stimulation by serotonin would lead to inhibition of the inhibitory control of dopamine over PRL, thus causing increased PRL levels [22]. While the physiological control of PRL secretion is primarily exerted by the inhibiting action of dopamine, there are also other PRL-inhibiting factors in the CNS, including GABA, acetylcholine, norepinephrine, and somatostatin (SST) [38].

39.3 Somatostatin

In major depression, in addition to the levels of classical neurotransmitters, neuropeptide levels are also altered in certain brain regions. One of these neuropeptides is somatostatin (SST), which acts as both a hormone and a neurotransmitter. It has two active forms (SST-14 and SST-28) that are derived from the same precursor [24]. The SST-inhibiting factor was initially discovered as a neurohormone that inhibits growth hormone (GH) secretion from anterior pituitary somatotroph cells. For this function the hypophysiotropic cells are responsible, located in the anterior periventricular hypothalamic nucleus, which project to the median eminence and release the peptide in the capillaries of the hypothalamo-hypophyseal portal vessels, thus directly connecting the brain to the anterior pituitary. SST is

also a potent inhibitor of many hormonal secretions, including PRL. SST receptors (SST1R–SST5R) are G protein-coupled receptors responsible for the inhibition of adenylate cyclase, activation of potassium channels, and stimulation of tyrosine phosphatase. SST receptors are widely distributed within the CNS [3, 26, 45]. SST, besides its initially described neuroendocrine role, subserves neuromodulatory roles in the brain, influencing motor activity, sleep, sensory processes, and cognitive functions, and is altered in brain diseases like affective disorders, epilepsy, and Alzheimer's disease [82].

39.3.1 Somatostatin and Stress

The existing evidence supports an important modulatory role of brain somatostatinergic system in the stress response. Different types of stressors were shown to alter hypothalamic somatostatin mRNA levels or peptide release. Acute immobilization stress increased SST mRNA levels in the periventricular part of the PVN and decreased the peptide content in hypothalamic (supraoptic nucleus, PVN) and extrahypothalamic areas such as the locus coeruleus and nucleus of the solitary tract in rats [2]. Exposure to a novel environment such as the elevated plus maze activates SST neurons in the basolateral amygdala [10]. Handling and thereby disturbing the rats' environment increased SST release from the median eminence [1]. Likewise, social stress, such as maternal separation, elevates SST levels in nerve terminals of the median eminence in young lambs [59]. The role of SST signaling in modulating the stress response is also supported by the brain distribution of SST receptors and their regulation under stress conditions. At the receptor level, the acute exposure of rats to a potential predator led to an upregulation of SST2 mRNA expression in the amygdala and the anterior cingulate cortex [54]. This evidence supports previous studies which showed that SST2 knockout mice displayed anxiogenic response to environmental stress [83]. Despite these reports, very interesting results have been reported recently, indicating that populations of interneurons in the adult rat hypothalamus switch between dopamine and SST expression in response to exposure to short- and long-day photoperiods [21]. The shifts in SST/dopamine expression are regulated at the transcriptional level, are matched by parallel changes in postynaptic D2R/SST2R/SST4R expression, and have profound effects on behavior. Increased dopamine signaling during short days results in the decreased release of CRF from target neurons and consequently leads to a decrease in CRF and corticosteroids in the plasma and a decreased number of SST cells. These conditions are associated with a decrease in stress behaviors in nocturnal rodents. The converse is observed with decreased dopamine signaling during long-day conditions.

39.3.2 Somatostatin, Depression, and Antidepressant Treatment

There is evidence that SST may be involved in emotional processes such depression. Intracerebral administration of SST to animals induces anxiolytic and antidepressant-like effects in behavioral tests [24]. Moreover, the effects of SST2R and/or SST3R receptor agonists in the elevated plus maze and forced swim tests were equivalent to the effects of anxiolytic and antidepressant drugs, which indicate the potential role of these receptor subtypes in the action of antidepressants. The antidepressant effect may result from positive SST effects on serotonin release, while the anxiolytic-like effects of SST seem to result from the general inhibitory effects of this neuropeptide [55]. Although results obtained by Tripp et al. suggest an impaired excitation/inhibition balance in MDD potentially mediated by decreased GABA content, recently obtained results indicate downregulation of the SST levels in the anterior cingulate cortex, dorsolateral prefrontal cortex, and amygdala [36, 70, 79] of MDD patients. SST is coexpressed and coreleased with GABA in the interneurons in various regions of the human brain, including the anterior cingulate

cortex. Thus, lower levels of SST (downregulated by ~30 % at the mRNA level and ~20 % at the SST precursor level) may provide a starting point to further characterize the effects of its changes on MDD [79]. Chronic administration of ADs influences the SST levels and its receptors in the rat brain [58].

Recently, it has been shown that imipramine upregulates SST release in the mouse hypothalamus [55]. Moreover, SST expression has also been shown to be positively regulated by brain-derived neurotrophic factor (BDNF). Recent studies using transgenic mice models suggest a complex mechanism of low constitutive and activity-dependent BDNF function, particularly affecting SST/NPY-expressing GABA neurons in the amygdala [36]. SST was also investigated in the context of its influence on sleep quality as a potential therapeutic approach to sleep disturbances in MDD, although the results were inconsistent. The results obtained in our recent experiments using the CMS model indicate that the levels of SST receptors are influenced both by chronic stress and antidepressant treatment. Interestingly, in the cingulate and primary motor cortex, we observed a statistically significant decrease in SST binding sites in the group of animals that are nonresponsive to imipramine treatment as compared to rats that responded to this drug. Because some studies have indicated that imipramine increases the level of SST in the cingulate cortex, the reduction of SST receptors in the rats subjected to CMS that do not respond to imipramine may indicate its involvement in the mechanisms of drug resistance of these animals [26].

SST and dopamine interaction is well described in the literature. It has been reported that dopamine administration regulates SST [62] and that selective dopamine receptor agonists increase SST receptor density in the striatum [39]. Likewise, SST positively modulates dopamine release in the striatum [12, 75]. Numerous studies have linked potential role of SST and dopamine systems in mood regulation. Reduced levels of SST and dopamine metabolites were shown in the cerebrospinal fluid [52, 65] and in the subgenual anterior cingulate cortex [79] of depressive patients. Intracerebroventricular administration of SST results in antidepressant-like effects in the forced swim test in rats [23]. More recent studies demonstrate transmitter switching between dopamine and SST receptor expression induced by exposure to short- and long-day photoperiods which can affect depressive-like behaviors [21].

It also appears that antidepressants interact with the dopamine and SST pathways. Chronic desipramine treatment selectively potentiates SST-induced dopamine release in the nucleus accumbens and the striatum in rats [57]. Chronic SSRI treatment results in a reduction in SST levels [58]. Imipramine upregulates SST release in the mouse hypothalamus and elicits antidepressant-like effects in the tail suspension test [55].

The molecular basis of this functional interaction between the somatostatinergic and dopaminergic systems may result from the physical interaction of SST and dopamine receptors. In recent years the concept that the heterodimerization of G protein-coupled receptors (GPCRs) is important for the action of neurotransmitters has been widely studied. A number of studies have indicated that GPCRs can associate and form heteromers that exhibit unique pharmacological and functional properties. At physiological level, heterodimerization of receptors has several important implications. It appears to be essential for the correct biosynthesis of the receptors but also results in the diversification of GPCRs' functioning in terms of ligand binding, signaling, and trafficking. Therefore, heterodimers have functional properties that are distinct from the corresponding monomers. There is strong evidence that GPCR heterodimers may play a crucial role in psychiatric disorders associated with altered neurotransmitter signaling, including depression [73].

Dopamine and SST receptors are members of the GPCR superfamily and display homo- and heterodimerization. Both families show about 30 % sequence homology and appear to be structurally related [61]. Immunohistochemical studies established that dopamine D2 receptors colocalize with SST receptor subtypes in

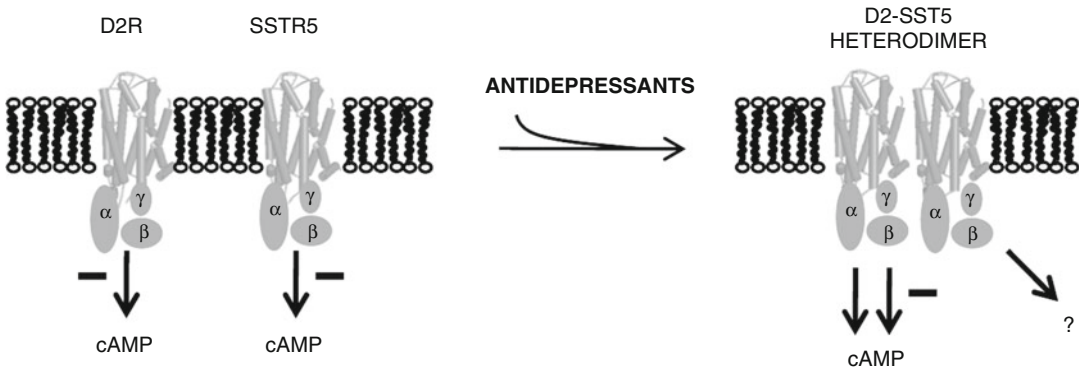

Fig. 39.3 Antidepressant treatment promotes heterodimerization of dopamine D2 and somatostatin SST5 receptors. The formation of the D2-SST5 heterodimers enhances the inhibitory effect on cyclic AMP production and may lead to the activation of unknown signaling pathways

medium-sized spiny interneurons in the striatum [4, 61] and pyramidal neurons in the cerebral cortex [61], what suggested the possibility of their direct interactions. It has been demonstrated that these receptors, when coexpressed in the same cell and in the presence of specific ligands, interact at the membrane level, forming functional heterodimers.

FRET studies revealed that D2 and SST5 receptors undergo ligand-dependent heterodimerization [61]. Treatment with agonists of either receptor or co-treatment with agonists of both receptors led to an increase in heterodimers' formation. The pharmacological properties of the D2-SST5 heterodimer were found to be distinct from those of individual receptors. The heterodimers exhibited a greater affinity for the binding of each agonist and exert a synergistic effect on the transduction pathway [61].

Recent studies indicated that antidepressant drugs promote heterodimerization of dopamine D2 and somatostatin SST5 receptors (Fig. 39.3) [72]. The efficiency of FRET was markedly increased in HEK293 cells coexpressing both receptors, after incubation with desipramine and citalopram. The mechanism of this influence might result from modulatory effect of ADs on membrane fluidity, what in turn might enhance receptor-receptor interactions. These results may provide interesting evidence of possible role of D2-SST5 heterodimerization in the mechanism of action of antidepressant drugs. The enhanced heterodimerization of dopamine D2 and somatostatin SST5 receptors should be taken into consideration for improving the occurrence of side effects induced by antidepressant therapy (Fig. 39.3).

The heterodimerization of GPCRs is regarded as a widespread phenomenon that regulates receptor functions. Knowledge about the formation of the GPCR heterodimers is of great importance for the development of therapeutical solutions. Since heterodimers can display unique pharmacological and functional properties, the novel therapeutics targeting these unique heterocomplexes may be expected to show reduced side effects and hence improve the treatment. The new class of chimeric compounds, which combine structural elements of both SST and dopamine in the same molecule and target simultaneously SST5 and D2 receptors (dopastatins), are currently under investigation [28]. They are shown to achieve a better control of hormonal hypersecretion in pituitary adenomas. Excessive secretion of the same hormones (such as prolactin, growth hormone, and adrenocorticotropic hormone) is also observed in depressive patients. Therefore, chimeric drugs acting on D2-SST5 heterodimers could be used in improvement of therapy of depression.

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The Role of Vasopressin in Anxiety and Depression

40

Julio Cesar Morales-Medina, Shannah K. Witchey, and Heather K. Caldwell

40.1 Overview

While acute stress can be beneficial and allow an organism to cope with short-term imaginary or real threats, chronic stress often has profound and deleterious effects on physiology and behavior. When maintained for long periods of time, stress can result in disruptions in immune response, neuronal dendritic remodeling, and memory deficits that can resemble some of the symptomatology observed in subjects with various emotional disorders including anxiety and depression [45, 110, 135]. Chronic stress produces these behavioral, immunological, and neurological deficits through a cascade of hormonal and neuroendocrine responses that target the hypothalamic-pituitary-adrenal (HPA) axis [102]. Activation of this axis is initiated by the release of corticotropin-releasing hormone (CRH) from the hypothalamus. However, the neuropeptide arginine vasopressin (Avp), which is co-expressed in the aforementioned neurons, can modulate the release of CRH and is an important modulator of the HPA axis [6].

Abnormal activation of the HPA axis is observed in a large number of patients diagnosed with anxiety and depression [74, 150]. Unfortunately, these disorders have a huge economic and societal cost and are expected to become the second leading cause of disability worldwide by 2020 [113]. Thus, the elucidation of the biological origins of anxiety and depression, as well as the search for novel therapeutic agents, is of the upmost urgency for individuals suffering from these disorders, their families, and society. Since the Avp system is known to interact with the HPA axis, has effects on anxiety and depression, and is an emerging therapeutic target, this chapter will review the most recent scientific work on the relationship between Avp and anxiety and mood-related disorders.

40.2 Vasopressin

Avp is an evolutionary ancient neuropeptide involved in both peripheral physiological regulatory processes, as well as the central modulation of emotional and social behaviors [30, 82]. Peripherally, Avp acts as an antidiuretic hormone, which helps regulate various functions including

J.C. Morales-Medina, PhD (✉)
Research Center for Animal Reproduction, CINVESTAV-Universidad Autónoma, Tlaxcala 90000, México
e-mail: jcmm.cinvestav@gmail.com

S.K. Witchey
Laboratory of Neuroendocrinology and Behavior, Department of Biological Sciences, Kent State University, Kent, OH 44242, USA

H.K. Caldwell, PhD
Laboratory of Neuroendocrinology and Behavior, Department of Biological Sciences, Kent State University, Kent, OH 44242, USA

School of Biomedical Sciences, Kent State University, Kent, OH 44242, USA

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renal water reabsorption and cardiovascular homeostasis [77, 93]. Centrally, Avp can act as either a neurotransmitter with direct release from axon terminals, contributing to the synaptic mode of rapid information processing [91], or as a neuromodulator with non-synaptic release (in coordination with HPA axis and limbic areas) from dendritic, somatic, and nonterminal axonal regions of the neuronal membrane to act upon distant targets [91, 101]. It is Avp's role as a neuromodulator that is thought to contribute to the regulation of emotional traits such as anxiety, stress-coping, and sociality [75, 91, 114].

40.2.1 Structure

Avp and its sister hormone oxytocin (Oxt) are nonapeptides that are a part of a peptide family that is evolutionarily conserved across phyla [32, 34]. Avp and Oxt are the result of a gene duplication that occurred more than 600 million years ago [1], and within mammals Avp is highly conserved in its sequence, usually differing from Oxt by only two amino acids. Avp is located on chromosome 2 in mice, chromosome 20 in humans, and chromosome 3 in rats and is synthesized as part of a larger preprohormone that is enzymatically cleaved within secretory granules [63, 107]. The preprohormone contains a signal peptide, the nonapeptide Avp, a neurophysin, and a glycoprotein. The gene itself is composed of three exons: the first exon encodes the 5′ noncoding promoter region, the nonapeptide, the tripeptide processing signal, and the first nine residues of the neurophysin; the second exon encodes for the majority of the neurophysin (residues 1–76); and the third exon encodes the remainder of the neurophysin (77–93/95 residues), including the carboxy terminal, as well as the glycopeptide of the Avp preprohormone [29, 56, 123]. Prepro-Avp is then cleaved to produce a signal peptide of 19 amino acids, a cyclic nonapeptide Avp, neurophysin 2, and copeptin. The neurophysin serves as a carrier for Avp and has been proposed to protect Avp from enzymatic damage and perhaps aid in packaging; however, no function has been defined for the glycoprotein copeptin [43].

40.2.2 Distribution

Comparative studies indicate that there is a conservation in the distribution of Avp cells and fibers across species [108], with Avp consistently found within the preoptic area (POA), the paraventricular nucleus of the hypothalamus (PVN), the suprachiasmatic nucleus (SCN), the supraoptic nucleus (SON), the bed nucleus of the stria terminalis (BNST), and the medial amygdala (MeA) [28, 47, 48, 130]. Avp fibers originating from these regions are widely distributed throughout the central nervous system, including but not limited to hypothalamic areas, the lateral septum (LS), the midbrain tegmentum, the periaqueductal gray, the locus coeruleus, the nucleus of the solitary tract, and the spinal cord [108]. In most species Avp is secreted from two types of cells, magnocellular and parvocellular neurons found within the PVN and SON.

40.2.2.1 Magnocellular Neurons

Magnocellular neurons are among the largest cells found within the brain and are capable of producing and releasing hormones. Avp is primarily synthesized in the magnocellular neurons of the PVN and SON of the hypothalamus. Axons of the magnocellular Avp neurons of the PVN project to the posterior pituitary where Avp is secreted into the peripheral circulation [11]. The secretion of Avp by magnocellular neurons regulates water conservation within the kidneys and depends on osmotic stimulation [94].

40.2.2.2 Parvocellular Neurons

Parvocellular Avp neurons are located within the PVN and have lower levels of Avp expression compared to the magnocellular neurons. However, their projections are more extensive, extending to the brain stem, spinal cord, and median eminence [9, 103]. The axons of the parvocellular neurons from the PVN terminate in the external layer of the median eminence where Avp is secreted into the pituitary portal circulation. Avp then is able to induce the secretion of anterior pituitary hormones, such as adrenocorticotropic hormone (ACTH) [11]. The secretion of Avp from parvocellular neurons is independent of osmotic regulation,

and stimulation of these neurons has been linked to stress and glucocorticoid release [3, 44]. In addition to the PVN, Avp is expressed within parvocellular neurons in extrahypothalamic regions of the brain including the MeA, BNST, and SCN [139]. The expression of Avp in these other areas of the brain is responsible for its neuromodulatory effects. For example, Avp-like neurons in the MeA and BNST projections extend to the LS and are implicated in the modulation of social and emotional behaviors [48, 114].

40.2.3 Receptors: Structure and Distribution

Avp has three receptor subtypes: the Avp 1a receptor (Avpr1a), which was first identified in the vascular system; the Avp 1b receptor (Avpr1b), which was first identified in the anterior pituitary; and the Avp 2 receptor (Avpr2), which was initially identified in the kidneys [17]. These receptors are widely distributed, but only the Avpr1a and the Avpr1b have been localized to the central nervous system (CNS), though all three are found peripherally. Of note, there is one study that describes Avpr2 expression in the brain, but these findings are controversial and have not been corroborated by other investigators [78]. All three receptor subtypes belong to a family of heptahelical guanine nucleotide-binding protein-coupled receptors (GPCRs). The Avpr1a and Avpr1b receptors activate $G_{\alpha q/11}$ GTP-binding proteins, which in turn activate phospholipase C with the help of $G_{\beta\lambda,}$, whereas the Avpr2 is coupled to the Gs and adenylate cyclase pathway [76]. All three receptors can cause changes in several intracellular messengers, such as cAMP, Ca2, inositol 1,4,5-trisphosphate, and diacylglycerol [76, 105, 144, 145].

The Avpr1a is widely expressed throughout the brain and in peripheral tissues; though, this review is focused primarily on its central expression, please see [16, 22, 71] for more information about its peripheral expression. The Avpr1a is found throughout the brain stem, cerebral cortex, hippocampus, hypothalamus, striatum, and olfactory bulbs [115, 116, 143] and has been studied extensively for its role in the modulation of social behaviors [2, 7, 8, 30, 32, 86, 142, 151].

The Avpr1b is mostly expressed in the anterior pituitary in the corticotrophs where it acts as an important modulator of the stress response. Reports of the exact distribution of the Avpr1b within the central nervous system are conflicting. Initially, in rats, Avpr1b transcripts and immunoreactive cell bodies were reported in the olfactory bulb, piriform cortical layer II, septum, cerebral cortex, hippocampus, PVN, SCN, cerebellum, and red nucleus [66, 97, 133, 141, 146]. Later, with the use of a more specific riboprobe, Avpr1b was found to be prominently expressed within the CA2 region of the hippocampus with only a few neuronal cells labeled in the hypothalamus and amygdala [157]. Thus, it has been proposed that the early studies may have used antibodies or riboprobes that lacked specificity [157]. Peripherally, low levels of the Avpr1b have been found in numerous tissues; for review, please see [97, 86].

40.3 The Hypothalamic-Pituitary-Adrenal Axis and the Limbic Circuit

The hypothalamus, the pituitary gland, and the adrenal gland make up the HPA axis, also referred to as the stress axis, which is important for the maintenance of homeostasis. The HPA axis is key to the regulation of the stress response, anxiety, and mood. The limbic circuit is comprised of five brain regions and their inner connections: the limbic cortex, the hippocampal formation, the amygdala, the septal area, and the hypothalamus [65]. The limbic circuit structures function to control emotional responses, memory, and social cognition [35, 132]. These two systems, the HPA axis and the limbic circuit, dynamically interact with one another to balance the physiological and behavioral response to stress, with different structures of the limbic system either inhibiting or stimulating the HPA axis dependent on the type of stressor [65].

The HPA axis stimulates corticosteroid release through either direct or indirect activation. Its direct

activation is usually in response to a threat, which is recognized by either somatic, visceral, or circumventricular pathways [64]. An example of direct activation of the PVN is via the ascending nociceptive pathways (e.g., pain) [118]. The indirect activation, or anticipatory activation, can occur in the absence of a direct threat, in preparation for future homeostatic threat, and can be generated by either innate defensive predisposition (e.g., aversion to predators) [53] or memory of an event or object (e.g., fear conditioning) [147]. In this case it is the limbic circuit that stimulates the HPA axis. While the limbic circuit has almost no direct connections to the PVN, it innervates the BNST, which then projects to the PVN [40]. The HPA axis relies on these BNST connections to relay the information from limbic structures and aid in the interpretation of the external cues [64]. The MeA and the hippocampus are the limbic regions that connect to the BNST [37], though their effects oppose one another. The MeA primarily uses excitatory signals to simulate the HPA axis [26], whereas the hippocampus inhibits the HPA axis [158]. However, whether direct or indirect, stimulation of the HPA axis results in the production of myriad hormones, including CRH, Avp, ACTH, and the glucocorticoids [81].

40.3.1 Interactions Between Adrenocorticotropic Hormone, Corticotropin-Releasing Hormone, and Vasopressin

In response to the activation of the HPA axis, neurons within the PVN release CRH and Avp, which are then carried to the pituitary gland by the hypothalamo-hypophyseal portal system. CRH is the most critical as it modulates the autonomic, immune, and behavioral effects of stress [36, 85] and stimulates the corticotrophs to release ACTH. After a stressful encounter, ACTH is rapidly released into the bloodstream and travels and ultimately binds with receptors in the adrenal glands, promoting the synthesis and secretion of the glucocorticoids (cortisol in humans and corticosterone in most rodents) [10]. This surge of ACTH is followed by a "shut-off" signal generated by glucocorticoids as well as other neuronal feedback mechanisms [81]. The significance of Avp release is that it synergizes with CRH resulting in a greater secretion of ACTH and then either peptide generates when administered alone [12, 57, 89, 128] (See Fig. 40.1).

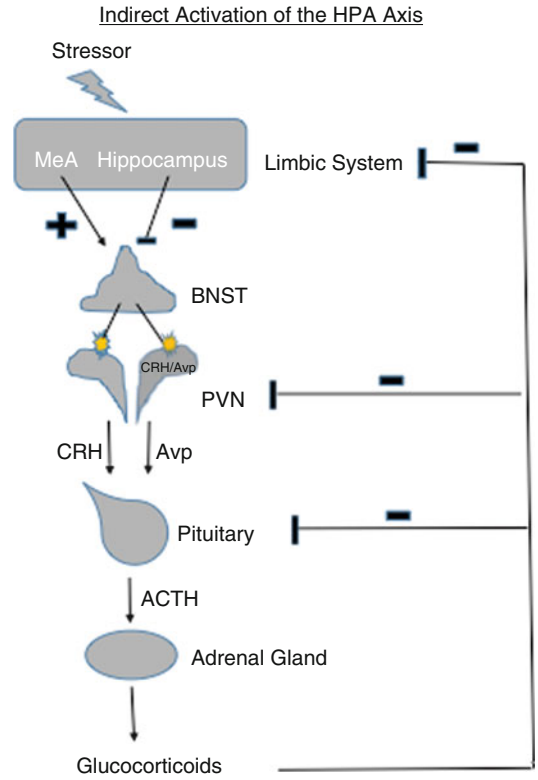

Fig. 40.1 In the presence of a perceived threat (stressor), such as an innate defensive predisposition or the memory of an event or object, different regions of the limbic system can be activated (e.g., the medial amygdala (MeA) or hippocampus). These regions then relay their signals, which can be excitatory or inhibitory, to the bed nucleus of the stria terminalis (BNST). From the BNST there are projections to the paraventricular nucleus (PVN), where parvocellular neurons that express both corticotropin-releasing hormone (CRH) and vasopressin (Avp) are stimulated. CRH and Avp then act synergistically to stimulate the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. ACTH, in turn, stimulates the release of glucocorticoids from the adrenal cortex. The glucocorticoids then exert negative feedback on all levels of the axis. In anxiety and depressive disorders, there is a dysregulation of this pathway

40.3.2 Co-expression of Corticotropin-Releasing Hormone and Vasopressin

Similar to Avp, CRH is primarily synthesized in parvocellular neurons within the PVN. CRH is also expressed by neurons in the amygdala, cerebrocortical area, septum, and hippocampus. In rodents, CRH labeling within the PVN is restricted to parvocellular neurons with approximately 50 % of these neurons co-localizing with Avp [154]. There is also co-localization of CRH and Avp within neurons of the median eminence, where CRH axons terminate on capillary walls [18]. Unlike rodents, in humans, all of the CRH-immunopositive neurons of the PVN co-localize with Avp [111]. Further, this co-localization in parvocellular neurons increases after activation of the HPA axis [119]. For example, following an acute stressor, maximum levels of heteronuclear RNA for Avp and CRH occur at 5 min and 2 h, respectively [88]. The amount of either CRH or Avp secreted into the hypothalamo-hypophyseal portal system also varies depending on the type of stressor [44, 84, 137].

40.3.3 Regulation by Glucocorticoids

While the secretion of glucocorticoids in the short term can be beneficial, prolonged exposure can cause serious metabolic, immune, and psychological dysfunction [104]. Glucocorticoids signal through two types of receptors: (1) glucocorticoid receptors and (2) mineralocorticoid receptors, for which they have high affinity and are capable of binding at low concentrations [127]. Glucocorticoid and mineralocorticoid receptors can be found throughout the brain (e.g., prefrontal cortex, amygdala, hippocampus, and other limbic structures), though mineralocorticoid receptors have lower levels of expression compared to glucocorticoid receptors [14]. Glucocorticoid receptors are only activated when glucocorticoids are at high concentrations, i.e., during a stressful event [46].

Following the activation of the HPA axis, glucocorticoids exert negative feedback on the pituitary and the brain to inhibit CRH and ACTH production and thus aid in the maintenance of homeostasis [41, 120, 121, 136]. The glucocorticoids are also known to act directly on the PVN, as demonstrated by electrophysiological studies indicating that glucocorticoids bind membrane receptors on CRH neurons in the PVN and elicit intracellular cascades that result in the production of endocannabinoids [50, 52]. The glucocorticoids also exert strong negative feedback on Avp release. In adrenalectomized animals, in which there is no negative feedback, both Avp transcription and CRH transcription increase, but Avp transcription increases more dramatically and is maintained longer than that of CRH [87].

40.3.4 Stress and the Modulation of the Vasopressin System

Depending on the stressor, the HPA axis can differentially impact Avp and Avpr1b expression [5, 124, 125]. Avp release significantly increases in the PVN in response to acute [156] and chronic [5] stress. In rats bred for high or low anxiety-related behavior, there is a correlation between increased Avp mRNA and Avp release from the PVN and the high-anxiety phenotype [155]. This increase of Avp in the PVN has been attributed to an impairment in the repression of the Avp promoter, resulting in Avp overexpression [112]. Stress increases Avpr1b expression in the pituitary [3–5], and mice lacking the Avpr1b have lower ACTH and corticosterone levels compared to wild-type mice following certain types of stressors [99]. The Avpr1b is also required for normal HPA axis response to water deprivation stress [129]. For a more complete review of the effects of stress on the Avpr1b, please see the paper by Roper and colleagues [131].

40.4 Vasopressin and Anxiety- and Depression-Related Behaviors in Animal Models

In this section we will review the commonly used behavioral tests that are employed to examine anxiety- and depression-related behaviors in

rodents. We will then go on to review how pharmacological studies, gene deletion studies, and studies using models of anxiety- and depression-related behaviors have been used to shed light on the role of the Avp system in these stress-related disorders.

40.4.1 Standard Behavioral Tests Used to Examine Anxiety- and Depression-Related Behaviors

Multiple approaches have been used to evaluate the contributions of Avp and its receptors to anxiety and depression. The role of Avp in mood disorders was pioneered by Gold et al. [60], who proposed that Avp function is decreased in depressed patients and increased in manic patients. Since then, pharmacological and genetic manipulations in animal models have provided critical evidence implicating Avp in the modulation of anxiety and depression. Animal models have and continue to be powerful tools to help to understand different pathologies, including emotional behaviors, and to test potential therapeutic agents [38].

Numerous behavioral tests have been validated to measure anxiety- and depression-related behaviors in mice and rats. In brief, we will describe the tests used to evaluate anxiety- and depression-related behaviors, which are based on how animals respond to treatment with pharmacological agents. The elevated plus maze (EPM), the open field test (OFT), the light-dark box test (LDBT), and the social interaction test (SIT) are used to measure anxiety. The forced swim test (FST) and the tail suspension test (TST) have been used to measure depression-like behaviors [25, 38, 39]. The EPM is based on a rodent's natural aversion to open spaces and uses this innate conflict between exploration and the aversion to elevated open places to assess anxiety-related behaviors [96]. Rodents prefer to spend their time in closed arms compared to the center or the open arms. However, treatment with anxiogenic or anxiolytic agents can shift where they spend their time, with anxiogenic agents increasing their time in the closed arms and anxiolytic agents increasing their time in the open arms, relative to controls. In the OFT, animals naturally tend to stay near the periphery of the field (i.e. thigmotaxis), and high levels of anxiety lead to decreases in the ratio of "time spend in the center/time spend in the periphery" [25]. The LDBT is similar to the EPM or OFT since it is based on the innate aversion of rodents to bright areas and is sensitive to treatment with anxiogenic and anxiolytic agents [24]. Finally, the SIT is considered an ethological based test because it mimics the state of anxiety experienced in generalized anxiety disorder, as it places rodents in an unfamiliar environment and then examines how they interact with conspecifics; it is also sensitive to both anxiogenic and anxiolytic agents [54, 55].

The FST is one of the most widely used behavioral tests to detect possible antidepressant effects in rats and mice [38, 99, 121]. Rodents develop an immobile posture in an inescapable cylinder filled with water, and antidepressants' treatment results in longer periods of time in escape-related behaviors. Meanwhile, the TST is considered a version of the FST for mice since the test readout is similar [39]. In the TST, mice are suspended by their tails, and the amount of time they are immobile is recorded, with acute antidepressant administration causing a reduction in immobility times [39].

40.4.2 Pharmacological Studies

Numerous groups have administered Avp or selective agonists and antagonists of the Avpr1a and the Avpr1b to investigate their potential anxiolytic- and antidepressant-related effects in rodents. A summary of these findings can be found in Table 40.1. In an early study by Liesbsch and colleagues [94], it was found that Avp administered into the LS had no effect on entries into the arms on the EPM. However, a couple of years later, Appenrodt et al. [13] administered a slightly higher dose of Avp into this same brain region and observed an increase in the percentage of time in the open arm of the EPM, suggestive of an anxiolytic-related effect of Avp.

Avp signaling through Avpr1b and the Avpr1a appears to be what is modulating this anxiety-related behavior, as acute administration of the nonselective Avpr1 antagonist d(CH2)5Tyr(Me) Avp into the LS increases the percentage of time rats spend in the open arm of the EPM [90, 95]. Peripheral administration of the Avpr1b antagonist SSR149415 or the Avpr1a antagonist JNJ-17308616 to rats also increases the number of entries in the open arms of the EPM [23, 62, 69]. The Avpr1b-specific effects on anxiety-related behaviors have been narrowed down to the basolateral amygdala (BLA), as site-specific microinjections of SSR149415 into the BLA, but not the central amygdala (CeA), MeA, or LS, produce anxiolytic effects in this behavioral test [134, 141].

Studies that have manipulated Avp signaling through the Avpr1b in the SIT and LDBT continue to support the hypothesis that normal Avpr1b stimulation results in increases in anxiety. Specifically, when SSR149415 or the novel Avpr1b antagonist, TASP0233278, is administered to rats, there are significant increases in active social contacts [73]. Treatment with SSR149415 also results in increases in the amount of time spent in the light compartment of the LDBT [62]. Taken together these data suggest that blockade of the Avpr1b results in anxiolytic effects, which suggests that normal Avpr1b signaling is likely anxiogenic.

Activation of the Avpr1b also appears to be important in depression-related behaviors. When SSR149415 is administered either orally or site-specifically into the CeA, BLA, MeA, or LS of rats and mice, immobility times are reduced in the FST and TST [62, 134, 141], suggestive of a strong antidepressant-related effect. These findings have been confirmed with the newer Avpr1b antagonists TASP0233278 and TASP0390325 [73]. This acute administration also suggests that peripheral or striatal blockade of the Avpr1b does not modulate depression-related behaviors. Thus, the site of the antidepressant actions of the Avpr1b differs from its anxiogenic actions, with Avp signaling via the Avpr1b in the LS and amygdaloid complex being antidepressant and Avp signaling via the Avpr1b in the BLA being anxiolytic. Unfortunately, little to nothing is known about the possible antidepressant properties of Avp signaling through Avpr1a, but given the findings (mentioned above) using the Avpr1a antagonist JNJ-17308616 in the EPM, its role should be evaluated.

40.4.3 Gene Deletion Studies

A handful of studies have used rats and knockout (KO) mice to investigate the role of Avp and its receptors in anxiety and mood-related behaviors. A summary of these findings can be found in Table 40.2. Avp-deficient rats (Brattleboro) that were spontaneously derived from Long-Evans rats have a point mutation in an Avp precursor resulting in its inability to enter the secretory pathway [140] and have resulted in a model of diabetes insipidus. When tested for anxiety- and depression-related behaviors, Brattleboro rats display increases in the time spent in the open arms of the EPM [15] and reductions in immobility time in the FST [15, 106]. However, it should be noted that Mlynarik et al. [106] found no genotypic differences in these rats when they were tested in the EPM. Taken together, these studies support the hypothesis that reductions in Avp induce an "antidepressant" phenotype. In addition, Brattleboro rats continuously administered the Avpr2 agonist, desmopressin, display no differences in anxiety-or depression-related behaviors in the EPM or FST [15], providing further support that the Avpr2 does not modulate any of the central effects of Avp.

Studies that have used Avpr1a KO mice to examine anxiety- and depression-related behaviors have not been conclusive. In a couple of studies, Avpr1a KO male mice were found to display increases in the time spent in the open arms of the EPM, in the center of the OFT, and on the light side of the LDBT [20, 51]. Rather surprisingly, female Avpr1a KO mice present a normal phenotype in these behavioral tests [21]. In contrast, Wersinger et al. [152] found no difference between Avpr1a KO and control mice in the EPM, OFT, or FST in either males or females. So, whether or not Avp signaling through the

Table 40.1 Data from pharmacological studies

Ligand	Target	Species	Strain	Dose	Route	Duration	Test	Effect	Reference
Avp	Agonist Avpr1a, Avpr1b, Avpr2	Rat	Wistar	0.20 ng	LS	Acute	EPM	↑ percentage time in open arm	Appenrodt et al. [13]
				0.25 ng	LS	Acute	EPM	NE	Liebsch et al. [95]
d(CH2)5Tyr(Me) Avp	Antagonist Avpr1	Rat	Wistar	5 ng	LS	Acute	EPM	↑ percentage time in open arm/↑ percentage entries in open arm	Liebsch et al. [95]
JNJ-17308616	Antagonist Avpr1a	Rat	Sprague-Dawley	3–100 mg/Kg	IP	Acute	EPM	↑ percentage time in open arm	Bleickardt et al. [23]
				3–30 mg/Kg	IP	Acute	EPM	↑ time in open arms	Bleickardt et al. [23]
V1B-30 N	Antagonist Avpr1b	Rat	CD	1–30 mg/Kg	IP	Acute	Pup vocalization assay	↓ number of calls	Hodgson et al. [70]
		Mice	CD1	3–30 mg/Kg	IP	Acute	FST	NE	
		Rat	CD	1–30 mg/Kg	IP	Acute	FST	NE	
SSR149415	Antagonist Avpr1b	Rat	Sprague-Dawley	0.1–100 ng	CeA	Acute	EPM	NE	Salome et al. [134]
		Rat	Sprague-Dawley	0.1–10 ng	BLA	Acute	EPM	↑ time in open arms	
		Rat	Sprague-Dawley	0.1–100 ng	MeA	Acute	EPM	NE	
		Rat	Sprague-Dawley	0.1–100 ng	CeA	Acute	FST	↓ immobility	
		Rat	Sprague-Dawley	0.1–10 ng	BLA	Acute	FST	↓ immobility	
		Rat	Sprague-Dawley	10–100 ng	MeA	Acute	FST	↓ immobility	
		Rat	Sprague-Dawley	1–100 ng	LS	Acute	EPM	NE	Stemmelin et al. [141]
		Rat	Sprague-Dawley	1–100 ng	LS	Acute	FST	↓ immobility	
		Rat	Sprague-Dawley	100 ng	Striatum	Acute	FST	NE	
		Rat	Sprague-Dawley	3–30 mg/Kg	PO	Acute	FST	↓ immobility	Griebel et al. [62]
		Rat	Sprague-Dawley	3–30 mg/Kg	PO	Acute	EPM	↑ percentage in open arm entries	

		Mice	Undefined	1–30 mg/Kg	PO	Acute	LDBT	↑ time in bright box	
		Rat	CD	3–30 mg/Kg	IP	Acute	Pup vocalization assay	↓ number of calls	Hodgson et al. [69]
		Guinea Pig	Hartley	3–30 mg/Kg	IP	Acute	Pup vocalization assay	↓number of calls	
		Rat	CD	3–30 mg/Kg	IP	Acute	EPM	↑ percentage in open arm entries	
		Mice	CD1	3–30 mg/Kg	IP	Acute	FST	NE	
		Rat	CD	3–30 mg/Kg	IP	Acute	FST	NE	
		Mice	CD1	3–30 mg/Kg	IP	Acute	TST	↑ immobility	
		Rat	Sprague-Dawley	0.1–0.3 mg/Kg	PO	Acute	SIT	↑ interaction time	Shimazaki et al. [138]
TASP0233278	Antagonist Avpr1b	Rat	Sprague-Dawley	0.3–3.0 mg/Kg	PO	Acute	FST	↓immobility	Iijima et al. [73]
				0.3–3.0 mg/Kg	PO	Acute	SIT	↑ interaction time	
TASP0390325	Antagonist Avpr1b	Rat	Sprague-Dawley	0.1–1.0 mg/Kg	PO	Acute	FST	↓immobility	

↑ increase, ↓ decrease, *BLA* basolateral amygdala, *CeA* central amygdala, *EPM* elevated plus maze, *FST* forced swim test, *IP* intraperitoneal, *LS* lateral septum, *LDBT* light-dark box test, MeA medial amygdala, *NE* no effect, *PO* oral, *SIT* social interaction test, *TST* tail suspension test

Table 40.2 Data from animals with genetic mutations of the vasopressin system

Genetically modified animal	Type of deletion	Species	Strain	Sex	Paradigm	Effect	References
Avp KO (Brattleboro rat)	Germline	Rat	Long Evans	Unknown	FST	↓ immobility	Balazsfi et al. [15]
Avp KO (Brattleboro rat)	Germline	Rat	Long Evans	Unknown	EPM	↑ percentage time in open arm	Balazsfi et al. [15]
				Male	FST	↓ immobility	Mlynarik et al. [106]
Avpr1b KO	Germline	Mice	129/SvJ-C57Bl/6	Male	EPM	NE	Mlynarik et al. [106]
				Male	EPM	NE	Wersinger et al. [153]
Avpr1b KO	Germline	Mice	129/SvJ-C57Bl/6	Male	OFT	NE	Caldwell et al. [33]
					EPM	↓ number of entries in open arms	Caldwell et al. [31]
					EPM	Tendency toward a ↓ in percentage in time in open arm	
Avpr1a KO	Germline	Mice	129/Sv-C57Bl/6J	Male	OFT	NE	Caldwell et al. [31]
					FST	NE	Egashira et al. [51]
					EPM	↑time in open arms	
Avpr1a KO	Germline	Mice	129/Sv-C57Bl/6J	Male	SIT	↓ interaction time	Egashira et al. [51]
				Male	EPM	↑ time in open arms	Bielsky et al. [20]
				Male	Light-dark test	↑ time in light box	
				Male	OFT	↑ time in center of arena	Bielsky et al. [20]
				Female	EPM	NE	Bielsky et al. [21]
				Female	Light-dark test	NE	
				Female	OFT	NE	Bielsky et al. [21]
				Male	OFT	NE	Caldwell et al. [33]
				Male	OFT	NE	Wersinger et al. [157]
				Male	EPM	NE	Wersinger et al. [157]
				Male	FST	NE	
				Female	OFT	NE	
				Female	EPM	NE	
				Female	FST	NE	

↑ increase, ↓ decrease, *FST* forced swim test, *EPM* elevated plus maze, *LDBT* light-dark box test, *NE* no effect, *OFT* open field test, *SIT* social interaction test

Avpr1a affects anxiety-related behavior is still up for debate.

In Avpr1b KO mice, there appear to be no profound differences in anxiety- or depression-related behaviors in the EPM, OFT, or FST [31, 33, 153]. In the EPM, Avpr1b KO mice do show a reduced number of entries in the open arm, which might suggest a weak anxiogenic phenotype [31]. These data differ from the pharmacological data, but since these mice are traditional KOs that have been lacking the Avpr1b gene throughout development, it seems plausible that there has been some sort of developmental compensation.

40.4.4 Studies Using Viral Vectors

Viral vectors have been recently employed to site-specifically manipulate genes in order to explore the role of Avp receptors in anxiety. Pioneering work by Bielsky and colleagues [19] found that overexpression of the Avpr1a in the LS, delivered and upregulated by a viral vector, results in reductions in the amount of time spent in the light compartment in the LDBT, which has been interpreted as an anxiogenic effect. Further research is needed to assess the temporal contributions of the Avpr1a and the Avpr1b to anxiety- and depression-related behaviors.

40.4.5 Models of Anxiety- and Depression-Related Behaviors

While acute pharmacological or germline genetic manipulations have predictive validity for anxiety- and depression-related behavior, animal models that mimic some of the features observed in subjects that suffer from depression or anxiety disorders present face or construct validity. Thus, antagonists of the Avpr1a and Avpr1b have been used in four robust animal models of anxiety and mood disorders in rats: (1) high anxiety-related behavior (HAB), (2) olfactory bulbectomy (OBX), (3) chronic mild stress (CMS), and (4) the Flinders sensitive line (FSL). A summary of the findings from the use of these models can be found in Table 40.3.

The HAB and FSL rats represent genetic contributions to anxiety- and depression-related behaviors. Wistar rats bred over generations for extremes in high (HAB) and low (LAB) anxiety-related behavior are used as a model of trait anxiety- and depression-related behaviors [92]. HAB rats are more vulnerable to stress, prefer more passive stress-coping strategies, and have a hyperreactive HPA axis. In HAB rats, administration of d(CH2)5Tyr(Me)Avp into the PVN results in an increase in the number of entries into the open arm of the EPM [155], suggesting an anxiolytic-related effect. In addition, several studies suggest that the Avpergic system may be involved in increases in anxiety-related behavior observed in HAB rats. Specifically, two separate studies report that HAB rats have increases in *Avp* mRNA levels in the PVN, as assessed by in situ hybridization [80, 155], and the administration of paroxetine, a serotonin reuptake inhibitor (SRI), reduces this expression [80]. Landgraf and Wigger [92] observed that single nucleotide polymorphisms (SNPs) in HAB rats may be involved in the Avp hyperdrive observed in these animals.

The FSL exhibits increases in REM sleep, exaggerated responses to a cholinergic agonist, augmented immobility time in the FST, and reduced social contacts [117]. Using the FSL rats, the Avpr1b antagonist SSR149415 reverses the abnormally long immobility times that are observed in these animals and increases active social contacts [117].

OBX is a well-known animal model of depression-related behavior as it induces a constellation of behavioral, immunological, and neurological deficits similar to human depression [83, 109]. One of the most important hallmarks of this animal model is its possible face validity, as OBX animals show improved depression-related behavior only after repeated administration of antidepressants, which is similar to what is observed in humans [109]. Two separate studies using mice found that OBX mice repeatedly administered SSR149415 have reductions in their hyperemotional scores in two different

Table 40.3 Data from pharmacology studies in animal models of anxiety and depression

Ligand	Target	Model	Species	Strain	Dose	Route	Duration	Test	Effect	Reference
SSR149415	Avpr1b	OBX	Rat	Sprague-Dawley	10–30 mg/Kg	IP	Acute	OF	NE	Breuer et al. [27]
					10–30 mg/Kg		14 days		↓ hyperlocomotion	Breuer et al. [27]
				Wistar	10–30 mg/Kg		Acute	Emotional score	NE	Iijima and Chaki [72]
					10–30 mg/Kg		14 days	Emotional score	↓ hyperemotional score	Iijima and Chaki [72]
		CMS	Mice	Not defined	10–30 mg/Kg		3 days		Improve physical state scale	Griebel et al. [62]
					10–30 mg/Kg		9 days	EPM	↑ open arms entries	Griebel et al. [62]
		FSL	Rat	Wistar	3–30 mg/Kg		14 days	FST	↓ immobility	Overstreet et al. [117]
					3–30 mg/Kg		14 days	SIT	↑ interaction time	Overstreet et al. [117]
TASP0390325	Avpr1b	OBX	Rat	Wistar	0.1–0.3 mg/Kg	PO	Acute	Emotional score	NE	Iijima et al. [73]
							14 days		↓ hyperemotional score	Iijima et al. [73]
d(CH2)5Tyr(Me) Avp	Antagonist Avpr1	HAB	Rat	Wistar	5 ng	PVN	Acute	EPM	↑ percentage in open arm entries	Wigger et al. [155]
Desmopressin	Agonist Avpr2	Brattleboro	Rat	Long Evans	10 ng/h	Subcutaneous	14 days	EPM	NE	Balazsfi et al. [15]
								FST	NE	

↑ increase, ↓ decrease, *CMS* chronic mild stress, *EPM* elevated plus maze, *FSL* Flinders sensitive line, *FST* forced swim test, *IP* intraperitoneal, *HAB* high anxiety rat, *NE* no effect, *PO* oral, *PVN* paraventricular nucleus of the hypothalamus, *OFT* open field test, *OBX* olfactory bulbectomized, *SIT* social interaction test

strains and these reductions are not observed following acute treatment [27, 72]. Further, the newer Avpr1b antagonist, TASP0390325, administered to OBX animals, produces similar behavioral effects to those described above [73].

The CMS is often considered to have construct validity for anxiety and depression. In this model, a series of different stressors are presented over a long period of time resulting in increases in corticosterone production and the development of anhedonia, as observed by reductions in sucrose preference, as well as other signs of depression-related behaviors [68, 79]. In CMS animals, SSR149415 increases the number of entries to the open arms of the EPM [117]. Taken together these studies strongly suggest that antagonism of the Avpr1b not only produces antidepressant-related effects in control animals but can reverse behavioral despair in animal models of depression- and anxiety-related behavior. These studies also suggest that the Avpr1b may be a potential target for the treatment of anxiety and mood disorders.

40.5 Vasopressin and Human Subjects with Anxiety and Depression

Recently, the Avpr1b antagonist, SSR149415, was administered to patients suffering from generalized anxiety disorder (GAD) or major depressive disorder [61]. This clinical trial found that while SSR149415 treatment was not effective in individuals with GAD, it did have antidepressant effects after 8 weeks of treatment in subjects with major depressive disorder. Unfortunately, this is really no different than commonly prescribed antidepressants and therefore may be of limited therapeutic benefit. So, while manipulation of the Avp system might ultimately prove to be an effective treatment target in humans, this work is still in its infancy.

While there are no data on Avp's effects in patients with anxiety disorders, there is evidence of abnormalities in the Avp system of depressed patients, though the data are mixed. Measurements of neuropeptides in plasma or cerebrospinal fluid (CSF) have been used as a potential marker of state or trait of depression [126]. While many studies report elevated Avp plasma concentrations in depressed patients [42, 59, 67, 74], there is not complete agreement in the scientific literature. For instance, Gjerris et al. [58] found no differences in plasma levels of Avp between depressed subjects and healthy controls. Thus, subsequent studies have tried to identify what specific traits associated with depression correlate with Avp concentrations. For example, elevated plasma Avp is observed in depressed subjects with psychomotor retardation during the day and elevated motor activity during the night [148]. Further, a subgroup of depressed subjects shows a positive correlation between memory performance and Avp concentrations, which might suggest that Avp has a "protective" effect against the cognitive deficits often seen in depressed subjects [147]. These studies suggest that the initial hypothesis made by Gold in 1978 was not wholly correct that Avp levels were decreased in depression and increased in mania. While it does seem that different subgroups of depressed subjects present abnormalities in the Avpergic system, further research is needed to evaluate whether changes in Avp concentrations can be used as a marker of depression, define whether high concentrations of Avp are beneficial or detrimental in depressed subjects, and determine if the changes in peripheral Avp have any direct effects on the brain.

Since depression is a multifactorial disorder, there are a large array of susceptibility genes and epigenetic and environmental factors that contribute to its etiology [102]. Thus, genetic association studies have been used in an attempt to link changes in the Avp system to depression. Dempster et al. [49] evaluated the effects of SNPs in the *Avpr1b* in subjects with childhood onset mood disorders. This study revealed that changes in the amino acid Lys65Asn produce a change in the intracellular protein domain; the authors speculate that this alteration modifies the activation of phospholipase C and ultimately disrupts the ability of corticotropin to affect Avp binding. Additional SNPs in the *Avpr1b* have been studied

by van West et al. [150] in two populations of depressed subjects (Belgian and Swedish). These studies found that the SNP Avpr1b-s3 is found at a higher frequency in depressed subjects in the Swedish sample, while the SNP Avpr1b-s5 is found at a higher frequency in control, Belgian, subjects. In both samples, the haplotype defined by alleles A-T-CA-G of the SNP Avpr1b-s1-s2-s3-s4-s5 is overrepresented in control subjects, again suggesting that Avp signaling may contribute to a protective phenotype. These genetic association studies suggest that genetic differences in the Avpr1b may contribute to different forms of depression in humans.

40.6 Concluding Remarks and Future Directions

The studies presented in this review support the assertion that the Avp system is important to anxiety and depression, with Avp being intimately intertwined with the HPA axis; which is known to contribute to anxiety- and depression-related behaviors [12, 57]. Of the different Avp receptors, the Avpr1b is consistently implicated in the modulation of anxiety and depression. Pharmacological and genetic studies in animal models support a role for this receptor in the stress response and suggest that its stimulation can result in anxiolytic- and antidepressant-like effects [62, 98, 99]. Consistent with the animal literature, data from human studies suggests that alterations in the Avp system may contribute to depression, as Avp concentrations tend to be high in depressed patients [59, 74], and polymorphisms of the *Avpr1b* are associated with resilience to the development of mood disorders [49, 150]. Thus, it appears that more research is needed to elucidate the specific roles of this receptor in these stress-related disorders. Perhaps if more is known about its actions, then it may emerge as a viable therapeutic target.

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Ketamine: The Glutamatergic Antidepressant and Its Efficacy

41

Derek K. Tracy, Caroline Caddy, and Sukhwinder S. Shergill

41.1 Introduction: From Anaesthetic to Street Drug

41.1.1 The Initial Synthesis and Use of Ketamine

Hallucinogens profoundly alter consciousness, perceptions and emotions, and they have been used recreationally and entheogenically – which is to say as part of spiritual and cultural practices – in various forms for thousands of years. They can be subdivided into so-called 'classical' psychedelics (such as lysergic acid diethylamide (LSD), psilocybin, dimethyltryptamine (DMT) and mescaline) that exert their effects through agonism of serotonergic 5-HT_{2A} receptors [9] and dissociative hallucinogens that include ketamine (as well as, amongst others, phencyclidine (PCP), also known as 'angel dust'), which acts primarily as glutamatergic *N*-methyl-D-aspartate (NMDA) receptor antagonists [52]. Ketamine is an arylcyclohexylamine: the first such compound, 1-(1-phenylcyclohexyl)amine (PCA), was synthesised in 1907, with the biochemical class exemplars of ketamine and PCP commercialised by Parke Davis half a century later. Ketamine was approved for use as an anaesthetic and induction agent by the Food and Drugs Administration in the United States in 1970 and was widely used initially as a surgical anaesthetic that was short acting with no respiratory depression. However, its strong post-operative dissociative effects ultimately limited its use and led to its gradual decline in use in routine surgical and medical practice. Clinically it remains an anaesthetic frequently used in veterinary medicine (and, in some circumstances, paediatric surgery), leading to its common pejorative nickname of 'horse tranquiliser'.

41.1.2 Illicit Use: Effects and Risks

Ketamine's potential for illicit misuse was recognised early on, and it is a proscribed drug in most jurisdictions. However, it has generally been seen as having less potential for harm and abuse than PCP and therein has typically attracted a lower

D.K. Tracy (✉)
Oxleas NHS Foundation Trust, London, UK

Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK
e-mail: derek.tracy@oxleas.nhs.uk

C. Caddy
Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK
e-mail: caz_caddy@hotmail.co.uk

S.S. Shergill
South London and Maudsley, NHS Foundation Trust, London, UK

Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK
e-mail: sukhi.shergill@kcl.ac.uk

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illicit 'rating' and lesser judicial punishments for possession and distribution than the more notorious 'angel dust'. Desired subjective effects of what drug consumers often call 'ket', 'K' or 'special K' include a sense of dissociation from the environment and body with a feeling of an absence of time; auditory and visual hallucinations and illusions; excitement, euphoria and feeling 'high'; and novel bodily sensations such as perceptions of weightlessness. Less pleasant experiences can include a sense of loss of control, confusion, impaired memory and thought disorder; and uncomfortable physical sensations such as tachycardia, nausea and vomiting, blurred vision and impaired speech are reasonably common. At higher doses the infamous 'K-hole' can occur, with an enormous degree of dissociation and derealisation that can vary from deeply profound subjective states of universal harmony and connectedness with other worlds, to hellish nightmare states.

As well as producing 'desired' illicit effects through NMDA antagonism, ketamine increases dopamine activity in the striatal reward pathway, which imbues it with the potential for addiction [111]. However, there are limited data on the prevalence of ketamine dependency: a recent large survey [109] estimated that only 17% of 1,285 ketamine consumers met criteria for a dependence syndrome; though other work has noted ketamine users' frequent reports of compulsive behaviour such as taking the drug without stopping until they had no more [73]. Thus craving the drug appears common, but actual withdrawal symptoms are not [21]. The direct risk of death from ketamine toxicity is relatively low: in the United Kingdom between 1993 and 2006, there were only four deaths wherein it was the only drug detected [96]. In medical settings, inadvertent administration of single doses of ketamine up to 100 times higher than recommended has been reported in paediatric populations without adverse outcomes [35]. However, intoxication can lead to careless and risky behaviour, and the highest mortality risk from ketamine is accidental death. Longer-term illicit use is associated with physical problems including gastrointestinal and urinary tract complications. Ulcerative cystitis of the bladder – sometimes referred to as 'Bristol bladder' – and renal damage can occur, with increased frequency and urgency of urination, dysuria and haematuria [19]. This typically resolves with drug cessation, but persisting and permanent damages – usually dose dependent – have been recorded. Gastrointestinal problems are common, including abdominal pain and cramping (often called 'K-cramps'), nausea and altered liver function [114].

41.1.3 Ketamine as a Psychosis-Inducing Drug?

Ketamine has been noted to adversely affect memory in some, which is likely related to the alterations to the role of the antagonised NMDA receptor that is ordinarily involved in the cognitive processes of long-term potentiation (LTP) and depression (LTD). Impairments have been demonstrated in episodic, semantic and working memory, as well as procedural learning [72]. Ketamine has psychotomimetic properties – especially at higher doses – and it can lead to both negative and positive symptoms of psychosis in healthy volunteers [52] and a worsening of illness in those with schizophrenia [69]. Some frequent and heavy users of the drug have been shown to demonstrate an apparent prodrome or schizotypal picture [57]: however, unlike, for example, cannabis, a clear causal relationship between ketamine consumption and the development or incidence of psychotic disorders has yet to be firmly established.

41.2 From Harmful Back to Helpful? Ketamine's Effects as an Antidepressant

In recent years there has been a huge surge in interest in the pharmacological properties of ketamine following the recurring observation that it can have positive effects on low mood (Fig. 41.1). Particular excitement has garnered on the facts that such improvements are incredibly rapid when compared to traditional

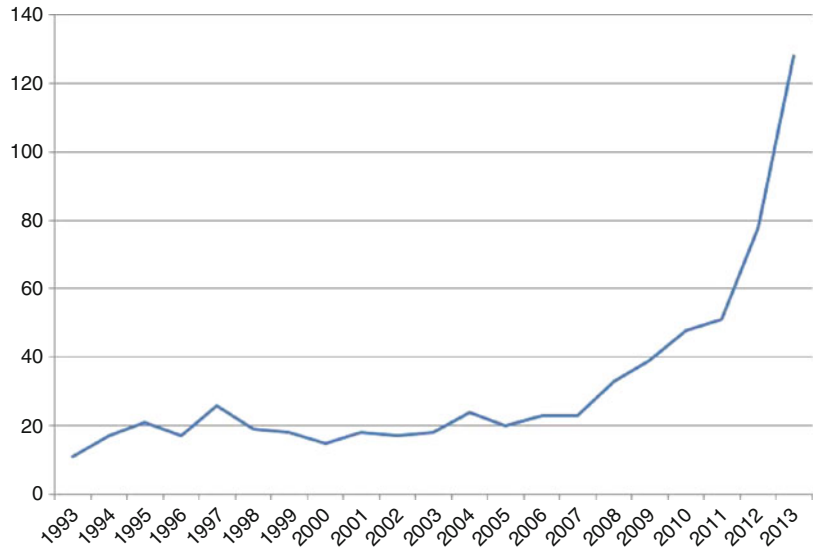

Fig. 41.1 The number of annual publications on ketamine and depression over a 20-year period from 1993 to 2013 (Source: Pubmed)

antidepressants – taking hours rather than weeks – and that such effects must be through novel mechanisms as ketamine does not interact with the 'usual' antidepressant pharmacological targets of serotonin or noradrenaline. The tantalising possibility of a novel and rapidly acting class of agent, as well as better understanding of the neuropathology of depression, has been tempered only by opposing concerns about ketamine's darker side of illicit use, dependency and potential psychosis.

However, the balance of such debates appears to be shifting, at least very recently and in academic settings, towards more and more clinical trials of ketamine's effectiveness. No doubt that this is coloured by the huge global burden of major affective disorder: the personal, societal, clinical and financial tolls they arouse, not to mention the frequent inadequacy of existing pharmacological and psychological interventions. The World Health Organization (WHO) determined that major depressive disorders (MDD) were the third leading cause of global disability in 2006 – already occupying that unenviable position in first-world countries – and that they would become the greatest cause of burden by 2030 [113]. In reviewing the current literature on ketamine as an antidepressant, three major domains arise: ketamine as a sole pharmacological agent, ketamine plus a second drug and ketamine in ECT or surgery.

41.2.1 Ketamine Only

41.2.1.1 Studies Without Control Groups

Several open-label studies have evaluated the effectiveness of ketamine as the sole pharmacological agent in MDD without having a control drug or placebo with which to compare results. Whilst the outcomes in these trials were generally very positive, the results must be therefore contextualised by inevitable methodological concerns. Five such works have been primarily concerned with ketamine-induced changes to protein synthesis [67, 93] or functional activity [15, 91, 92], in the brain, with clinical markers of depression secondary findings, which may in part explain their open-label nature. All of these trials demonstrated statistically significant improvement in mood at the predefined 230-min post-infusion time point. Larkin and Beautrais [56] administered a less common 0.2 mg/kg single bolus of ketamine over 1–2 min to 14 participants with MDD and suicidal ideation in an open-label

trial in an emergency department that had the novel aim of evaluating the practicality of such a 'real world' paradigm. Antidepressant effects occurred rapidly, with 92.3 % meeting response criteria in less than 4 h, whilst the average MADRS score fell from 40.4 to 11.5.

Work [82] has attempted to see if a family history of alcohol dependency altered response to ketamine. The rationale behind this was twofold: firstly there are high rates of comorbidity between this and depression, with both psychosocial and genetic factors involved in this complex mix [22], and, secondly, excess alcohol leads to changes in the glutamatergic system, specifically with NMDA receptors. Interestingly this study found that those with positive family histories for alcohol dependency showed statistically significant improvements to ketamine infusion over those who did not (as measured by the MADRS, HDRS and BDI scores).

Aan het Rot et al. [1] gave six ketamine infusions over a 12-day period to ten participants with MDD who had demonstrated a response to the drug in an earlier trial on suicidality [85]. Ninety percent of individuals responded to the first infusion, and by the study's completion, 100 % and 89 % met response and remission criteria, respectively, for their depressive disorder. This team added a further 14 participants to their original 10 and in a larger study [75] gave six ketamine injections over a 12-day period. Almost all ($n = 21$) completed this regime, and the response rate by the final day was 71 %. In this work an initial response to ketamine was predictive of a later favourable outcome. More recently Shiroma et al. [99] have adopted similar methodology, administering six ketamine infusions over 12 days. Shiroma and colleague's results were similar to that of Aan het Rot and Murrough, showing that 92 % of participants met response criteria after six infusions and 67 % met remission. Rasmussen et al. [88] also administered several ketamine infusions to individuals with major depressive disorders, though over a much shorter timeframe, with up to four doses in 100 min. Eight out of ten participants showed a 50 % or greater reduction of depressive scores, as measure on the MADRS; and five effected a remission state (defined as ≤9 on the MADRS), with two of this remaining well at a 4-week follow-up. This work also demonstrated improvements in suicidal ideation that was significantly correlated with improvements in mood.

Two open-label trials were primarily concerned with suicidal ideation rather than depression per se, and both reported significant findings. The data of Price et al. [85] showed considerable reductions in the MADRS-SI, with 21 of 26 participants – all of whom had treatment-resistant depression – scoring 0 or 1 on this scale. Diazgranados et al. [25] demonstrated improvements in suicidal thinking in 33 individuals with MDD within 40 min of infusion. Suicidal ideation, in the context of MDD, improved within 40 min of infusion, which was sustained to the 4-h final assessment. Price et al. [84] conducted a randomised control trial to further explore the effect of ketamine on suicidal ideation, comparing ketamine with midazolam. Midazolam, a benzodiazepine and anaesthetic, was selected in this particular study due to the similar characteristics it holds to ketamine, its short half-life and fast onset of action. Fifty three percent of participants who received ketamine scored 0 on all explicit suicide measures (BSS, MADRS-SI and QIDS-SR) at 24 h, in comparison to 24 % who received midazolam.

A novel study by Irwin et al. [43] investigated the effect of ketamine in the treatment of both anxiety and depression. This study examined the use of oral administration of ketamine as opposed to the more typical intravenous method, with participants receiving 0.5 mg/kg of ketamine orally over a period of 28 days. Results demonstrated a significant response in depressive symptoms after day 14 of administration and a significant response in anxiety symptoms after day 3. The significant responses recorded remained until day 28 of the trial upon the final administration of ketamine.

There have been two notable studies to date, by the same research group, that have administered IV ketamine, without a control condition, to individuals with bipolar depression. Permoda-Osip et al. [81] and Rybakowski et al. [90] found similar results, demonstrating a significant reduc-

tion in depressive symptoms after 24 h. These significant responses were maintained 14-day post-infusion in both studies, with Permoda-Osip recording a 22 % response rate and 40 % remission rate and Rybakowski recording a 52 % response rate and 48 % remission rate. Within both of these studies, participants remained on their mood stabilisers.

Two studies have investigated the effectiveness of ketamine alone with a mixed sample that consisted of both bipolar depression and MDD [23, 55]. The studies differed in their method of ketamine delivery, but both demonstrated a positive and significant antidepressant effect of the drug. Diamond et al. adopted the commonly used administration of 0.5 mg/kg via IV, with participants receiving either three or six infusions over a 3-week period. Response rates of 29 % were recorded, with 50 % of these individuals fitting the criteria for remission. In Lara and colleagues' study, participants received a very low dose sublingual (VLDS) of ketamine orally (10 mg from a 100 mg/ml solution for 5 min), repeatedly administered every either 2–3 days or weekly. Seventy seven percent of individuals demonstrated response or remission in depressive symptoms by the final administration of ketamine. This study additionally found that some individuals retained the antidepressant response of ketamine after stopping treatment, which, the authors posited, might be attributable to induced neuroplastic changes.

Whilst the majority of clinical trials to date have administered ketamine via intravenous methods, one recent study evaluated its use via intramuscular injection, which is potentially a less dangerous method of delivery, as peak plasma levels are reached very quickly following any intravenous drug delivery. Chilukuri et al. [18] evaluated the comparative effectiveness of IV and IM ketamine on 27 individuals with MDD. Participants were randomised to receive ketamine in one of three different forms: 0.5 mg/kg IV, 0.5 mg/kg IM or 0.25 mg/kg IM. Results demonstrated that 2-h post-infusion HAM-D scores were found to fall by 59 %, 60 % and 57 %, respectively, which was sustained for 3 days post infusion.

41.2.1.2 Studies with a Control Group

Several trials have evaluated ketamine (0.5 mg/kg) against a control placebo saline infusion. Whilst such work is methodologically superior to the open-label data, the majority was with a cross-over design, with individuals receiving both conditions, and it can be argued that participants are therefore not really blinded as ketamine's dissociative effects are quite distinctive. Studies have varied to include only those with a major depressive disorder or to also include those with a bipolar picture.

An early study [11] of eight individuals with MDD and one with bipolar depression found that ketamine reduced HAM-D depression scores by, on average, almost half, 72 h after administration. Zarate et al. [120] compared ketamine with placebo on 17 individuals with MDD, crossing over so that one injection of each was given 2 weeks apart. Ketamine showed a significant benefit at the 24-h post-injection time point, at which point just over 70 % on the active treatment met response and almost 30 % remission criteria. A similarly designed, though smaller, study [110] of ten individuals with MDD also found rapidly occurring antidepressant effects from ketamine that were significantly greater than that of the placebo, though, as discussed later, the primary purpose of this work was to assess changes in occipital amino acid neurotransmitters. A more recent study by Sos et al. [100] adopted a parallel methodology comparing ketamine to a placebo on 30 individuals with MDD, using a cross-over design with 1-week intervals between injections. Results demonstrated ketamine to have a superior response to placebo at days 1, 4 and 7 post infusion.

Of work solely exploring effects in bipolar depression, positive findings for ketamine over placebo have been demonstrated [24, 119] and were as impressive as the data from MDD, with response rates of between 71 % and 79 % and remission rates of 29–31 %. The pattern was similar with a very quick response to treatment, though the follow-up periods were brief: peak responses were typically seen between a few hours and a couple of days after injection; but time to relapse was, disappointingly, almost as

quick, with less than a third of those who met remission criteria still at this point a week later. Ballard et al. [8] conducted a large-scale study evaluating the improvement in suicidal ideation following ketamine in a sample of 133 individuals with bipolar depression and MDD. Ketamine infusion led to a significant reduction in suicidal ideation, as measured by the SSI, compared to placebo.

A novel study by Murrough et al. [74] used a randomised control trial to evaluate the efficacy of ketamine compared to midazolam, selected due to the parallels in its behavioural features, thus potentially circumventing the common criticism that many RCTs are not truly blinded due to distinctive active-drug effects. This study was large scale, with a total of 72 participants diagnosed with MDD receiving either 0.05 mg/kg of ketamine or 0.045 mg/kg midazolam. Twenty-four hours post infusion, 64 % of the ketamine group had met response criteria, compared to 28 % in the midazolam group. There remained a statistically significant difference between the groups until 7-day post-infusion.

Intranasal (IN) administration has been explored by Lapidus et al. [54], in which individuals with MDD were randomly assigned to receive either 50 mg of intranasal ketamine or saline. IN is an interesting potential mechanism of drug delivery that has the potential to avoid some of the inherent dangers, as well as patient acceptability, that follow on from injecting medications. Results in this work showed a significant improvement in MADRS scores after 24 h in the ketamine group compared to placebo. Forty four percent of participants in the ketamine group reached response criteria, compared to 6 % in the saline group.

41.2.2 Ketamine and a Second Drug

As striking as the high and rapid rates of improvement demonstrated by those administered ketamine are the hugely disappointing precipitous relapses that typically follow. Several studies have therefore explored co-prescribing a second agent with ketamine to see if gains made can be sustained by the additional drug. The most commonly utilised agents have been the anticonvulsants lamotrigine and riluzole, which have the pharmacodynamic actions of inhibiting Na + channels and glutamate exocytosis, blocking the NMDAR but facilitating AMPA and enhancing GluR1 and 2 receptor membrane expression. The pharmacological rationale was that such agents are therefore both neuroprotective and potentially synergistic with the mechanisms of action of ketamine. Furthermore, both have been shown to have individual efficacy in the treatment of bipolar depression [27], and riluzole has been noted to increase the production of BDNF that is increasingly considered a vital neurotrophic growth factor in depressive disorders. However, despite both the clinical and pharmacological rationales, outcome data for such polypharmacy has been very disappointing, with little support to date for such prescribing.

A methodologically novel study by Mathew et al. [70] used a two-stage methodology to compare both lamotrigine and riluzole as it was hypothesised that each drug might have differing actions and effects. Twenty six medication-free individuals with TRD were infused with ketamine after being given either lamotrigine 300 mg or a placebo 2 h prior to this. This first step was aimed to evaluate whether or not lamotrigine might limit any psychotomimetic side effects of ketamine and/or augment initial antidepressant effects. Participants showing a response to this phase of the trial (defined as $\geq$50 % decrease in MADRS scores 72 h post infusion) were entered into the second arm, which was a 32-day double-blind randomised controlled phase entailing either riluzole (flexibly dosed at 100–200 mg/day) or placebo. In the first stage there was a significant mean reduction in depression scores at 24 h, with response and remission rates of 65 % and 50 %, respectively. Response rates at 72 h, which was the criterion for moving to the second phase, were 54 %. The co-prescribed drugs had no effect in their respective roles: lamotrigine did not affect side effects or augment ketamine efficacy; and the time to relapse was a mean of 24 days for the riluzole cohort and 22 days for placebo, the difference

being non-significant. A 4-week study of 42 patients with MDD [42] showed the typical pattern of a rapid response in depressive symptoms following an initial ketamine infusion at 0.5 mg/kg, but there was no difference in time to relapse – which was a mean of 13 days – between those thereafter on riluzole (100–200 mg/day) compared with those placed on placebo. Just over a quarter of participants remained well at the 4-week study endpoint, which is somewhat higher than average in such studies, but as noted this was regardless of any co-prescribing. A substudy by the same group [31] indicated that riluzole also did not confer any benefit in the initial management and treatment of depressive symptoms.

41.2.3 Ketamine as an Antidepressant in ECT, TMS or Surgery

Interest in the comparative natures of ketamine and ECT has been evident for some time, and it is not difficult to see why. They are both very rapidly acting and appear appropriate for emergency and potentially life-threatening scenarios; and, on the downside, by themselves neither appears to have particularly sustained actions. They are further linked in that ketamine was initially marketed and used as an anaesthetic, and of course ECT is applied under the aegis of general anaesthesia. The obvious logical question was therefore whether they might be co-applied to get an additive or even synergistic effect. Pharmacologically, despite being a second-line anaesthetic due to its dissociative after effects, propensity to raise blood pressure through catecholamine release, and prolongation of the QTc interval [33], ketamine is – unlike most other anaesthetics – pro-convulsive, which also might enhance ECT, as well as being putatively neuroprotective to ECT-induced hyper-excitability and cognitive impairment through relative attenuation of Glu release [68].

Two different methodologies for combining ECT and ketamine have been attempted. The first is to augment a standard ECT + anaesthetic protocol with the drug and the second is to utilise ketamine as the sole anaesthetic agent. Three trials to date have evaluated ketamine augmentation, and the results have varied between them but overall have not demonstrated a particularly convincing effect. Loo et al. [61] showed initial – though not sustained – benefit to its addition, whilst Abdallah et al. [2] failed to demonstrate such gains. The first study applied thrice weekly ultrabrief pulse-width unilateral (right-sided) ECT under the general anaesthetic thiopentone to 51 individuals with TRD randomised to either augmentation with a subanaesthetic dose of ketamine (0.5 mg/kg) or saline placebo after the main anaesthetic. Despite its characteristics as a pro-convulsive, ketamine did not affect seizure duration. The ketamine group did show a small but statistically significant clinical improvement in depressive symptoms during the first week of treatment and the 1-week follow-up measurement, but this gain had disappeared by the end of the treatment course. Jarventausta et al. [45] randomised patients with MDD to either receive ketamine (0.4 mg/kg) followed by propofol or saline followed by propofol. Concurrent with Loo et al. [61], Jarventausta and colleagues found a significant reduction in depression scores for both groups, but there was no significant difference found between them. The final study [2] had a similar RCT design, though it also included individuals with bipolar depression, and the six-session, 2-week, ECT protocol was different in that it could be applied either uni- or bilaterally. The trial was terminated early due to a lack of between-group clinical differences, and the authors postulated that the GABAergic potentiation and AMPA blocking effects of the barbiturate anaesthetic might have pharmacologically countered the actions of ketamine.

Some studies have evaluated the use of ketamine as the main anaesthetic agent in ECT, comparing it to a more typical general anaesthetic. A single-session bilateral ECT study by Wang et al. [112] randomised 48 participants with MDD to one of three anaesthetic protocols: propofol, ketamine (0.8 mg/kg) and a combination of ketamine and propofol anaesthesia. This allowed testing both of ketamine's potential

additional antidepressant effects as well as evaluation, in the combination group, of whether propofol might ameliorate any adverse cardiovascular events cause by ketamine. HDRS scores improved earlier, up to day 3 post-ECT, in the two ketamine groups compared with the propofol-alone group, but this difference was lost by day 7, whilst the dual-anaesthesia group showed fewer physical and psychological (post-anaesthetic hallucinations) adverse events than ketamine alone. A longer prospective open-label study of 8 ECT sessions over a 4-week period in 31 participants with TRD [78] administered either ketamine or propofol as the anaesthetic agent, with drug allocation according to patient preference in discussion with the anaesthetist. Those anaesthetised with ketamine showed statistically significant improvements in HDRS scores compared with those who received propofol after the second and fourth sessions, but these gains were thereafter lost. The final two studies simply evaluated the effectiveness of ketamine compared to a general anaesthetic in participants with MDD. Yoosefi et al. [118] conducted a randomised, double-blind study comparing ketamine and thiopental over six sessions of ECT. They found a significant improvement in depression in both the ketamine and thiopental group. However, results showed a significant difference in improvement of depressive symptoms before the second ECT session in the ketamine group, compared to those receiving thiopental. Rasmussen et al. [87] conducted a randomised but unblended comparison of methohexital and ketamine and found no significant difference groups on depression measures.

Kranaster et al. [51] retrospectively evaluated the records of 42 patients with TRD who had undergone ECT anaesthetised with either ketamine or thiopental. No differences in depression severity were noted between the groups. Those treated with the former required significantly fewer sessions in total, had lower HAM-D scores and had higher MMSE scores post treatment. However, those treated with ketamine also needed more anaesthetic interventions to manage increased rates of hypertension.

An interesting and unique study carried out by Ghasemi et al. [34] compared the effect of repeated doses of ketamine to ECT in individuals with treatment-resistant MDD. Participants received either 0.5 mg/kg of ketamine or ECT on three treatment days, each of which was separated by 48 h. Following the first treatment, 11 % of individuals in the ECT group met the criteria of response, compared to 78 % in the ketamine group. These response rates were maintained through to 1-week post-treatment, where 89 % of those in the ECT group responded and 100 % in the ketamine group.

Finally with regard to neuromodulation more generally, repetitive transcranial magnetic stimulation (rTMS) has emerged in recent years as a painless tool to alter cortical activity without anaesthesia [105, 106], with data to support its clinical use in several psychiatric conditions including depression. A case report [12] has been published of its efficacy in combination with ketamine in a 23-year-old woman with TRD: whilst circumspection in inevitably required in such literature, it seems likely that, as with ECT, there will be a growing exploration of such combination treatments in the near future.

41.2.3.1 Surgical Use of Ketamine as an Anaesthetic in Depressed Patients

To date one trial has explored the effectiveness of using ketamine as a general anaesthetic in surgical patients with depression. Kudoh et al. [53] compared ketamine combined with propofol and fentanyl to the dual combination of propofol and fentanyl in 70 orthopaedic patients with concomitant depression, with the further inclusion of a control group of 25 non-depressed surgical patients received the three-drug combination. In the depressed cohort, those receiving the ketamine combination showed statistically significant improvement in their mood 1 day post-operatively, as well as significant reduction in post-operative pain scores (a recognised complication of surgery in depressed patients).

41.3 Understanding the Pharmacology of Ketamine: From Psychotic to Antidepressant

Dissociative hallucinogens such as ketamine work primarily as uncompetitive antagonists at the ionotropic glutamatergic NMDA receptor (NMDAR) through blockade of its open channel [40]. The NMDAR ordinarily requires two co-agonists – glutamate and glycine – to open, and it can be distinguished from the other ionotropic glutamate receptors (AMPA and kainate) by its high permeability to Ca^{2+}. Influx of calcium leads to activation of various intracellular secondary messenger systems that alter cell functioning and structure, including synaptogenesis [59], and it is through these that it is thought to effect its roles in higher cognition such as learning and memory [108]. Although NMDAR antagonism is the defining feature of dissociative hallucinogens, they also affect several other receptor classes including the opioid, dopaminergic and adrenergic and have been described as neurochemically 'dirty drugs' [71]. It has a half-life of 2–3 h and is metabolised in the liver by the cytochrome P450 family of enzymes, with an active metabolite, norketamine. Peak plasma concentrations occur between 0.5 and 4 h for oral administration and between 30 s and 10min for intravenous administration.

The psychotomimetic actions of dissociatives have been noted since the time of their initial synthesis [63], and this information has been used over the years to try build up a better neurobiological model of psychoses. NMDA antagonism disrupts a homeostatic process that ordinarily limits the amount of sensory information reaching the prefrontal cortex (PFC). This physiological negative feedback loop – the so-called cortico-striatal-thalamic-cortical loop – has glutamatergic projections from the PFC that, via GABA interneurons, tonically inhibit ascending thalamic pyramidal pathways. Ketamine interruption of this results in far greater quantities of sensory data reaching the PFC, producing psychosis-like effects. This model changes occur in schizophrenia, albeit with the critical difference that in schizophrenia the feedback loop is pathologically interrupted through the primarily dopaminergic effects of mesolimbic hyperdopaminergia.

Whilst modelling interruptions to the cortico-striatal-thalamic-cortical loop can produce a psychosis paradigm, it does not account for any putative antidepressant effects of the drug. In recent years there has been a flurry of research in various modalities trying to elucidate mechanisms that might be consistent with either the known pathology of depression or established therapeutic effects of existing medications. Research has included studying ketamine's effects on intracellular biochemical and molecular processes, glutamatergic neurotransmission, neuroendocrine systems and neuroimaging – including functional magnetic resonance imaging (fMRI), positron emission tomography (PET) and proton magnetic resonance spectroscopy. To date the sum of findings across these fields does not provide a full or consistent account of ketamine's therapeutic actions, though several plausible mechanisms have been – at least partially – elicited. Whether there is ultimately a single therapeutic mechanism or it is a combination of these or as-yet undiscovered factors remains to be determined.

41.3.1 Ketamine's Intracellular Effects

NMDAR antagonism has a secondary effect of increasing the relative activation of the other major ionotropic Glu receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, or AMPA (a third ionotropic receptor, kainate, is also likely to be effected, but its total numbers are lower, and topographical distribution in the brain differs: at this time its role, if any, in the ketamine story is relative unexplored). Interestingly longer-term use of traditional antidepressants has also been shown to enhance relative AMPA to NMDA activity [26], though it is uncertain as to whether or not this is a therapeutic or incidental finding. Whilst ketamine will therefore, through blockade

of one but not the other fast ionotropic Glu receptors, alter patterns of neuronal activity, as discussed later, this also raises the possibility that the differential activation will thus also cause changes to intracellular functioning. Consideration of neurotransmitter-induced intracellular changes tends to focus on metabotropic receptors, but it is nevertheless clear that as well as their role in 'fast' neuronal communication, activation of postsynaptic AMPA receptors also produces changes in intracellular protein expression. Interestingly, amongst the proteins so affected are brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR), which have established roles in neuronal growth and differentiation and synaptogenesis. Reduced cortical and hippocampal [28] and serum [29] levels of these proteins are associated with depression and a return to normal with functional recovery from depression [97], though it has not yet proven possible to determine the causality of such changes or to utilise them as reliable illness biomarkers.

Ketamine has been shown to increase expression of these three proteins [46, 50, 117], though in chronic misuse of the drug, it can lead to reductions in BDNF and nerve growth factor (NGF) [48]. Genetics work [7] has demonstrated that BDNF knockout mice failed to show improvement in depression-like behaviour when administered ketamine, but that mouse controls did benefit from drug treatment. This study also showed that ketamine's inhibition of spontaneous NMDAR-mediated currents leads to deactivation of eEF2 (the kinase eukaryotic elongation factor 2) with very rapid but temporary subsequent increases in BDNF translation: the authors argue that the longer-term therapeutic effects are due to resultant secondary changes in synaptic plasticity. Interestingly, in this study ketamine's effects were due to enhanced plasticity in resting neurons' spontaneous – as opposed to evoked – neurotransmission, supporting a hypothesis that these two forms of glutamatergic signalling are physiologically segregated.

Whilst BDNF has been the most studied cellular protein in depression more generally [97], in research with ketamine there has been more of a focus on the serine/threonine protein kinase mTOR. Amongst mTOR's roles, it affects the translational control of proteins necessary for the formation and functional maturation of dendritic spines [41]. Such changes have been shown to be very rapid, with mTOR-induced phosphorylation and activation occurring within half an hour of ketamine administration, followed thereafter by increases in the maturation, density and function of PFC neuronal spines [59]. This study, which utilised an animal depression model, also demonstrated that use of a selective AMPA receptor inhibitor to block mTOR signalling inhibited both synaptogenesis and behavioural improvement in the rodents, supporting necessary therapeutic roles for both mTOR and functional AMPA receptors. Such changes in neuronal growth patterns also fit with existing data on atrophic brain changes and altered glutamatergic neurotransmission in chronic stress-depression paradigms [83].

The ubiquitous protein kinase glycogen synthase kinase 3 (GSK-3) has been demonstrated to regulate a wide range of intracellular signalling pathways, and modulation of GSK-3 is considered to be one of the mechanisms through which the psychotropic medication lithium acts [14]. GSK-3's actions are rapidly inhibited by ketamine, and in work by Beurel et al. [13] that involved depression-model mice, this inhibitory action was shown to be essential for the rapid antidepressant effects of the drug. Countering this, however, another mouse study [66] found that GSK-3 antagonism did not alter ketamine's antidepressant effects. An importance for ketamine-induced changes to GSK-3 was also identified in work by Bellet et al. [10], utilising a quite different paradigm that explored cell-culture circadian gene transcription patterns. In this work ketamine produced a reduction in the circadian transcription of genes driven by the critical heterodimeric CLOCK/BMAL-1 complex, and, further, these actions were reduced through antagonism of GSK-3B. The involvement of circadian genes is interesting given the well-established diurnal pattern of mood changes seen in many depressive disorders, as well as the potentially precipitating and perpetuating problems caused by interrupted sleep patterns in these conditions. Animal studies have further demonstrated

that ketamine can change both NMDA and AMPA's circadian rhythmicity [20], as well as inhibiting suprachiasmatic light induction [4], a crucial centre for driving temporal gene transcription and neuroendocrine functioning patterns.

Another protein affected by ketamine is the endoplasmic reticular (ER) membrane σ1 (sigma-one) receptor, which has been shown to have decreased expression in several neuropsychiatric conditions [38]. σ1 is implicated in the regulation of intracellular calcium, as well as producing more complex biochemical changes through downstream effects on the inositol-1, 4, 5 trisphosphate (IP3), phospholipase C (PLC)-gamma, protein kinase C (PKC) and the Ras/Raf/MAPK pathways. The range of effects is large and complex, with σ1 receptors, having so-called chaperoning roles in the ER, wherein they promote the correct folding of synthesised proteins as well as facilitating transfer of those which have become degraded to proteasomes for lysis [77], promoting neuronal plasticity through synaptogenesis and neurite growth [39]; and work on σ1 knockout mice has shown they activate antioxidant responses and therein have a role in cellular protection against oxidative stresses [79]. Ketamine is an agonist at the σ1 receptor [101], though its effects upon it are poorly understood at this time. However, work by Ishima and Hashimoto [44] showed that the NMDA antagonist ifenprodil – which binds to both σ1 and σ2 receptors – potentiated nerve growth factor-induced neurite outgrowth and that such effects were blocked by concomitant administration of a specific σ1 or IP3 but not an σ2 antagonist. It is therefore possible, though at this time speculative, that ketamine agonism at the σ1 receptor has similar positive effects on neurites to ifenprodil, and interestingly the antidepressant fluvoxamine has also been found to be an agonist at this receptor [5].

41.3.2 Ketamine's Effects on Glutamatergic Neurotransmission

Ketamine acts on glutamate (Glu), but it has been the cortical monoaminergic systems of serotonin and noradrenaline that have been central to the pharmacology and neurobiological understanding of depression for over half a century. All 'traditional' antidepressants all act on these latter systems, increasing synaptic levels of one or both of their neurotransmitters through inhibiting their reuptake into the presynaptic neuron, stimulating their release through inhibition of presynaptic autoreceptors or inhibiting their enzymatic synaptic breakdown. These facts led to the emergence of the monoaminergic hypothesis of depression that, although heavily criticised, has remained a central tenet in the pharmacotherapy of depression. A contemporary version of this model posits that whilst most antidepressants effect change through increasing synaptic levels of serotonin and/or noradrenaline, therapeutic effects are far more complex involving downstream intracellular signalling pathways that are altered by these monoamines with resultant changes in gene transcription and protein synthesis [14, 80].

The focus on serotonin and noradrenaline has meant that less attention has been paid to glutamate, even though it is a far more ubiquitous neurotransmitter and there are good data supporting its dysfunction in depression [94]. High synaptic levels of Glu are excitotoxic in the brain, and this is avoided through a tightly regulated recycling of the neurotransmitter by supporting glial cells that enzymatically convert it to glutamine, which in turn is thereafter reuptaken by neurons and hydrolysed back into Glu for repeated use. Proton magnetic resonance spectroscopy ([1H]-MRS) has been utilised to evaluate changes in the glutamatergic system produced by ketamine, though to date such work has been limited and results somewhat conflicting. Salvadore et al. [93] were able to show that a lower Glx:Glu ratio (Glx being a composite of glutamate and glutamine) was associated with a greater antidepressant response to ketamine which demonstrated both that abnormal glutamatergic functioning (whether neuronal or in support glial cells or both) may underlie some of the illness pathology and that the drug therapeutic effects might occur through re-regulation of the same. However, another study [110] did not identify any such relationship between ketamine use in those with depression and alterations to amino acid neurotransmitter

content, albeit that this latter study focused on changes only in the occipital cortex.

41.3.3 Ketamine's Effects on Other Neurobiological Systems

Links between depression and obesity have been well established epidemiologically, with considerable attention paid to overlapping psychological and socioeconomic factors that link them, undoubtedly in a bidirectional causal relationship [65]. However, more recently there has been a growing awareness that adipose tissue is far from the erstwhilst considered inert storage medium, but is an endocrinologically active and dynamic tissue, secreting adipokines including leptin, adiponectin and resistin. These hormones have roles in hunger and satiety through complex negative feedback homeostatic loops involving, amongst other regions, the hypothalamus. Their expression is stimulated by glucocorticoids, such as occurrences in stress states, and they promote cytokine production, and therein enhance inflammatory responses [115]. Hominin evolutionary models [30] support complex, and incompletely understood, links between hunger and satiated states with more multifaceted physiological processes; and links with immunity and altered appetite, mood and immune responses have been similarly noted for some time. Research continues into putative depression-mediating role for various inflammatory markers, and more recently, interest has accrued in the potential effects of the neurologically active adipokines.

Most work has specifically explored leptin, and both excessive and depleted leptin levels have been linked with depressive disorders [116]. Rodent studies have demonstrated that rats exposed to chronic stress have reduced leptin levels and, further, that administration of leptin can have antidepressant effects [60, 62]. Mice without the long form of the leptin receptor (Lepr), which is selectively distributed in the PFC and hippocampus, have been shown to demonstrate normal growth and body weight but depression-like behaviour and NMDA-induced long-term synaptic depression in the hippocampus when compared to healthy control littermates [36]. Whilst ketamine itself has not been directly tested, the mice were very sensitive to antidepressant-like effects of the selective NMDA receptor GluN2B (NR2B) antagonist Ro25-6981 but resistant to leptin, inferring a possible depressopathogenic role for defective Lepr signalling in glutamatergic neurons and long-term synapse depression mediated through NMDA GluN2B receptors. Furthermore, the therapeutic actions of NMDA antagonists might be through regulation of normal leptin-Glu functioning.

41.3.4 Ketamine's Effects on Patterns of Cortical Activation

Exploration of more global levels of cortical activation has evidenced the existence of two large anti-correlated networks of intrinsic and extrinsic functioning. The former is a functionally dominant non-goal-orientated resting state, the so-called default mode network or DMN, whilst the latter is an attentional and goal-driven network. The connections between these modular hubs can dysfunction in various mental illnesses [86, 107], and in depression a 'dorsal nexus', which consists of the bilateral dorsal medial PFC, has significantly greater functional connectivity with the DMN [98] that is correlated with a pathological increase in introspection, self-reflective processes and rumination [37]. Both traditional antidepressants [58] and ketamine [95] have been shown to re-regulate this dysfunctional DMN connectivity pattern. In this latter work utilising ketamine, the authors posit that at least some of these drug therapeutic effects might therefore be due to a more global re-regulation of illness-induced disconnections, particularly in glutamatergic limbic-cortico-striatal-pallido-thalamic circuitry implicated in pathological mood states.

At this time our understanding of the mechanism or mechanisms behind ketamine's pharmacological actions as an antidepressant remains incomplete, though several interesting areas have been explored. The drug blocks NMDA receptors, which leads to a relatively enhanced role for the unaffected AMPA receptors, and this in turn

alters more global patterns of connectivity. Within glutamatergic cells numerous downstream changes have been observed, including changes to cellular protein transcription, alteration of several signalling pathways and alterations to intracellular receptors. As yet which of these – or which combination of these – various factors ultimately results in improvement in mood has yet to be elucidated, and there are an almost bewildering number of permutations in which the various changes could interact. It is fascinating to compare and contrast how some of the noted intracellular changes chime with those produced by existing antidepressants, which would appear to add face validity to such mechanisms having therapeutic roles whilst considering that one of the tenets of the monoaminergic hypothesis of depression is that current therapies take time to effect change due to complex intracellular changes, yet ketamine, in many of these studies, affects similar changes in minutes to hours. If ketamine and traditional antidepressants have, at least some, common intracellular endpoints, they would appear to affect them through quite different mechanisms.

41.4 Conclusion: The First Glutamatergic Antidepressant of Many?

41.4.1 How Strong Are the Existing Data?

The existing data on ketamine infusion supports a rapid antidepressant effect in MDD and bipolar depression for the majority of those with a TRD, either when given alone or in augmentation of a second therapy including ECT: however, the augmentation data generally shows little benefit for the additional therapy. As well as a primary effect on depressive states, there are impressive data on a suicidal thinking. High response rates have been recorded in most studies, including double-blinded RCTs – often over 70 % a day or so post infusion. This is, in the context of TRD, frankly astonishing data, especially as such effects are seen so rapidly after administration. One only has to contrast with outcomes from traditional monoaminergic antidepressants where response rates of 65 % following 6–8 *weeks* of treatment are noteworthy [32, 104]. However the obvious rejoinder is that such responses were not maintained, even in studies with repeated dosing, and the evidence for longer-term use of ketamine is currently limited at best. Considerable individual variation was seen in some trials, and, for example, in Murrough et al. [75], duration of antidepressant response ranged from 4 days to >83 days. The parallels between ketamine and ECT have been noted, and the nascent work is interesting, though limited in scope at this time, and not all studies showed positive results. Ketamine's role as an anaesthetic is, due to its side-effect profile, generally considerably limited these days, but the question of potentially targeting surgical patients with depression remains an interesting one.

Most studies reported dissociative and psychotomimetic effects following ketamine infusion. Dissociative effects typically occurred immediately following ketamine infusion at around 40 min, but typically returned to normal by 80 min. The side effects of perceptual disturbances, drowsiness, confusion, hypertension, tachycardia and dizziness were also commonly reported. Adverse effects were not followed up in any of the identified studies, and only short-term effects were recorded.

Methodologically, many current studies are severely limited in regard to their sample size, a problem that continues to hinder many pharmacological studies more generally [49]. A sample size of 102, 51 in each group, would be required within RCT methodology to detect a moderate effect size of 0.5, with a power of 80 % and 0.05 significance [102]. However, no study to date has included a sample size in this region, with the highest sample provided in a non-RCT design of 70. This therefore limits the generalisability of this research, and psychiatric research is littered with examples of studies with statistically significant findings but which are fundamentally underpowered, and the seeming support of follow-on work essentially acts as a type of publication bias [47]. Caution must therefore be taken in interpreting and applying these results, though several

authors identified the difficulties in blinding the administration of ketamine given its frequent highly notable temporary symptoms of dissociation and perceptual change and indeed potential euphoria

41.4.2 The Clinical Use of Ketamine: Practical and Ethical Challenges

The data on our current licensed antidepressants are in themselves depressing. Where they work – and they commonly do not – therapeutic improvement is measured in weeks and months. The burden of depression scarcely needs reiterating, and clinicians are only too familiar with the acute and potentially time-sensitive problems of seeing patients who are suffering from major depression and, all too frequently, strong suicidal ideation in a crisis [16]. At such times the limitations of our existing pharmacological treatments are all too stark. The very rapid and high rates of success of ketamine – albeit seemingly temporary for most – offer tantalising possibilities in the treatment of MDD and bipolar depression. The drug would appear to have the potential to be literally life saving if given to the right person at the right time. The apparently limited time frame that ketamine works for in most is disappointing, but nevertheless this opens a window of several weeks to instigate more traditional treatments, and considering the necessary psychosocial interventions and supports could be hugely valuable for many patients, relatives and clinicians. Crisis intervention work is typically predicated on acknowledging that mental health problems are likely more engrained and needing longer-term work but that short acute interventions can also have huge value through the peak of a problem [17]: How valuable an addition to the armamentarium might ketamine prove in such circumstances?

Most research has been on patients who were, by definition, treatment resistant to standard pharmacological interventions. This is scarcely surprising and indeed surely makes the positive results in the ketamine trials all the more remarkable. However, this does raise the as-yet unanswered question as to how treatment-naïve individuals might respond to ketamine. Furthermore those presenting in crisis or emergency to mental health services, including with acute suicidality, oftentimes do not have a diagnosable depression, but could have other issues, for example, a borderline personality disorder, and clarifying such diagnoses in times of crisis can be complex [89]. How might such individuals react to ketamine? We currently do not know. Fitting with the search for biomarkers and individualised medicine in psychopharmacology and psychiatry more broadly, there are emerging data, for example, to indicate that hippocampal volume [3], higher BMI and family history of alcohol misuse [76] and – in rodent studies – alterations in ketamine dose [6] might be important factors in predicting outcomes, though other factors such as vitamin B12 and folate levels have been shown not to have any predictive value [64]. Finally, whilst augmentation with lamotrigine or riluzole had a pharmacodynamic logic, this was not borne out clinically, and there is a dearth of information on combining ketamine with more commonly utilised medications such as SSRIs to see if these could help sustain initial gains.

For clinicians the practical considerations about instigating ketamine treatment are likely to be considerable. Legislative and ethical criteria and protocols will vary between jurisdictions, but common concerns are likely to be quickly raised, not least about the potential for the medication to cause harm through addiction or psychosis. What seniority or grade of doctor might be allowed to make a decision about prescribing – and emergency crisis presentations are commonly 'out of hours' during the middle of the night – and who will monitor this? Protocols would need to consider eligibility and exclusion criteria: What about those with a current or past history of substance misuse or a depression with psychotic features? Might it be possible, and would it be ethical, to administer treatment to some acute suicidal individuals against their wishes under the aegis of legally enforced detention or to those incapacitous to make decisions about their own care? There are also inherent dangers to clinical staff and services if it is publicly known that, for exam-

ple, an emergency room will dispense and inject individuals with ketamine if they arrive and state that they are in crisis. Even assuming presentations are always clinically appropriate, and there are training needs: many psychiatrists will have become less familiar with intravenous administration of medication; many psychiatric wards have become deskilled on the monitoring of such individuals; yet busy acute medical wards might feel understandably frustrated at holding and monitoring patients that are 'not theirs'. Research has tested the viability of administering a standard ketamine protocol in an ER [56], so there is a model to support this, but undoubtedly there will be challenges in setting up parallel schemes.

A rebuttal to these justifiable concerns is that the existing data support ketamine's safety and acute efficacy in a significantly unwell population, and this is without even beginning to consider the opportunity costs of having individuals not fully treated, including the personal and financial costs of hospital admission and incalculable factors such as suicides. A reasoned argument can be made that with the data we already have, it is unethical to withhold ketamine from some of our most vulnerable patients.

Most existing ketamine trials have been of short duration, and any longer-term role for this drug and side effects and tolerability over greater time frames is currently unclear. A particular concern will be the longer-term rates of addiction and potential precipitation of psychotic states, and, to take as a parallel existing data on those misusing illicit ketamine, the drug can induce considerable psychopathology, albeit with the numerous caveats that evaluation of such populations requires [103]. Recent work [99] has shown that a 'standard' six-session ketamine protocol does not cause any acute neurocognitive impairments, and indeed most show improvements, though these are linked to reduction in depression, but more work is needed in this area. The majority of research administered ketamine in a saline drip over 40 min or so at a dose of 0.5 mg/kg, though this was not the only schedule, with, for example, a bolus administration of 0.2 mg/kg over 1–2 min, and some trials gave several infusions over a period of days-weeks.

Ketamine research has provided new neurobiological evidence both to support aspects of the flawed but dominating monoaminergic hypothesis and to offer novel insights. Will these longer-term concerns critically and terminally kill ketamine as a treatment? No doubt larger trials with greater duration follow-up are required, and evaluation needs to occur in a broader range of participants. The jury is currently out on whether it will just be a prototype for the development of future glutamatergic antidepressants and in furthering our understanding of the neuropathology of depression or soon to join our prescribing medications.

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42 Acetylcholinergic Nicotinic Receptors as Pharmacological Targets for Cognitive Enhancement: Emerging Evidence from Psychosis Populations

Derek K. Tracy, Valentina Casetti, Arann R. Rowe, Louise Mercer, and Sukhwinder S. Shergill

Abbreviations

ACh	Acetylcholine
BPRS	Brief psychiatric rating scale
CANTAB	Cambridge neuropsychological test automated battery
CPT	Continuous performance test
CPT-IP	Continuous performance test – identical pairs version
DSST	Digital symbol substitution test
GAF	Global assessment of functioning
HRT-SD	Hit reaction time variability
KMMSE	Korean Mini-Mental State Examination
LOF	Level of functioning
LTD	Long-term depression
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
MATRICS	Measurement and treatment research to improve cognition in schizophrenia
MCCB	MATRICS consensus cognitive battery
nAChR	Nicotinic acetylcholine receptor
NMDA	N-methyl-D-aspartate
PANSS	Positive and negative syndrome scale
PCP	Phencyclidine
RBANS	Repeatable battery for the assessment of neuropsychological status
RCT	Randomised controlled trial
SANS	Scale for the assessment of negative symptoms

D.K. Tracy (✉) • V. Casetti
Crisis, Inpatient, and Rehabilitation Services, Oxleas NHS Foundation Trust, London, UK

Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, The Institute of Psychiatry, King's College, London, UK
e-mail: derek.tracy@oxleas.nhs.uk

A.R. Rowe • L. Mercer
Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, The Institute of Psychiatry, King's College, London, UK

S.S. Shergill
The National Psychosis Unit, South London and Maudsley NHS Foundation Trust, London, UK

Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, The Institute of Psychiatry, King's College, London, UK

42.1 Introduction

> The only time I really think is when I smoke, and I quit smoking years ago. Jarod Kintz

Emil Kraepelin popularised the concept of a 'dementia praecox' [91] or 'premature dementia', prior to Bleuler's coining of the term 'schizophrenia' ('split mind') in 1908. For the better part of the following century, the focus in this illness has been on positive symptom domains of hallucinations, delusions and so forth, but there has been a recent renewal of interest in the initially described cogni-

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tive deficits and indeed calls by authorities in the field to reconceptualise schizophrenia as a primarily cognitive disorder [84]. Current antipsychotic medications work principally through antagonism of postsynaptic dopamine (DA) receptors. This is reasonably effective in mesolimbic DA projections – treating positive symptoms of psychosis – but has made little impact on cognitive and negative illness domains, though it is these latter factors that are better correlated with problems in 'real-world' social functioning such as employment, interpersonal relationships and community activities (Bowie et al. 2006). The acetylcholinergic system has an important role in normal cognitive functioning, and its therapeutic modulation in this regard has had a high visibility through drugs used in dementia. There are emerging data indicating that this system is also a target for novel psychopharmacological agents to ameliorate cognitive symptoms of several other psychiatric disorders. This chapter will give an overview of the neurophysiology and pathology of the acetylcholinergic system in cognition and schizophrenia, thereafter will review the efficacy of existing nicotinic modulating drugs targeting this domain and will conclude by discussing the potential directions and research required with this novel group of compounds.

42.1.1 The Acetylcholinergic System in the Brain

42.1.1.1 The Neurotransmitter and Its Receptors

Acetylcholine (ACh) was the first neurotransmitter so identified. It was initially labelled *vagusstoff* – the substance of the vagus nerve – by the German pharmacologist Otto Loewi, who shared the 1936 Nobel Prize in Medicine and Physiology with Sir Henry Hallett Dale for its discovery and elucidating its action, therein determining how neurons communicated chemically across the synapse. ACh occurs widely throughout both the central nervous system (CNS) and peripheral nervous system (PNS). It is an ester of acetic acid and choline – the latter being wholly obtained through dietary sources as it cannot be synthesised by neurons – formed via the enzyme choline acetyltransferase. After release from the synapse, it is hydrolysed by acetylcholinesterase into choline and acetyl coenzyme A and recycled into neurons. Inhibition of this enzyme is the primary pharmacological mechanism of action of memory-sparing drugs in dementia.

Acetylcholinergic Receptors

There are two broad classes of ACh receptor: the metabotropic muscarinic (mAChR) and ionotropic nicotinic (nAChR) [82]. The metabotropic or G-coupled mAChR is named after its activation by muscarine, a chemical synthesised by the mushroom *Amanita muscaria*. mAChRs are widespread throughout the CNS, occurring both pre- and postsynaptically, with five subtypes (M1–M5) divided into two major groups according to their intracellular activities: M1, M3 and M5 being coupled to intracellular G_q/G11 proteins that activate phospholipase C and generate inositol-1,4,5-trisphosphate (IP3); M2 and M4 are coupled to $G_{i/o}$ proteins that inhibit adenylate cyclase, reduce intracellular cyclic AMP (cAMP) and enhance the opening of potassium channels in the cell. mAChRs are considered to have important roles in cognition, and whilst they will be mentioned where relevant, a full discussion of their physiology is outside the remit of this chapter: the interested reader is referred to the review by Wess [180].

The nAChR, discovered in 1905 [93], has been the paradigmatic exemplar of physiology receptor studies since the early days of patch-clamp work, helping to determine the principles of neurochemical transmission at the neuromuscular junction [126]; and it has achieved somewhat of a notoriety through its role in nicotine addiction (the binding of which led to its naming). The nAChR is part of a cys-loop ligand-gated receptor superfamily that has been highly conserved on an evolutionary basis, and it is seen in both complex and simple life forms [130, 168]. Eleven genes encode the 17 known variations of nAChR [96], and these are grouped into four overarching families (labelled I–IV). Each nAChR is pentameric, which is to say it consists of five transmembrane elements, constructed from varying combinations of subunits – α1-10, ß1-4, γ

(foetal), δ and ε – of differing ion permeability, arranged around a central water permeable pore [186]. Receptors have, depending on their precise structure, between two and five ACh binding sites that occur at the interface between adjacent α subunits (except for α5 subunits which do not possess ligand binding sites) [139]. Notably, nAChRs have a distinct and separate allosteric binding site where ligands can either increase (positive allosteric modulation or 'PAM') or decrease (negative allosteric modulation – progesterone being the most common example for nAChRs [12]) receptors' activity in the copresence of ACh [26, 137]. Allosteric modulators work by changing the energy required to transition the receptor from the resting to active state and therein alter (positively or negatively) the apparent affinity for an agonist, the concentration-activation curve and the maximum response amplitude [78]. As a target for exogenous ligands, allosteric modulation has the putative attraction of augmenting signalling without fundamentally changing their normal physiological pattern, though benzodiazepines (which are PAMs at the GABA receptor) show that such a hypothesis is not inevitably correct.

The varying nAChR subtypes are characterised by different properties in terms of ligand pharmacology, activation and desensitisation of kinetics and cation permeability. At the neuromuscular junction, nAChRs constituted of α1, β1, γ, δ and ε subunits predominate; whilst in the brain the most common subtypes are the heteromeric α4ß2, followed by the homomeric α7, nAChRs [82, 136], and both of these latter two receptor types considered to have important roles in cognition [80, 190]. Other subtypes of nicotinic receptor occur in the brain, including colocalisation of receptors with α2, α3 and α5, but their roles are less well established [187].

α4β2 nAChRs constitute approximately 90% of CNS nAChRs [141] and are composed of α4 and β2 subunits that can be expressed in two different stoichiometries, each with two ACh binding sites: $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$, with the former having a relatively lower Ca^{2+} permeability and higher affinity for ACh and nicotine than the latter [82]. Compared to the α7 receptor, α4β2 nAChRs have a higher affinity (nM as opposed to the latter's μM) for ACh and nicotine [185]. The homomeric α7 nAChR, which displays a high sensitivity to the alkaloid methyllycaconitine and the snake venom toxin α-bungarotoxin, is undoubtedly a biochemically simpler and phylogenetically more ancient receptor [30], with its five α7 subunits contain an equal number of ACh binding sites [176]. It has a lower affinity for ACh and nicotine than the α4β2 nAChR and more rapidly returns from a desensitised to resting state than other nAChRs. Its ion channels have a notable preferential cation permeability to Ca^{2+} that, as discussed later, affords its distinct functional abilities [43].

Receptor Topography

Different nAChR subtypes vary in their topographical location across the brain; they differ in their occurrence on different parts of the neuron (whether synaptically, on the cell body or the dendrites); and their distribution changes through the human life span, attaining their greatest numbers during the perinatal period, when the brain is undergoing its maximal period of synapto- and dendritogenesis, before decreasing considerably to mature adult levels [123]. In the peripheral nervous system, nAChRs are found at neuromuscular junctions and autonomic ganglia where their primary role is to mediate fast synaptic transmission. However in the CNS whilst they also occur pre- and postsynaptically, they are more typically involved in non-synaptic functions, such as influencing cell excitability and release of other neurotransmitters including glutamate (Glu), GABA, dopamine (DA) and noradrenaline (NA); altering synaptic plasticity [78]; and modifying non-neuronal cells on which they occur, including astrocytes [131]. Their localisation on some presynaptic terminals, such as in the prefrontal cortex (PFC) and ventral tegmental area (VTA), allows some specific patterns of neurotransmitter release that are independent of that neuron's depolarisation [107]. nAChRs are particularly common in brain areas important to cognition, learning and memory, such as the hippocampus and the frontal cortex [100, 190], with lower levels in the thalamus and basal ganglia [145].

The varying pharmacodynamics of the α7 and α4β2 nAChRs in response to ACh release and

their common co-localisation afford areas enriched with both, such as the VTA and hippocampus, complex and nuanced neuromodulations through ACh signalling [139], and with nAChR signalling it is the pattern and timing of induced neuronal activity that have been determined to be critical factors as well as 'just' receptor activation or inactivation. The richness and flexibility of potential responses have led to ACh being referred to as a neuromodulator rather than 'only' a fast ionotropic neurotransmitter, with nAChRs able to act to variously enhance local and neuronal circuitry responses [143].

42.1.1.2 ACh Binding

There are four main functional states of activation of nAChRs: resting, active, desensitised and inactive [32]. Acute exposure to an agonist induces a transient activation of the receptor, with opening of the channel and influx of depolarising Na^+, K^+ and Ca^{2+} cations – the precise influx pattern differing between receptor subtypes – followed rapidly by closing to a nonconducting state. Continued exposure to an agonist leads to receptor desensitisation, which is a closed state of temporary unresponsiveness to agonist binding; whilst 'inactive' is a more prolonged state that can occur in certain situations [25]. In common with most rapidly acting ionotropic systems in the brain, the receptor has a relatively low affinity for the endogenous ligand, ACh, which allows for very rapid dissociation and termination of postsynaptic signalling [78]. However, importantly, the precise kinetics between these functional states varies further depending upon the receptor subunit construction, the binding ligand and the presence of allosteric modulation of the receptor. α4β2 nAChRs open with high probability followed by desensitisation, whilst α7 nAChRs open with low probability, and their desensitisation is more easily reversed [171]: such differences have significant consequences, as will be discussed later, in both addiction to tobacco and the application of novel pharmacotherapeutics. As well as having a direct effect on cellular excitability, acetylcholine binding can create plateaued or persistent spiking action potential in neurons, even in the absence of glutamatergic stimulation, a process that appears driven by mAChR activation of a calcium-sensitive nonspecific cation current and cellular influx of Ca^{2+} [45]. The precise physiological purpose of this is uncertain, but such changes to cellular excitability would appear to have prima facie connections with attentional and memory-encoding models discussed later.

Calcium Influx and Intracellular Changes

Calcium has well-described roles in intracellular secondary messaging pathways including, in the neuron, synaptic plasticity [85]. Whilst non-α7 nAChRs, including α4β2, mobilise intracellular Ca^{2+} through stimulation of voltage-operated calcium channels (VOCCs), α7 nAChRs' unique ion channel kinetics give it a particular role in effecting calcium influx through the receptor pore: this in turn further increases intracellular Ca^{2+} levels through activation of calcium-induced calcium release (CICR) from internal neuronal stores [39, 82]. The receptors' pharmacodynamics mean that desensitisation to further agonist activity will prevent excitotoxicity from excessive Ca^{2+} influx [58].

Increases in intracellular Ca^{2+} activate a range of secondary messenger systems including Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII) and mitogen-activated protein kinase (MAPK) pathways [19]. These signal transduction pathways are involved in the regulation of numerous neuronal processes critical to the pattern and complexity of dendritic fields in individual neurons, which also effects broader patterns of functional neuronal circuitry [3, 22, 56, 155].

The CaMKII pathway regulates several intracellular kinase systems including karilin 7 and Rac 1, and these have important roles in maintaining the actin cytoskeleton via induction of dendritic spines and activity-dependent dendritic stabilisation [142, 188]. nAChR signalling also modulates protein kinase A and C (PKA and PKC), which in turn alters phosphatidylinositol-3-kinase and cytoskeletal functioning. Further intracellular modulatory roles including regulation of cell adhesion molecules and cell scaffolding proteins that regulate synapse formation and stabilisation [123].

42.1.1.3 Acetylcholine's Roles in the Brain

Acetylcholine is released from two main sources in the brain: the major ACh pathways that project to distal locations and local interneurons. As noted earlier, the main effects are non-synaptic but rather change other non-cholinergic neurons' excitability, plasticity and pattern of firing (typically from tonic to burst firing), nuancing and enhancing cortical patterns of connectivity. Whilst the following section is divided into the subcategories of memory, attentional processing and reward and behavioural systems, this is somewhat of an artificial divide insofar as there are rich connections between most of these brain regions, as well as neurophysiological and neurocognitive overlap in these functions. Furthermore, mAChRs have a recognised role in cognition, though this is less explored in this current work.

Memory

The link between the cholinergic system and memory is well established. Its dysfunction, along with amyloid plaques and neurofibrillary tangles, is one of the hallmarks of Alzheimer's dementia; and enhancement of the acetylcholinergic system through inhibition of the enzyme acetylcholinesterase has been the pharmacological cornerstone of most memory-sparing medications. Furthermore, impairments to memory and attention from 'anticholinergic' side effects of many medications have long been recognised [53, 179]. Experimentally, lesion studies to the cholinergic system have been shown to impair memory, and normal functioning is necessary for the acquisition of new memories. More recently abnormalities in the nAChR have been demonstrated – and thus the potential role of pharmacological intervention variously discussed – in several neuropsychiatric conditions with altered cognitive states/traits including intellectual disability [119], autistic spectrum disorders (ASD) [38, 88], attention deficit hyperactivity disorder (ADHD) [97, 181] and, as will be discussed here in detail, schizophrenia. Indeed, given the evidence that nicotine administration improves cognition in otherwise healthy non-smokers, even administration of nicotine agonists as so-called nootropics (namely, pharmacologically enhancing 'normal' performance in healthy individuals) has been debated [108].

The basal forebrain is the major source of cholinergic projections in the brain, and of its constituent parts, it is the nucleus basalis of Meynert (NBM), named after its discoverer [121], that is, the primary origin of CNS ACh innervation, and a critical contributor to normal functioning of memory, brain plasticity, arousal and perception [92]. The gradual degeneration of the NBM is a hallmark of both Alzheimer and Lewy body dementia [63]. The NBM contains approximately 200,000 neurons per hemisphere [55] and receives a wide range of inputs, including from the orbitofrontal cortex and the parahippocampal and cingulate cortices; limbic input from the amygdala, hypothalamus and nucleus accumbens; monoaminergic serotonergic and noradrenergic input; and cholinergic input from the pedunculopontine nucleus. Efferent projections, which are largely unmyelinated, extend through the substantia innominate to project widely through medial and lateral (with capsular and perisylvian subdivisions) pathways to the cortex and subcortical regions including the amygdala, caudate nucleus, putamen and thalamus [59].

The medial septal nucleus (MSN) is very closely linked with the hippocampus through the septohippocampal pathway and is the main source of hippocampal ACh (though it also projects GABAergic and glutamatergic neurons). The hippocampal formation – which includes the dentate gyrus, the hippocampus, the subiculum and the entorhinal cortex – is critically involved in memory and learning, and the roles of its acetylcholinergic inputs in modulating synaptic plasticity in the induction of long-term potentiation (LTP) and long-term depression (LTD) have received considerable attention. The MSN has an especially important role maintaining the frequency of oscillations in the hippocampus in the theta (5–12 Hz) range, which appears to facilitate synapse formation and thus memory processing and learning [138]. Lesions to this pathway impair hippocampal-dependent learning [1, 122]; localised injections of scopolamine impair memory encoding [149]; and deep brain stimulation

of the MSN has been shown to improve spatial working memory after traumatic brain injury [98] and in rodent dementia models [81].

The hippocampus contains both α4β2 and α7 nAChRs. Rodent studies indicate that a large number of α7 nAChRs present are necessary for the initial development and subsequent maintenance of appropriate CA1 dendritic patterns [125]. nAChR activation facilitates both glutamatergic input and the signalling of GABAergic interneurons on which they reside. GABAergic activation inhibits Glu release on CA3 pyramidal neurons and therein their response to secondary stimuli [100], a phenomenon described in more detail later in this chapter when discussing the P50 auditory stimulus paradigm. α4β2 nAChRs occur on dendritic spines of glutamatergic and GABAergic pyramidal cells [4, 167]. Activation of both types of nAChR in glutamatergic neurons enhances postsynaptic responses and cellular plasticity [40]. Glutamatergic functioning in the hippocampus also appears to be indirectly modulated by astrocytic nAChRs altering synaptic Ca^{2+} levels [165] and triggering AMPA receptor recruitment [177]. Furthermore mAChRs have a role in hippocampal encoding through the earlier described phenomenon of plateaued or persistent depolarisation spiking to afferent input through activation of calcium influx currents, and they also suppress recurrent endogenous tonic excitatory activation [73].

Overall there is a complex dynamic interplay between ACh, the different nAChRs and receptive glutamatergic and GABAergic cells in the hippocampus that remains incompletely understood, demonstrated by the finding that, for example, hippocampal ACh levels are high during acquisition of spatial learning but low during its consolidation [35]. The precise timing of neuronal depolarisations, facilitated by ACh synchronisation, also appears critical in effecting the induction of the specific synaptic alterations and patterns of LTP and LTD, though this has yet to be fully elucidated [64].

Attentional Processing

The acetylcholinergic system has a vital role in attentional processing, with evidence from neuroimaging and pharmacological [11, 36] and human and animal lesion [31, 67] studies. Attentional processing is physiologically complex, involving interplays between sensory perception, parietal integration of information and frontal lobe appraisal. It is sometimes divided into 'top-down' higher cognitive processes of sustained choosing (and ignoring) between competing stimuli, and the right PFC has a particular role in cue detection and maintaining focus and 'bottom-up' detection and assimilation of cues, utilising particularly a ventral frontoparietal network [36, 73]. The precise mechanism through which ACh mediates this has not been fully elicited, though a so-called pre-attentional model is commonly posited wherein cholinergic inputs will predispose cortical information processing towards a favoured cue.

As noted, most ACh in the PFC arises from the NBM, and this facilitates thalamocortical glutamatergic synaptic functioning and therein appropriate thalamic sensory activation of connected cortical regions [77]. Three further cholinergic-induced processes appear to assist this: mAChRs inhibiting recurrent cortical neurons, selective activation of GABAergic inhibitory interneurons and inhibition of layer V cortical neuronal output, the net result of which is a reduction in the unwarranted spread of cortical activity and a greater relative attention to sensory input [73]. The mesocortical dopaminergic system is also involved in working memory and attention: DA cell bodies have, in particular, dense α4β2 nAChR expression, activation of which facilitates DA release [94, 117]. Furthermore, in the PFC both α4β2 and α7 are expressed on GABAergic inhibitory interneuronal soma, with α7 further located on presynaptic glutamatergic neurons that also stimulate DA release [182]. The precise circuitry and mechanism through which these factors all combine and interact to enhance PFC attentional processing is not fully known, though there is evidence that changes in DA firing to a burst pattern overcomes nicotinic inhibition of inputs to layer V of the cortex, modifying a so-called signal to noise ratio in the neurons that might be important in this regard [116]. Similar to changes in the hippocampus, the precise timing and

synchronicity of firing patterns appears to be an important factor in altering neuronal connection strength [173].

α7 nAChR knockout mice show significant deficits in sustained attention, olfactory working memory and procedural learning [189]. Several studies have further demonstrated that α7 receptor agonists improve visual attention and social and object recognition in rodents [144], and these enhancing effects were blocked by the selective α7 nAChR antagonist methyllycaconitine [147]. Similarly administration of α7 nAChR agonists can also reverse the attentional impairments induced by phencyclidine (PCP), an N-methyl-D-aspartate (NMDA) receptor antagonist widely used in animal models of cognitive deficits in schizophrenia [24] – see Chapter 41 on *Ketamine*: *the glutamatergic antidepressant* for additional information on this topic. In line with these results, α7 nAChR agonists can also reverse the attentional impairments induced by phencyclidine (PCP), a N-methyl-D-aspartate (NMDA) receptor antagonist widely used in animal models of cognitive deficits in schizophrenia [169]. Administration of partial α7 agonists DMXB-A and tropisetron improved sensory gating deficit in DBA/2 mice, which spontaneously exhibit an impairment in the P20-N40 auditory evoked potential (AEP) that models the human P50 deficit [72, 158]. Activation of α7 receptors mediates P50 evoked response inhibition by stimulating GABAergic interneurons to release GABA, which binds to $GABA_B$ receptors; these, in turn, decrease their release of glutamate and thus suppress the response to second stimuli [114].

Reinforcement Learning and Response Selection

As well as the aforementioned forebrain cholinergic system, several other cholinergic projections and regions of greater cholinergic innervation are worthy of mention. The subcortical striatum, which is largely composed of the caudate nucleus and putamen, primarily receives input from the cortex and outputs to the basal ganglia and has a significant role in the coordination of movement. The vast majority of its cells are GABAergic medium spiny neurons, but there are also cholinergic interneurons, which, although only accounting for 1–2% of cells, have a role in motor stereotypy, reinforcement learning and association plasticity: factors all critical in adaptive response selection [5]. Their precise synchronised patterns of multiphasic firing produce large inhibitory synaptic currents [127] and signal either the unpredicted occurrence or the omission of a predicted occurrence, of a motivational stimulus (or cues for that stimulus). In conjunction with coincident firing, changes in midbrain dopaminergic systems encode the value and expected probability of an event [42] and regulate striatal output through GABAergic inhibition. Data support this process having a role in the salience of environmental cues with drug misuse [115].

Reward processing, reinforcement behaviour and higher cognitive functioning are also affected by acetylcholinergic interplay with the dopaminergic system. In the mesolimbic system, there is a variable pattern of nAChR localisation, with rich α4β2 expression on dopaminergic cell soma in the ventral tegmental area (VTA) that projects to the nucleus accumbens (NAcc). This dopaminergic pathway has both inhibitory GABAergic and stimulatory glutamatergic inputs, which have primarily α4β2 receptors on the former and α7 on the latter: these are ordinarily in a dynamic interplay, and precisely timed cholinergic activation can facilitate altering dopaminergic firing patterns from a tonic to a burst pattern of activity [110]. The addictive effects of nicotine are thought to occur primarily through alteration of the higher affinity α4β2 nAChRs in this reward pathway, and this will be reviewed in more detail in a later section.

Arousal

The pedunculopontine nucleus is a midbrain structure with a diverse range of functions. It has rich connections with the basal ganglia, thalamus and spinal cord and receives input from the cortex. It has cholinergic projection in its pars compacta and has roles in arousal, behavioural state control and movement planning [10]. The ascending mesolimbic cholinergic system, a division of the ascending reticular activating system, projects from cholinergic neurons in the laterodorsal

tegmental nucleus and projects to wide range of cortical and limbic regions and is involved in sustained attention and in producing alerting and aversive arousal and emotional states [18]. Along with the pedunculopontine nucleus, this has a role in the synchronisation of REM sleep.

42.1.1.4 Acetylcholine and Schizophrenia

Schizophrenia is increasingly considered in terms of lying along a psychosis spectrum, with three major symptom domains: 'positive' symptoms that include hallucinations, delusions and so forth; 'negative' symptoms that include amotivation, social withdrawal and a lack of spontaneous speech; and cognitive symptoms that include memory, attention and executive functioning difficulties [172]. An international prospective 25-year follow-up study demonstrated that outcomes in schizophrenia are very heterogeneous [69]. Routine treatment typically results in a reduction in positive symptoms, though work on first-episode patients show that about half have persisting symptoms a decade after first assessment [17]. With regard to longer-term outcomes, a meta-analysis by Jaaskelainen et al. [79] demonstrated that fewer than one in eight patients will attain criteria for recovery – which was defined as showing improvement in both clinical and social domains, with at least one of these persisting for 2 years.

Over three quarters of patients with schizophrenia report some cognitive problems [148], and longitudinal studies have clearly shown them to be highly indicative of longer-term functional outcomes [60, 70, 89, 129], with evidence of impairment of social and role functioning even before illness onset [23]. Cognitive symptoms often occur before positive symptoms and frequently remain after the treatment of the latter. Data from the large Dunedin birth cohort [118] showed that premorbid cognitive deficits and later cognitive deterioration, which was shown to occur in a wide range of faculties including IQ, executive functioning, processing speed and learning, delineated psychosis from other mental disorders such as depression. Data show that patients are, on average, one standard deviation below age-corrected means, whatever the test battery applied [74], and a recent meta-analysis of almost 250 papers evaluating cognition in schizophrenia demonstrated that this occurred across all cognitive domains [44].

Whilst there are data to indicate that existing antipsychotics can improve neurocognitive function, such findings typically demonstrate reasonably limited effect sizes [46, 71, 87]. Despite initial enthusiasm about newer second-generation antipsychotic novel pharmacodynamics – particularly their specific serotonergic $5HT_{2A}$ presynaptic antagonism that would disinhibit mesocortical dopamine release – big naturalistic trials such as EUFEST [33] and CATIE [86] have not shown any substantial superiority over older medications in treating cognitive symptoms, especially when the first-generation compounds were given at lower doses (thus avoiding the confounder of the need to treat extrapyramidal side effects with anticholinergic agents). Undoubtedly the varying merits of these two classes of drugs continue to be hotly debated, and a recent meta-analysis – albeit in trials on first-episode psychosis – has swung back behind the newer drugs having some superiority in treating cognitive symptoms, though the authors acknowledge that there might be an issue of sponsorship bias as most studies finding this were pharmaceutical industry sponsored, whereas independent studies typically did not [192]. However despite the variations between study methodologies, participants and outcome data, the bottom line at this time is that existing antipsychotics are, at best, a very limited treatment option for cognitive symptoms [21, 164, 174]. Indeed there is also the worry that they might *worsen* cognitive deficits through several mechanisms: antagonism of mesocortical dopamine receptors (clearly far more so for older atypical or first-generation antipsychotics), leading to reduced PFC functioning; sedative effects from blockade of the histaminergic system; and, critically, through blockage of cholinergic functioning. Both first- ('typical') and second-('atypical') generation antipsychotics have been demonstrated, in a rodent model, to be non-competitive inhibitors of both the α7 and α4β2 nAChRs at concentrations consistent with plasma levels

typically obtained in schizophrenia, with few differences between the individual drugs [62].

A fundamental problem with regard to cognitive symptoms has been that outside specific neurocognitive research literature, it has been a somewhat neglected field, and a recent systematic review of treatment resistant psychosis [163] noted that none of the included studies measured cognitive functioning. Whether this represents the 'hidden' nature of cognitive deficits or is a frank reflection of our inability to treat them is uncertain, but both factors are probably contributory. However the tide appears to have begun to turn in this regard, and a couple of recent welcome developments to refocus efforts on evaluating cognition in schizophrenia have been the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) (matricsinc.org) and the Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) initiatives. The MATRICS aims to standardise research and evaluation of therapies in seven cognitive domains significantly affected by schizophrenia: verbal learning and memory, visual learning and memory, working memory, attention and vigilance, processing speed, reasoning and problem solving and social cognition. CNTRICS is a parallel and complimentary programme that draws together cognitive neuroscientists and other researchers working in animal and human models particularly focusing upon biomarker and preclinical translational technologies to identify testable cognitive constructs and hypotheses [124].

Sensory Gating as a Cognitive Biomarker?

Sensory gating is the ability to filter sensory stimuli responses: deficits in this process have been consistently described in schizophrenia [133]. Auditory sensory gating, and any abnormalities therein, can be verified through the hippocampal P50 auditory evoked potential (AEP) (Bramon et al. 2004), as measured by electroencephalogram (EEG). This experimental paradigm delivers two acoustic clicks 500 ms apart: an EEG response is demonstrated approximately 50 ms after each stimulus, with, ordinarily, a shortened (gated) diminished amplitude response provoked by the second click. It is considered that such gating is a physiological response to diminish repetitive and unimportant stimuli. In schizophrenia there is commonly a failure to inhibit the response to the second stimulus, and so frequent is this finding that some authorities have argued that it might serve as an illness biomarker [50]. Abnormalities have been further demonstrated in about half of relatives of those with schizophrenia without the illness, indicating a heritability to this [66, 132], and deficits have been shown to be correlated with impairments in attentional processing.

Work by Freedman et al. [48] initially showed that such auditory gating deficits were positively correlated with a locus at chromosome 15q14, a finding that has been replicated in numerous studies since [160, 162]. This region contains the CHRNA7 gene, which encodes the α7 receptor, and Leonard et al. [101] showed that individuals with schizophrenia further have single nucleotide polymorphisms (SNPs) in CHRNA7's promoter region that reduce its expression. Post-mortem studies have demonstrated the decreased expression of nAChRs in schizophrenia, though the structure of the receptors present appears normal. Such reductions have been demonstrated in the hippocampus, in the reticular nucleus of the thalamus, in the dentate gyrus and in the frontal cortex of individuals with schizophrenia [29, 47, 65, 112, 120]. Rodent studies have shown that appropriate functioning of hippocampal α7 nAChRs on inhibitory GABAergic interneurons is essential for normal auditory gating [6] and also that reduced receptor expression deficits could be partially ameliorated by agonism of the receptor [161].

The importance of P50 as a physiological measure is that it can be used to demonstrate a sensory deficit that is related to both cognitive processing and the α7 nAChR; and changes to it are thus putatively changes in these underlying neurobiological processes. Indeed it has been shown that the abnormal P50 response in schizophrenia can be altered through nicotine use [113], and it has therefore been argued that the high rates of smoking in schizophrenia might therefore represent attempts to re-regulate such deficits

and improve cognitive performance [133]. However whilst smoking cigarettes has been shown to improve sensory gating, such effects appear to last no more than half an hour or so, though chronic smokers show reduced deficits overall compared to non-smokers [27]. Animal models for sensory gating have shown improvements in DBA/2 mice, which spontaneously exhibit an impairment in the P20-N40 auditory evoked potential that models the human P50 deficit, after administration of partial α7 agonists DMXB-A and tropisetron [72, 158].

Nicotine and nAChRs

Nicotine, named after the French Ambassador, Jean Nicot De Villemain, who introduced it to Europe [15], is an alkaloid synthesised by several plant species, including the tobacco plant, acting as a natural insecticide. The positive impact of nicotine on attentional processing and a range of cognitive tasks is well established [100], and this has been shown in animal models and healthy smoking and non-smoking volunteers, as well as in those with schizophrenia [75]. Withdrawal for nicotine hinders cognitive performance, which is resolved upon recommencement, and data have shown that transdermal nicotine patches can ameliorate abstinence-related deficits [90]. Nicotine is an agonist at nAChRs, though its pharmacodynamics vary from those of ACh: it has a 100-fold greater affinity for nAChRs with α4 subunits than those with α7 nAChR, and concentrations of nicotine achieved by smoking are insufficiently high to desensitise the α7 nAChR. Acute exposure leads to intricate changes to nAChRs, including desensitisation and reduction in α4β2 receptor function, whilst chronic use facilitates receptor trafficking, leads to changes in subunit stoichiometry, reduces nAChR degradation and results in receptor upregulation [28, 51]. Smoking also increases α4β2 receptor levels in the brain in schizophrenia, though it is currently unclear if this is a therapeutic mechanism for enhancing cognition. However the pattern is different to that ordinarily found in the brain of otherwise healthy chronic smokers, though patients, as noted, are generally starting from a position of having pathologically fewer receptors [16].

The well-recognised phenomenon of nicotine dependency is in part due to changes in receptor numbers and functioning but also is considerably affected by drug modulation of the dopaminergic mesolimbic reward processing system. As noted earlier, the VTA, which projects to the NA, has primarily α4β2 on its soma, and nicotine binding at the mesolimbic dopaminergic nAChRs will therein alter dopaminergic tone from a tonic to a burst firing pattern with increased DA release [111]. Furthermore this dopaminergic pathway has both inhibitory GABAergic and stimulatory glutamatergic input, which have primarily α4β2 receptors on the former and α7 on the latter: these are ordinarily in a dynamic interplay, but the specific variation in nicotine pharmacodynamics means that nicotine concentrations obtained from smoking will quickly desensitise the high-affinity α4β2, but not the α7, further increasing DA release through a decrease in inhibitory input and a simultaneous increase in excitatory input [182]. The interplay with the dopaminergic mesolimbic system – particularly the increase in DA release by smoking – is clearly of interest in the field of psychosis studies, where pathological overactivity is the cardinal feature of the much criticised but still intact (in a modified fashion) dopaminergic hypothesis of schizophrenia. However despite the pharmacodynamic rationale, there is no evidence that smoking exacerbates positive symptoms in those with schizophrenia: it is unclear if this is due to an insufficient activation of the pathway or, in those with schizophrenia, the fact that most will be on dopamine blocking drugs that will negate such effects. The mesocortical dopaminergic pathway similarly has a significant number of α4β2 receptors on dopaminergic soma in the VTA, and some of nicotine's precognitive effects may come from modulation of the mesocortical dopaminergic pathway [76, 152].

Smoking in Schizophrenia

There is an exceptionally high prevalence of smoking in people with schizophrenia, and they are more likely to commence and less likely to give up smoking than the general population [95, 178]. Figures generally show that about 70 % of those with schizophrenia smoke, of whom two

thirds are heavy smokers (>25 cigarettes/day) [34, 193]; cigarettes with higher nicotine content are chosen more often [54]. The public health implications are considerable, with life expectancy in schizophrenia between 15 and 20 years less than that of the general population [166], and it has been estimated that tobacco smoking accounts for about half of this [52].

Various explanations have been posited for these findings, including greater psychosocial deprivation [183]; a reduction in antipsychotic medication side effects, particularly extrapyramidal side effects (EPSE) through induction of hepatic enzymes [9]; and a shared genetic predisposition with variation in the 15q13-q14 chromosomal region that encodes the α7 gene CHRNA7 [153] also having been shown to have an association with nicotine addiction. However the greater rates of smoking have also been speculated to be an implicitly learned behaviour to self-medicate deficits in cognition (Olincy and Stevens 2007). Interestingly, premorbid smoking has been shown to be associated with a later onset [109] and a delayed advancement [191] of schizophrenia, whilst smoking has been consistently found to improve cognitive performance [68, 103]. A recent review by Wing et al. [182] noted that most improvements in attentive and cognitive performances induced by nicotine in those with schizophrenia were in processes associated with dopamine release, which fits with a broader dopaminergic hypothesis of schizophrenia. However tying in with this is the confounder in such work is that, as noted earlier, antipsychotic medication may induce iatrogenic cognitive difficulties through direct anticholinergic effects (as well as antihistaminergic sedative effects). Work that has addressed this issue directly would indicate that tobacco-induced improvements are probably through several parallel mechanisms, with a reduction in medication side effects being one of them [104]. Another issue in smokers is that some cognitive deficits demonstrated might be due to withdrawal and nicotine abstinence, and therefore smoking is actually just returning cognitive functioning to normal (whether a healthy control or an individual with schizophrenia and stable deficits). There are data to support this principle – in part – for those with schizophrenia, with time of abstinence/withdrawal adversely affecting performance, though not healthy controls [2]. Clearly however, besides such effects being modest, the therapeutic use of nicotine is severely limited by addictive potential, tachyphylaxis and other side effects [68].

42.1.2 The Current Evidence from Clinical Trials

Nicotinic receptor-modulating drugs that aim to improve cognitive functioning can currently be divided into three broad classes: those that are α4β2 agonists, those that are α7 receptor agonists and those that act as positive allosteric modulators (PAMs) rather than being direct agonists.

42.1.2.1 The α4ß2 Receptor Agonist: Varenicline

One α4ß2 receptor agonist has been tested as a potential cognitive enhancer in schizophrenia, varenicline, and it has a complex pharmacology: it is a partial agonist of α4ß2, α3ß4 and α6 and a full agonist at α7 nAChRs. However its affinity for the α7 nAChR is 100th that of the α4ß2. Its actions on the α4ß2 receptor have led to its use in assisting quitting smoking, as it has less than half of nicotine's efficacy at the receptor [151]. To date clinical outcomes in schizophrenia have been very disappointing, with no trials showing statistically significant overall improvements in clinical symptomatology or cognition, though secondary analyses have demonstrated within-test variation and some improvement in particular cognitive subdomains.

Shim et al. evaluated a dose of 1 mg twice a day, compared with placebo, in an 8-week RCT of 120 participants with schizophrenia [157]. Half were smokers, and their randomisation was stratified between the two groups. As noted above, none of the varenicline studies showed positive overall drug effects, but in this work secondary analyses showed active compound superiority on the Digital Symbol Substitution Test (DSST) ($p = 0.013$, $d = 0.13$) and the Wisconsin

Card Sorting Test (WCST) non-perseverative error ($p=0.043$, $d=0.21$). Hong et al. [76] had a similarly designed – and similarly negative in overall neurocognitive outcome measures – 8-week RCT of 69 participants (40 of whom were smokers). The dosing was slightly different, titrated to 0.5 mg twice daily, and the trial did have positive findings with regard to drug-induced normalisation of P50 sensory gating in non-smokers by the trial end point ($p=.006$). Work by Waldo et al. [175] was prematurely terminated due to three participants dropping out after developing self-limiting but intense psychiatric symptomatology. No significant results of the compound were noticed in those who remained in the study, but the final sample size of six makes interpretation of this work problematic. Roh et al. [150] had a unique methodological design, utilising two drugs and including patients with schizophrenia ($n=30$) and healthy controls ($n=41$). Their double-blind RCT had a crossover design, assessing the effects of the nAChR *antagonist* mecamylamine (10 mg) and varenicline (1 mg) compared with placebo. All participants took a single dose of mecamylamine, varenicline or placebo in a random order 1 week apart. The main neurocognitive measure was sustained attention measured through the hit reaction time variability (HRT-SD) on the continuous performance test – identical pairs version (CPT-IP). As would be predicted, mecamylamine worsened test performance compared with both placebo and varenicline in those with schizophrenia, but this was not the case for healthy controls. However there were no differences between varenicline and placebo in the patient population.

42.1.2.2 α7 Receptor Agonists

Four different α7 receptor agonists have been evaluated as cognitive enhancers in schizophrenia: encenicline (EVP-6124), tropisetron, DMXB-A and TC-5619. The individual trials have varied considerably: drug dosing ranging from a double-dosing study to a 12-week follow-up; participant numbers from 12 to 185; and some including only non-smokers, some only smokers and some a mix. Consistent with such inconsistency, overall data are mixed: four trials produced statistically significant improvements in cognition compared with placebo; all four that assessed P50 changes showed normalisation of electrophysiology (though not always with demonstrable changes in cognition); and five of the six that assessed clinical symptomatology demonstrated significant improvement. The most common side effects were sleep disturbances and gastrointestinal problems.

Encenicline (EVP-6124)

Encenicline, previously known as EVP-6124, is a co-agonist at the α7 receptor and also requires the presence of ACh. It has a half-life of approximately 55 h, and it is also an agonist at the ionotropic serotonergic $5HT_3$ receptor. Preskorn et al. [146] evaluated encenicline in 21 stable individuals with schizophrenia or schizoaffective disorder, including non-smokers ($N=3$) and smokers ($N=18$) – though participants were not allowed to smoke for an hour and a half before cognitive testing. Testing was in three groups, each treated for 3 weeks: 0.3 mg/day encenicline ($N=8$), 1 mg/day encenicline ($N=9$) and placebo ($N=4$). No differences were elicited between the two doses, though the combined active treatment groups showed a trend in improvement over placebo. The CogState Battery was utilised, and drug-induced positive differences were shown in the domains of visual memory, working memory, executive function, social cognition and combined score (for combined score: $d=0.37$ at day 1, $d=0.34$ at day 21). Electrophysiological measurements of P50 did not show any change, though other markers – namely, MMN and P300 – showed normalisation with medication. Symptomatology was assessed but did not show any improvement.

Tropisetron

As well as being an α7 nAChR partial agonist, this compound is also a serotonergic ionotropic $5\text{-}HT_3$ receptor antagonist, and this second property has led to its more traditional use as an antiemetic agent, both post-operatively and in those undergoing chemotherapy. Results from the three double-blinded RCTs that have evaluated tropisetron are mixed.

An 8-week trial by Shiina et al. [156] of 40 individuals with schizophrenia failed to demonstrate positive effects of 10 mg of tropisetron (compared with placebo) over the study's 8-week test period, though P50 measurements were normalised in the active treatment arm. However secondary analyses found differences between smokers and non-smokers, with the latter ($n=11$) doing better on the CANTAB's delayed matching to sample task and rapid visual information processing task: somewhat confusingly however, the first neurocognitive improvement was in the control group, the second in the active treatment arm. Contrasting with this Zhang et al. [192] showed statistically significant improvement – as measured by the RBANS – in non-smoking patients on both 20 mg ($p=0.002$) and 10 mg ($p=0.006$) doses over 10 days, compared with placebo, and similarly showed P50 normalisation. Finally Noroozian et al. [128] undertook a different approach in their 8-week study, taking clinical outcomes as their primary measure. A significant reduction on the PANSS total ($p=0.012$) and negative symptom subscale ($p<0.001$) was demonstrated in the active groups (5 mg/day) compared to placebo group, though participant smoking status was not recorded.

DMXB-A

3-(2,4-Dimethoxybenzylidene)anabaseine (DMXB-A) is an α7 nAChR agonist with a very short half-life of just 2 h. One research group has investigated its effectiveness in two crossover trials on non-smoking patients with schizophrenia. In 2006, Olincy et al. [134] evaluated a double-dose regimen consisting of either 75 or 150 mg of DMXB-A, followed 2 h later by half the initial dose, and compared this with placebo administration, in 12 participants. The two active treatments showed a statistically significant improvement over placebo in cognition – as measured by the RBANS – and normalisation of P50 electrophysiological measurements, with no difference determined between the doses. Follow-up work by the same team [49] evaluated two dosing regimens, 75 mg twice daily and 150 mg twice daily, compared with placebo. Thirty-one participants with psychosis spent 4 weeks in each arm, having a wash-out week between. Interesting, in this larger and longer-duration study, no significant differences were found between DMXB-A and placebo, whatever the dose. However the authors noted that there was a considerable (and statistically significant) test repetition effect that may have hindered the eliciting of differences, and secondary analysis of the first study arm alone found that DMXB-A increased the T score over placebo for attention/vigilance at both doses (75 mg $p=0.05$, 150 mg $p=0.03$) and for working memory at the 75 mg dose ($p=0.03$). In each trial there was a significant improvement in symptomatology.

TC-5619

Lieberman et al. [105] conducted an international (the United States and India) study evaluating TC-5619 in 185 patients, almost half of whom were smokers, over a 12-week period during which time dosing of the active compound increased over 4 weeks (1 mg/day, 5 mg/day, 25 mg/day). TC-5619 was significantly ($p=0.036$) superior to placebo, as measured by the Groton Maze Learning Task of the CogState Battery, by week 4; but whilst the effect size persisted until the end of the study, a significant difference over placebo did not. Average improvements were greater in smokers at weeks 4 ($p=0.028$) and 12 ($p=0.004$).

42.1.2.3 Positive Allosteric Modulators (PAMs)

Positive allosteric modulators, or PAMs, enhance the efficiency of receptor-ligand binding rather than binding directly with the nAChR orthostatic site. This has a pharmacological appeal insofar as the α7 nAChR desensitises relatively rapidly to the high direct agonist levels potentially achieved by nicotinic medications, a phenomenon that is liable to adversely alter longer-term physiological functioning of the acetylcholinergic system [140], whereas PAMS will augment the usual temporo-spatial functioning of acetylcholine in the brain [61]. PAMs work either by enhancing receptor gating, thereby keeping the nAChR in non-refractory and responsive state (so-called type I PAMs), or reducing desensitisation of the nAChR (type II) [8].

Galantamine

Most work on the utility of nicotinergic PAMs in augmenting cognitive symptoms of schizophrenia has been with the compound galantamine, an acetylcholinesterase inhibitor (AChEI) best known for its use in the treatment of Alzheimer's dementia [41]. It further acts pharmacodynamically as a PAM of both the α7 and α4β2 nAChRs and increases mesocortical dopamine levels: the mechanism of action through which it effects such dopaminergic changes remains incompletely understood, but the result may be clinically significant given the pathological frontal lobe hypodopaminergic state typically seen in schizophrenia [57]. To date only one study has shown that galantamine effected a significant improvement in cognition, and none have demonstrated any change in clinical symptoms.

Schubert et al. [154] randomised 16 patients (15 of whom were smokers) to galantamine (titrated to 24 mg/day) and placebo in an 8-week RCT. Those on galantamine showed a significant improvement in cognition, measured by the RBANS, and, impressively, the 'attention' and 'delayed memory' subscales were effectively normalised. However these striking results have not been replicated by others. Indeed work by Dyer et al. [41] found galantamine (dose titrated to 16 mg twice a day) to be associated with *worsened* performance on several cognitive subdomains when compared with placebo in an 8-week RCT of 20 patients (all non-smokers). A 12-week RCT of both smoking and non-smoking patients undertaken by Lee et al. (2007) found that galantamine (dosed initially at 8 mg/day, increased to 16 mg/day after 6 weeks) produced a small increase in some aspects of cognitive function, though this was not significant. Scores on the Korean Mini-Mental State Examination (KMMSE) did not change significantly, though this is not a particularly sensitive marker of cognitive functioning. Buchanan et al. [20] evaluated galantamine 24 mg/day in 86 patients, almost half of whom were smokers, over a 12-week period. The treatment arm did not show any superiority over placebo, though the authors noted a considerable heterogeneity of treatment effect ($p=0.02$). Secondary analyses did show significant improvement in the active arm on some tests, namely, the WAIS-III digit symbol and verbal memory sub-measures, whilst smoking status had no effect on results. Lindenmayer and Khan [106] have completed the longest duration study of a nicotinic cognitive enhancer to date, comparing galantamine (titrated up to 24 mg/day over a month) and placebo in 32 individuals (smokers and non-smokers included but not co-varied) over a 6-month period. Participants were all on the long-acting injectable risperidone depot medication, as they had all been in an earlier unrelated study (Simpson et al. 2006). Those on the active treatment did not do any better than those on placebo, though the authors argued that their sample was a very well-defined and optimally treated group, which might have hindered the ability to show cognitive improvement.

CDP-Choline and Galantamine Combination

Deutsch et al. [37] evaluated galantamine (titrated to 24 mg/day) combined with CDP-choline (2000 mg/day) – which is a dietary source choline and also an α7 nAChR agonist – in 43 patients over 16 weeks. The combined active treatment did not show any significant cognitive effect over placebo (measured by the MATRICS battery), and there was no clinical change detected (measured by the PANSS). However the active group did demonstrate a significant improvement in functioning, as measured by the scale of functioning overall rating ($t=1.99$ [df=42, $p=.05$]).

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Winterer et al. [184] undertook a four-way crossover multicentre trial in 39 patients, all of whom smoked (though none were allowed to do so within 90 min of testing). Participants were randomised to have three of five possible doses of the active drug – 10 mg, 30 mg, 50 mg, 100 mg, 200 mg – and a single dose of placebo, each on 1 of 4 dosing days that had 1–2-week wash-out periods. Electrophysiological work did not elicit any significant effect on P50 sensory gating at any time point, though there was a trend towards this, and the authors considered that they were ultimately underpowered to detect such change.

42.2 Discussion

The burdens of psychotic illnesses are enormous, whether one measures this in terms of the impact on the individual; on families, carers and others around them; and in societal and economic losses. Long-term data are disheartening, with less than one in eight individuals recovering [170]. Existing drugs do show some variation in efficacy and side effects [102], but a far broader issue is the considerable difference in how any given individual will respond to a drug, though we currently cannot predict this and only fully assess this important fact retrospectively [69]. Despite all the changes in mental health care provision, psychosocial outcomes – which in many ways are the most meaningful domain for an individual – have barely shifted over the years, and much of this is due to our inability to manage cognitive symptoms. Strikingly, in the face of such data, a systematic review of treatment refractory illness [163] found that none of the included studies measured cognitive functioning. It seems probable that a sad combination of their less visible nature and an implicit recognition that we currently unable to offer any meaningful treatment have combined to produce this state of affairs. The potential for novel therapeutic compounds that could meaningfully alleviate these difficulties would be little short of revolutionary in the treatment of schizophrenia.

This chapter assessed the current evidence for nicotinic modifying compounds in this regard: α4ß2 and α7 receptor agonists and positive allosteric modifying drugs. At present, the best that one can say is that the current evidence is interesting but not convincing, with as many trials showing no improvement as show benefit. From a tolerance perspective, the compounds are generally acceptable to patients, with the major difficulties being gastrointestinal and sleep related.

What remains unanswered is whether the lack of particularly impressive findings is due to the inherent limitations of these compounds or whether it is due to confounding noise from inadequate study design. There is no doubt that the majority of studies have small participant numbers, and modest class size effects could be hiding as yet undiscovered (and demographically/clinically yet to be delineated) populations of full and partial responders [83]. Fitting with this, study follow-ups were brief and measured in days or weeks, and one could reasonably posit that this is not a sensible time frame in which to measure changes to cognition. If the drugs are effecting neural plasticity, with molecular changes to cell functioning, and outcomes ultimately measured by how this can be translated into brain changes in learning, surely a span of at least many months will be required to confidently ascertain effectiveness. Certainly most trials of existing psychotropics with proven efficacy would perform reasonably poor if only measured over a few days and weeks. Compounding this, the majority of studies were undertaken with patients with varying degrees of refractory illness stabilised psychosis, including cognitive deficits, but this must be contextualised by the recurring finding in schizophrenia research that the duration of untreated psychosis (DUP) is perhaps the most important prognostic indicator. Negative and cognitive symptoms typically progress through the course of schizophrenia, and by the time of entering these current trials, most individuals therefore have a presumed level of deficit that might be unresponsive to treatment [7]. The fascinating possibility is that much earlier intervention, even possibly in a prophylactic manner in those at high risk, might elicit far better responses in the longer term.

A further unresolved question is how the cloud of cigarette smoke might be fogging results. Data on nicotine's effects on cognition are clear, even if individual study outcomes vary; and long-term change to nAChRs from smoking is similarly established. Following on from this, what are the confounding acute effects of including patients who smoke, and how different is their brain chemistry and physiology? Whilst the memory and attentional enhancements seen from nicotine consumption appear to be through the α4ß2 nAChR, interactions with the α7 nAChR also occur. Existing trials have varied considerably both in their inclusion/exclusion and stratification of smokers: to date there is no discernable pattern of effect – some studies showing smokers

did better, some did worse – but the principle of their potential to hinder data interpretation is clear.

Another obvious issue when appraising the data is that most studies are using different neuropsychometric tests, which makes cross comparison (and meta-analytical review) far more difficult. Some of this might be a historical vestige, as in recent years the American National Institute of Mental Health has developed the MATRICS programme (www.matricsinc.org) that aims to standardise cognitive assessment in psychosis: however it remains to be seen how this will impact on future work and what it will mean for evaluating nAChR effectiveness. Nevertheless the potential for increased statistical power through grouped study analysis is tantalising, particularly when one considers that 'cognition' is not a unitary phenomenon and that several existing studies have shown improvements in some subdomains of testing but were underpowered to fully determine the meaning of such findings. For example, Preskorn et al.'s [146] data on α7 receptor agonists indicated that the drug tested might improve non-verbal higher cognitive functions; and Shiina et al. [156] showed sustained visual attention. Finally with regard to testing confounders, concern has been raised about a repetition effect altering results in serially applied test batteries, though it is currently uncertain as to how significant a factor this is.

The utility, or otherwise, of the P50 electrophysiological marker is another debated factor in nAChR trials. On the one hand it answers the common refrain that psychiatry has lacked meaningful biomarkers, and this test affords demonstrable physiological markers that can be measured and tracked and which have fundamental face validity with regard to associations with schizophrenia, attentional processing and the nAChR. Supporting this, most current studies that evaluated P50 in this context showed some normalisation with nicotinic modifiers. However the rebuttal is that re-regulation of P50 was often not accompanied by clinical change in cognition: so the drugs are being shown to do *something*, but what? And what use to an individual is the change in a neurophysiological marker? Fitting with the earlier point on study duration, it could be argued – though not yet proved – that we are therein seeing medication-induced changes to the attentional/memory systems of the brain, but we are not allowing sufficient time for improvement to occur.

At this time we remain faced by the enormous clinical burden and a lack of effective treatments. Data on any putative role for nAChRs is currently insufficient to recommend clinical usage but strong enough to warrant further trials. The questions that remain are whether the drugs might help some people, what cognitive domain(s) they most assist, when we should initiate them and how long will it take to show this. A notable untouched area is whether or not cognitive *training* should accompany cognitive enhancers: it seems plausible that improving brain plasticity might of itself not be enough and that the drugs might offer a window in which to implement psychosocial changes with better effect.

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43 The Glutamate mGluR5 Receptor as a Pharmacological Target to Enhance Cognitive Function: Emerging Evidence from Psychosis Models

Derek K. Tracy, Nicola Smallcombe, Farah Tiwana, Judith Fosbraey, Kyra-Verena Sendt, and Sukhwinder S. Shergill

43.1 Introduction

Schizophrenia is a severe mental illness that affects just under 1 % of the population [78]. It generally has a relapsing-remitting course that is characterised by serious cognitive and emotional dysfunction, with the onset typically beginning – often insidiously – in adolescence [26]. It manifests with so-called 'positive' symptoms, such as delusions and hallucinations; 'negative' symptoms that include flattened affect, alogia and avolition; and difficulties with cognition [117]. Whilst effective antipsychotic medications exist, they primarily work for positive symptoms (though not in all cases) and are frequently accompanied by a range of unpleasant side effects [65]. Adherence to treatment is commonly problematic [104]. As we noted in our earlier chapter of this book (Chap. 42), such medications are fundamentally ineffective against cognitive symptoms, though cognitive problems are most prognostic of longer-term functional outcomes.

For years, the dopamine hypothesis has been the dominant theory regarding the pathophysiology of schizophrenia, and research across a range of disciplines has provided consistent support for it [46]. Evidence from brain-imaging studies with antipsychotic-naïve patients of schizophrenia shows elevated levels of dopamine receptor occupancy [1], and almost all effective antipsychotic medications act by blocking dopamine receptors [87]. The initial development of the dopamine hypothesis evolved from a reverse engineering of these medications [10]. These discoveries were further supported by studies showing that agonism of dopamine receptors through the administration of amphetamines caused psychotic symptoms [66], and observations that the indole

D.K. Tracy (✉)
Crisis, Inpatient, and Rehabilitation Services, Oxleas NHS Foundation Trust, London, UK

Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK
e-mail: derek.tracy@oxleas.nhs.uk

N. Smallcombe • F. Tiwana • J. Fosbraey • K.-V. Sendt
Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK
e-mail: nmsmallcombe@hotmail.co.uk; farahtiwana@hotmail.com; jf430@exeter.ac.uk; kyra-verena.1.sendt@kcl.ac.uk

S.S. Shergill
The National Psychosis Unit, South London and Maudsley NHS Foundation Trust, London, UK

Cognition Schizophrenia and Imaging Laboratory, Department of Psychosis Studies, the Institute of Psychiatry, King's College London, London, UK
e-mail: sukhi.shergill@kcl.ac.uk

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alkaloid reserpine, which had been found to be effective in treating psychoses, produced its effects through dissipation of synaptic dopamine [11]. By the 1970s, the principle that the antagonism of dopamine receptors directly linked to the amelioration of psychotic symptoms was well established, along with the seemingly natural link that schizophrenia was therefore caused by excess dopamine [76]. However it soon became apparent that such a model was overly simplistic and did little to explain the differentiation between the three core symptom domains.

A seminal work by Davis et al. in the early 1990s provided a reconceptualisation of the existing hypothesis [20]. This review and conceptual piece evaluated advances across several domains including animal model data, post-mortem studies and cerebrospinal metabolite findings. It became clear that dopamine (its metabolites in the serum or cerebrospinal fluid) was not uniformly elevated in individuals with schizophrenia. In addition, the spotlight previously placed on D_2 receptors was challenged, as clozapine, the most efficacious antipsychotic and drug of choice for treatment refractory illness, had been demonstrated to have a reduced occupancy and low affinity at D_2 receptors [46]. These inconsistencies, along with discoveries of patients with relatively low mesolimbic dopamine levels but a similar disease burden and symptom severity and PET studies showing reduced blood flow in the frontal cortex, led to the conclusion that an excess of dopamine was not solely responsible for psychoses. Furthermore, clinical observation readily demonstrates the failure of many patients to respond to dopamine-blocking treatment.

43.1.1 The Emergence of Glutamate as an Aetiological Factor

It is now widely accepted that dysfunction of the glutamatergic system also plays a role in the aetiology of schizophrenia [82, 103]. The glutamate hypothesis of schizophrenia emerged initially from the finding that lower levels of glutamate were present in cerebrospinal fluid and post-mortem brain tissue of individuals with schizophrenia [59]. A parallel finding supporting a role for the glutamatergic system has been that the administration of glutamate antagonists such as ketamine and phencyclidine (PCP) resulted in schizophrenia-like symptoms, such as hallucinations [9].

Glutamate is the primary excitatory neurotransmitter of the central nervous system (CNS), mediating nearly 70 % of synaptic transmission [95, 106]. Glutamate acts on two families of glutamate-binding receptor proteins: the ionotropic receptors (iGlu) and the metabotropic receptors (mGlu). iGlu receptors mediate fast synaptic responses through ionotropic transmission and consist of NMDA, AMPA and kainate subtypes, whilst mGlu transmembrane receptors cause slower effects through alteration of intracellular secondary messenger systems [101]. Figure 43.1 gives an overview of the receptor subtypes.

43.1.1.1 Ionotropic Glutamate Receptors (iGluRs)

iGluRs are responsible for fast synaptic responses: receptors are tetrameric or pentameric complexes (both hetero- or homo-oligomeric subtypes occurring) permeable to K^+ and Na^+ and variably to Ca^{2+} [30, 95]. There are three main classes: N-methyl-D-aspartate (NMDA), α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainite (KA), all named after their originally noted selective agonists. Exogenous ligands typically do not differentiate between AMPA and KA receptors, and these two are thus often collectively termed ‘non-NMDA receptors’. Each class is composed of different subtypes: AMPA having GluR1-GluR4; KA GluR5-7 and KA1-2; and NMDA NR1, NR2A-D, NR3A-B and NR4 [58, 77], and these subunits are further diversified through RNA editing and alternative splicing [102].

There are high levels of NMDA receptors present within the CA1 region of the hippocampus, but also the forebrain and thalamus. In addition, a relatively lower level of NMDA receptor expression is observed within the cerebellum, spinal cord and basal ganglia. It produces a longer lasting and slower response than non-NMDARs and is considered critical for encoding new information essential to memory and learn-

iGlu			mGluR		
NMDA	AMPA	Kainate	Group I	Group II	Group III
NR1 (a-h) NR2 (A-D) NR3	GluR1-4	GluR5-7 KA1 KA2	mGluR1a-d mGluR5a,b	mGluR2 mGluR3	mGlu4a,b mGluR6 mGluR7a,b mGlu8a,b

Fig. 43.1 Glutamate receptor subtypes

ing [95]. Much of the animal and human data investigating NMDA (dys)function has focused on the NMDA antagonist ketamine [9], and its administration has been shown to produce dose-related psychotic symptoms, including negative and cognitive symptoms [62].

43.1.1.2 Metabotropic Glutamate Receptors (mGluRs)

Glutamate was originally shown to activate G-coupled protein receptors in the mid-1980s [27]. mGluRs are distributed heterogeneously throughout the central nervous system [27]. They mediate slow cellular conformational changes through secondary messenger pathways, and their roles are considered more variable than that of the iGluRs [58]. Amongst their roles, mGluRs have been proposed to be essential in learning and memory, and receptor dysfunction has been noted in various neuropsychiatric disorders including Alzheimer's disease, Parkinson's disease and schizophrenia [21, 31, 50, 95]. Increasingly mGluR dysfunction is being shown to be associated with the development of schizophrenia [90] though it is currently uncertain if this is in an epiphenomenal or causal relationship [72].

The receptors, in keeping with traditional G-protein structure, have seven transmembrane regions and contain a large extracellular N-terminus [37]. The eight different receptors ($mGlu_{1-8}$) show approximately 60–70 % between-group homology and only 40 % within-group homology, demonstrating a significantly greater degree of heterogeneity than most other traditional G-protein-coupled receptor families in the human CNS. They are functionally subcategorised into three groups differentiated by their intracellular signal transduction and specific G-protein coupling. Group I receptors (mGluR1a-d, mGluR5a,b) are predominantly postsynaptic and coupled with $G_{q/11}$ [30]: they are largely excitatory in nature, activating phospholipase C that leads to a release of intracellular Ca^{2+}, and also have a modest presynaptic presence and an ability to cause depolarisation through reduction in potassium currents. Groups II (mGluR2 and mGluR3) and III (mGluR4a,b, mGluR6, mGluR7a,b, mGlu8a,b) are primarily presynaptic and predominantly coupled with $G_{i/o}$ G proteins that decrease neuronal excitability through negative regulation of cyclic adenosine monophosphate (cAMP) [30, 72, 121].

A glutamatergic hypothesis of schizophrenia largely revolves around the concept of NMDAR hypofunction: specifically, subcortical reductions in NMDAR function on GABAergic neurons leading to dysregulation of a glutamate signalling,

with a loss of inhibitory tone resulting in a hyperglutamatergic state [72]. Whilst the NMDAR is thus crucial in this model of the development of schizophrenia, it is not considered to be the sole glutamatergic contributor. In particular, the unique interplay between the NMDAR and mGluR5 has led to the latter becoming a major focus for pharmacological experimentation [90].

43.1.2 The mGluR5

The mGluR5 has two main isoforms, mGluR5a and mGluR5b, and a lesser explored mGluR5d, which all vary in topographical location throughout the brain [18, 90]. As with other Group I mGluRs, they have a seven transmembrane region anchoring the receptor to the cell wall, with a large extracellular N-terminus, and an intracellular C-terminal domain [17]. They are coupled to $G_{q/11}$ proteins, activation of which leads to inositol phosphate stimulation and the mobilisation of calcium from its intracellular stores [18, 72]. The mGluR5 has a role in neural circuit patterning and synaptogenesis within the early stages of neurodevelopment [122], during which time the mGluR5a splice variant dominates [80, 97]. In adulthood, the primary version expressed is the mGluR5b, which is widely distributed throughout the cerebral cortex, nucleus accumbens, hippocampus, striatum, lateral septum and hypothalamus, as well as on some non-neuronal cells such as stem-progenitor cells, microglia, astrocytes and oligodendrocytes [52]. The mGluR5d is found in the hippocampus and cerebellum and has fewer interactions than the other splice variants, which has been proposed to be due to its shorter C terminus [70].

In the mature adult brain, mGluR activity is heavily regulated by various postsynaptic proteins, particularly scaffold proteins [98], and in this latter regard, the scaffold protein Homer has been shown to be especially important for both the signalling and localisation of mGluRs in general and mGluR5 in particular [110]. With regard to mGluR5, Homer has been shown to cause reduced coupling to postsynaptic effectors. There is much work currently being undertaken to understand the precise relationship between mGluRs and scaffold proteins [90], and proline-directed kinases have been shown [47] to negatively regulate mGluRs through phosphorylation of the Homer binding site. The mGluR5 has been shown to have a strong association with NMDA receptor [17], with data that it enhances its function [40] in part through the family of postsynaptic proteins SH_3 and multiple ankyrin repeat domains (SHANK) scaffolding proteins. In addition, mGluR5 has been shown to be a driving force in the changes in NMDAR subunit composition in response to learning experiences [74].

Genetic studies have linked the mGluR5 with schizophrenia [72], notably in a large study of Scottish individuals that demonstrated the microsatellite G64931, a constituent gene for the mGluR5, was found to be present in a higher allele frequency in patients than controls [23]. However, studies linking the mGluR5 directly to schizophrenia are relatively sparse and have tended to focus on other genes that affect mGluR5 indirectly. In particular, RGS4 – a negative $G_{q/11}$ protein modulator – has been highlighted as a susceptibility gene in several studies [63, 105, 107], and those encoding scaffold proteins such as SHANK and Homer have similarly been shown to be of importance within this patient group [112]. Post-mortem data have, to date, yielded little, with several such studies of patients with schizophrenia showing unchanged mRNA levels of mGluR5 in the hippocampus, prefrontal cortex, thalamus and striatum [42, 88, 94]. Volk et al. [119] reported mGluR5 expression in post-mortem prefrontal cortex human brain tissue from a sample of patients with schizophrenia, schizoaffective disorders and healthy controls: whilst mGluR5 expression was not altered in those with schizophrenia compared to controls, there was reduced mGluR5 expression in individuals with schizoaffective disorders. It may be that mGluR5 expression is reduced in schizoaffective patients due to its role in the more mood-related symptoms of schizophrenia such as asociality, though such a hypothesis is speculative at this time. Moreover, Timms et al. [111] studied families with multiple individuals affected by schizophrenia with the aim of

identifying genetic factors. In all families, there were mutations in at least one of the three genes associated with NMDA receptors. Additionally, one of the families shared a mutation of GRM5, which disrupted mGluR5 binding and normal functioning.

43.2 The Trials and Tribulations of Utilising Animal Models and Studies in Psychosis

Reliable, predictive animal models are a critical part of pathophysiological and pharmacotherapeutic research in schizophrenia [86]. Reproducing the entirety of a complex psychiatric illness in nonhumans is clearly not possible, but a large variety of rodent models have been developed to mimic specific, individual symptoms or aspects of the disease [123]. The overwhelming majority of studies to identify and test new molecular targets for novel drugs targeting cognitive impairments have comprised rodent models. Animal models have several advantages, including being a simpler, ethically more appropriate, significantly less resource-intense and more rapid method to test disease course than in a clinical population. They provide a unique, prospective method of manipulating and observing any causal interplay of environmental, genetic and molecular factors in the pathogenesis and physiology of the disorder [92]. Importantly, they provide various opportunities of invasive evaluations of neurobiological change characteristics of the disease and the development of new pharmacological agents not feasible in humans [51, 113].

Three different types of animal models are generally recognised within biological psychiatry: homologous models represent identical symptomatologies and disease courses to that occurring in humans; isomorphic models share phenotypic similarities, but differ in cause; and predictive models reliably describe the therapeutic activity of a particular drug [93]. In schizophrenia, these can be further differentiated into four main groups: genetic, developmental, drug-induced and lesion studies [51]. The studies included in this chapter all used developmental or drug-induced models of schizophrenia. The drugs used in these models were the uncompetitive NMDA receptor antagonists phencyclidine (PCP), (+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate (MK-801) and ketamine, the stimulant amphetamine and the neurotoxin methylazoxymethanol acetate (MAM).

The strength of an animal model of schizophrenia, and its translational potential into human studies, can be evaluated through a triad of face, construct and predictive validity: face validity describes the phenomenological similarity between the behaviour observed in the animal model and the associated symptoms in the clinical population; construct validity is the precision with which the animal model measures what it is anticipated to measure as a means of testing underlying disease aspects, whilst predictive validity reflects the ability to show the expected treatment effect, or lack thereof, of known therapeutic agents or novel drugs [93]. This last factor, predictive validity, has been proposed to be one of the most crucial initial criteria for determining the quality of a preclinical animal model in schizophrenia [123].

43.2.1 Validity of Developmental and Drug-Induced Models of Schizophrenia

43.2.1.1 Gestational MAM

Methylazoxymethanol acetate (MAM) is naturally occurring substance found in the seeds of cycad plants, which is an anti-mitotic and antiproliferative agent that methylates DNA [73]. Administration of MAM during gestation affects neurodevelopment and results in anatomical and behavioural deficits analogous to those found in schizophrenia, although this is highly dependent on the gestational day of MAM administration [68]. MAM administration on gestational day 17 is regarded at the optimal strategy. Gestational MAM has acceptable face validity for positive and cognitive symptoms and has construct validity regarding the structural and dopaminergic changes observed [51]. Its predictive validity has

not been evaluated due to the lack of research on pharmacological agents using this paradigm.

43.2.1.2 PCP Models

Phencyclidine (PCP) is an uncompetitive NMDA receptor antagonist which causes cognitive and neurochemical deficits similar to those found in schizophrenia, providing both some face and construct validity. However, the construct validity of PCP models is questionable as PCP is generally administered to adult animals and therefore does not reflect the neurodevelopmental origin of schizophrenia [51]. In light of this, some studies are now using PCP developmental models in which PCP is administered at the neonatal stage [16]. A strength of the PCP model is that it has translational relevance as findings from PCP models have been translated to primates. The predictive validity of PCP models is in question as the therapeutic effects of atypical antipsychotics on cognitive symptoms seen in PCP models are not found in clinical research [54].

43.2.1.3 MK-801 Models

MK-801, also known as dizocilpine, is an NMDA receptor antagonist. MK-801 models have good face validity for cognitive and negative symptoms of schizophrenia [85], as well as good construct validity through effects on neural connectivity and increased dopamine release in the medial prefrontal cortex and nucleus accumbens [100]. However, the predictive validity of MK-801 models is disputed, with critics arguing that the model is too permissive and may produce false positives for compounds with no clear efficacy in treating symptoms of schizophrenia [7].

43.2.1.4 Ketamine Models

Ketamine is an NMDA receptor antagonist which has a dissociative effect in humans. Administration in rats results in increased D2 receptor binding in the hippocampus, a decrease in glutamate binding in the frontal cortex and increased density of dopamine transporters in the striatum [5], giving this model good construct validity. Ketamine models also have strong face validity across all symptom domains and, in particular, replicate the cognitive impairments seen in schizophrenia, such as impaired performance in working memory tasks, altered social behaviour, stereotypy and sensory gating deficits [81]. Ketamine models have demonstrated predictive validity for positive [63], negative and cognitive symptoms [28].

43.2.1.5 Amphetamine Models

Amphetamine models of schizophrenia were developed after the observation that amphetamine causes psychotic-like experiences in humans through its excitatory action on the dopaminergic system. Amphetamine models only have face validity for positive symptoms of schizophrenia, as this model does not replicate cognitive or negative symptoms [51]. Amphetamine models have some construct validity due to the ability to induce mesolimbic dopaminergic hyperfunction but are less valid representations of glutamatergic deficits. As such, the predictive validity of the amphetamine model may be limited to drugs acting on the dopaminergic system, although amphetamine-induced hyperlocomotion is sensitive to other classes of drugs, including mGluR2/3 agonists.

43.2.2 Validity of Cognitive Tests in Animal Models

Over the past decade, several large-scale efforts have been fostered to improve the utility of animal models of cognitive deficits in schizophrenia for meaningful, clinically relevant human studies and the development of novel treatments for these debilitating and costly impairments. Notably, the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative, launched by the US National Institute of Mental Health (NIMH) in 2003, set out to address widespread barriers to the development of pro-cognitive pharmaceutical agents in schizophrenia by facilitating collaborations between the pharmaceutical industry, academia and governmental researchers. The main aim was to advance a standardised set of tools to

guide research into and evaluation of novel treatments in several domains of cognitive functioning severely affected in schizophrenia [38, 71]. Due to time constraints of the initiative, MATRICS focused on assembling a consensus test battery primarily consisting of well-established, mostly pen-and-paper assessments, neurocognitive functioning originating from the 1990s [57]. Over the following years, however, the field saw an enormous increase in technological advances in the areas of genetics and genomics, human neuroscience, physiology and neuroimaging, which, together with data from animal studies, has resulted in improved understanding of the neurobiological processes at the core of cognitive functioning in schizophrenia [49]. The rapid development of cognitive neuroscience in particular brought with it a novel set of tools and task paradigms to evaluate cognitive functioning in humans as well as animals and provided the basis for the creation of a new, complimentary initiative to MATRICS: the Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) consortium (http://cntrics.ucdavis.edu/). CNTRICS similarly aimed at creating a standardised set of tools to measure cognition and test new pharmacological agents for the treatment of cognitive deficits in schizophrenia, but concentrated on the use of neuroscientific, brain-based techniques and biomarkers within a preclinical translational framework. Drawing together an interdisciplinary group of cognitive scientists and other researchers from backgrounds in animal and human models, the improved identification of cognitive constructs and hypotheses as targets for novel treatments of cognitive deficits in schizophrenia lay at the heart of the initiative [83]. Cognitive constructs describe cognitive processes measured behaviourally and with clear, underlying hypothesised and measurable circuit-based mechanism [4]. Six different cognitive constructs of interest were chosen by CNTRICS within the domains of attention, perception, executive functioning, social and affective processes, working memory and long-term memory [83].

In addition to the identification of these cognitive constructs, the development of psychometric principles relevant to the advancement of tasks and neuroimaging tools measuring the relevant domains was chosen to be a second goal of CNTRICS. Emphasis was put on identifying benchmarks for the meaningful use of tasks in clinical trial settings, taking into account certain considerations required when working with a schizophrenia patient population displaying a level of cognitive impairment (e.g. avoiding excessive task length and complexity). Ultimately, CNTRICS aimed at the selection and further improvement of functional neuroimaging biomarkers and human cognitive neuroscience tasks with high levels of construct validity, which were able to measure the chosen constructs specifically and at the behavioural level. Importantly, the existence of homologous animal tasks used in preclinical research had to be ensured in order to fulfil the main goal of enhancing translational research of cognition in schizophrenia [12, 13, 79].

Using a contemporary cognitive neuroscience approach to enhance translational research in this field is supported by a number of distinct advantages. Crucially, the ability of measurements of various cognitive constructs to translate across mammalian species is one fundamental benefit. It is feasible to successfully employ neuroscientific cognitive tasks measuring domains of attention, perception and working memory, amongst others, which are all implicated significantly in schizophrenia-related impairments, using homologous paradigms in animals and humans. This results in the facilitated *identification* of molecular targets modulating cognition [12]. An illustrative example of this is an extensive body of research into the role of different neurotransmitter systems implicated in the construct of working memory, pinpointing new potential targets for drug discovery [2, 25, 64]. A second related advantage of this approach is the ability to *test* the molecular targets identified via translational animal/human paradigms, thereby increasing the likelihood of positive human clinical trial results of cognition-enhancing drugs [12]. Carter and Barch [12] further describe the benefit of using well-established cognitive testing paradigms within non-invasive

functional neuroimaging techniques [e.g. functional magnetic resonance imaging (fMRI) or magnetoencephalography (MEG)] to provide clarification of the nature of cognitive deficits in schizophrenia. This can facilitate the evaluation of novel pro-cognitive agents by elucidating their site and time course of action as well as confirming their potential to cross the blood-brain barrier. Lastly, the cognitive neuroscience approach enables the more specific identification of cognitive constructs associated with particular neurotransmitter functions or neural systems than traditional neuropsychological cognitive tasks, which often more crudely involve several cognitive processes simultaneously. A more precise isolation of targeted constructs appears likely to be more beneficial in aiding novel drug discovery [12, 69]. Ultimately and beyond the scope of the research arena, using a cognitive neuroscience approach like CNTRICS offers a valuable application to the clinic. The specific targeting of cognitive constructs of interest aids the testing of hypotheses to evaluate whether distinct aspects of cognitive dysfunction differ in their impact on patients' daily functioning [35]. Whilst the cognitive domains of working memory, attention and learning, for example, have already received consistent support for being strongly implicated in impaired daily functioning in schizophrenia [8, 36, 55], further clarification of different cognitive factors can inform the exploration of potential new therapeutic targets.

In sum, the CNTRICS initiative represents an example of urgently required efforts to enhance translational research in the area of cognitive deficits in schizophrenia, guiding the search for novel therapeutics to meet a crucial, unmet clinical need. Multiple spin-off initiatives have been developed in recent years and share similar, integrative aims (e.g. Cognitive Neuroscience Test Reliability and Clinical Applications for Schizophrenia (CNTRACS), http://cntracs.ucdavis.edu/root/publications*, and* Social Cognition and Functioning in Schizophrenia, Green and Penn (*SCAF*) [39]). Several types of animal model cognitive tests commonly utilised are described below.

43.2.2.1 Object Recognition

Object recognition is impaired in people with schizophrenia [29] and is therefore used as a measure of a drug's efficacy in treating the cognitive symptoms of schizophrenia. Object recognition tests are usually performed in rats and consist of a two-session procedure, in which recognition is indicated by reduced exploration of a familiar object relative to a novel object [91]. The task is comparatively easy to run and is ethologically valid, with good predictive validity [85].

43.2.2.2 Set-Shifting

The attentional set-shifting task is a measure of executive control and requires animals to adapt responding based on changes to the relevance of perceptual categories or dimension: it has been used across species, including humans, primates and rodents, with over 40 papers describing attentional set-shifting in rats [34]. The ability to focus attention on a relevant dimension and ignore irrelevant dimensions is impaired in schizophrenia, as measured by set-shifting tasks, and is mediated by the prefrontal cortex [14]. A limitation of this task is that there is currently no standardised version, and as such, there is a level of subjectivity in the interpretation of performance on this task; however, a strength is that it does not just model the cognitive processes observed in humans but measures the exact same processes. Its predictive validity remains somewhat unclear, as research into whether positive drug effects reported in rodents will translate into clinical studies is still underway.

43.2.2.3 Morris Water Maze

The Morris water maze is based on rodents' natural instinct to escape water and consists of a submerged platform in a pool of opaque water [84]. Rodents must use extra-maze clues to locate the platform, and performance is measured by the latency and path lengths to reach the platform. This test is designed to assess learning and memory and has a high reliability across a range of tank configurations and testing procedures: there is extensive evidence of its validity as a measure of hippocampally dependent spatial learning and reference memory, specificity as a measure of

place learning and the relative lack of motivational effects on the task from a range of experimental treatment effects [120]. Most pertinently to schizophrenia research, versions of the procedure exist across species, including humans, which have demonstrated that schizophrenia patients show profound learning and memory deficits in this task [124]; it has also been shown to have predictive validity for response to atypical antipsychotics [24].

43.2.2.4 Sucrose Preference

The negative symptoms of schizophrenia can be particularly difficult to model in animals. One of the more widely used tests is the sucrose preference task, in which decreased consumption or reduced preference for a sweet sucrose solution is purported to be a measure of anhedonia. The validity of this test has been questioned as there are confounding motivational effects due to the restriction of food and water during testing, and the variation in the procedure between different laboratories affects the reliability of this method [75]. Additionally, the reward deficits in schizophrenia are believed to reflect more than just anhedonia and may be more accurately described as the inappropriate valuation of rewards [22], which raises concerns regarding the face validity of the sucrose preference test. Despite this, the sucrose preference test is still widely used as a result of the lack of alternatives for replicating negative symptoms.

43.2.3 Limitations of Translational Studies in Schizophrenia

Translational research shortcomings include the self-evident difficulty of modelling the characteristic symptoms of psychosis that are uniquely human and unlikely to be meaningfully replicated in animal models [45, 92]. Attempts to identify and directly translate animal behavioural traits into symptoms of schizophrenia as laid out in standardised classification systems (e.g. DSM-V and ICD-10) range from challenging to virtually impossible [123], especially when the heterogeneity of the illness in humans is considered. Whilst rodents are usually genetically 'normal' and identical, as well as tested under species-specific ideal, low-stress conditions, patients with schizophrenia are often genetically and environmentally predisposed, with other complicating factors such as substance abuse and the use of multiple prescribed pharmacological agents [85].

One common criticism has been the failure of extensive preclinical rodent model studies to advance the development of novel therapeutics, in particular agents targeting the cognitive impairments characteristic of the disease [56]. Suggestions to tackle this so-called transitional bottleneck between preclinical and clinical research [48] have focused on improving the selection of viable preclinical models most likely to generate successful results in human research [33, 86]. Recent efforts in optimising schizophrenia cognition research have therefore primarily emphasised the selection of specific cognitive constructs as targets of interests for translational research. Several initiatives have been developed to improve the extent to which animal models can mirror the clinical picture of cognitive deficits in schizophrenia.

43.3 mGluR5 as a Therapeutic Target: Current Research Evidence

Modulation of the mGluR5 has been explored through investigation of orthosteric (drugs that bind and act at the endogenous receptor) and allosteric (drugs that bind at other sites) compounds, though thus far the creation of viable orthosteric modulators has been less successful. There are three main reasons for this: firstly, orthosteric agonists, which are glutamate analogues, have generally been unable to bypass the blood-brain barrier; secondly, the endogenous binding site is highly conserved, making it problematic to develop a very specific pharmacological agent; and finally, there is a more conceptual concern that direct agonists could lead to alteration to normal receptor physiological functioning, such as through desensitisation and altered

expression [17]. Consequently most work has looked at positive allosteric modulators (PAMs) that will only work in the presence of the endogenous ligand and – in principle at least – are therein less likely to lead to altered receptor physiology.

Early data have shown that positive allosteric modulators (PAMs) of the mGluR5 can reverse cognitive and behavioural deficits caused by administration of NMDA antagonists. Becker et al. [5] compared rats injected with saline solution and rats injected with ketamine, on measures of social activity and conditioned learning. It was observed that rats in the ketamine condition exhibited less non-aggressive social behaviour and were less receptive to conditioned learning. Also, a study by Brody et al. [6] tested mGluR5 knockout mice and controls with intact mGluR5 on measure of behavioural startle response to various acoustic stimuli. Prepulse inhibition (PPI) was found to be impaired, much like the sensorimotor gating deficits seen in patients of schizophrenia. Similarly, Kinney et al. [60] noted that whilst amphetamine administration induced PPI deficits in rats, such effects could be ameliorated by administration of a selective, partial mGluR5 agonist, CHPG ((RS)-2-chloro-5-hydroxyphenylglycine). Attenuating effects of mGluR5 PAMs were also reported by Homayoun et al. [43], when administration of MK-801 or 2-methyl-phenylethynyl-pyridine (MPEP), both mGluR5 NAMs, led to deteriorating social behaviour and problems with working memory and learning in rats. The behaviour observed in animals receiving mGluR5 NAMs or NMDA antagonists closely resembles negative and cognitive symptoms seen in clinical populations of psychosis, such as asociality, problems with sensorimotor gating and deficits in learning and memory.

43.3.1 CDPPB

3-Cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) is a selective positive allosteric modulator (PAM) of mGluR5. CDPPB has a 4.4 h plasma half-life with volume of distribution of 3.5 l/kg and clearance of 25.1 ml/min/kg and inhibits [^{3}H]methoxyPEPy binding to human mGluR5 CHO cell membranes [61]. Several studies have evaluated the effects of this compound in differing rodent models of schizophrenia, using MK-801, PCP and amphetamines to induce deficits.

43.3.1.1 Negative Symptoms

Vardigan et al. [118] investigated the effect of CDPPB on an MK-801-induced deficit in sucrose preference in rats, a rodent model of negative symptoms in schizophrenia, wherein the lack of sucrose preference is posited as a modelled reduction in the hedonic experience that is not confounded by disruptions in motor ability or taste. Animals were given CDPPB (3 mg/kg i.p., 2 mL/kg in 0.5% methylcellulose) or vehicle 30 min before injections of MK-801 (0.3 mg/kg i.p., 1 mL/kg in saline) or vehicle ($n=10$). Testing began 30 min following injections of MK-801. Acute treatment with CDPPB, comprising of a single injection of 3 mg/kg CDPPB, significantly reversed the MK-801-induced deficit in sucrose preference compared to controls ($p=0.045$).

43.3.1.2 Object Recognition

Two studies explored the effect of CDPPB on object recognition deficits. Horio et al. [44] examined the effect of CDPPB on performance in a novel object recognition task in mice after repeated administration of the NMDA receptor antagonist phencyclidine (PCP). The novel object recognition task consists of a training session and subsequent retention sessions: in the training session, mice were put into a black open-field box containing two objects and were allowed to explore for 5 min, with the time spent exploring the objects recorded, and the retention tests were carried out at 1-day intervals after the training session. During the retention tests, the mice were put in the same box used during training with one of the original objects and a novel object: again, they were given 5 min to explore freely and the time spent exploring the objects was recorded.

There was no significant difference in the exploratory preferences of the three groups

(control/PCP/CDPPB) ($F[2,25]=2,25$, $p=0.77$). PCP (10 mg/kg/day s.c. for 10 days)-induced deficits in exploratory preferences were not improved by a single administration of CDPPB (10 mg/kg/day, i.p.; $p=0.297$). However, PCP-induced deficits in exploratory preferences were significantly improved by subsequent subchronic (14 days) administration of CDPPB (10 mg/kg/day i.p.; $p \leq 0.001$), but not of CDPPB (1.0 mg/kg/day, i.p.). Similarly, CDPPB was found to dose dependently affect performance on a novel object recognition task after administration of MK-801 in rats [116]. 0, 3, 10 or 30 mg/kg CDPPB was administered intraperitoneally prior to the first exposure. Exploration of the novel object was significantly greater in the 10 mg/kg group than the vehicle group ($p<0.05$). In contrast, there was no difference between the 30 mg/kg treated group and the vehicle group ($p>0.05$).

43.3.1.3 Social Cognition

In a social novelty study, administration of CDPPB reversed deficits in social novelty discrimination (SND) [16]. Testing consisted of two consecutive juvenile presentation periods to an adult Wistar rat. During the first period, a juvenile was placed into the adult home cage, and during the second period, the same juvenile and a second novel juvenile were placed in the cage together with the adult. In period 1, SND was assessed by the time spent by the adult rat investigating the juvenile over 5 min. In period 2, the times spent by the adult investigating each juvenile were measured independently for 5 min. Two protocols were employed, each consisting of different cognitive deficits: the parametric deficit protocol weakened SND by a time delay, formed by a shorter first period of 5 min followed by a 30 min delay between period 1 and period 2; the pharmacological deficit protocol involved injecting MK-801 (0.08 mg/kg subcutaneously) or vehicle 30 min before period 1 (duration = 30 min) with no delay between period 1 and period 2. In a further experiment, rats were administered with 10 mg/kg PCP on postnatal days (PND) 7, 9 and 11 and then received either acute CDPPB treatment (0.63–10 mg/kg i.p.; 30 min before testing) or chronic CDPPB treatment (10 mg/kg per day for 12 days between PND 35–46 and PND 70–81) and tested on SND following the pharmacological deficit protocol. In the parametric deficit protocol, CDPPB (0.63–40 mg/kg i.p.; $n=5$–13) significantly modified the novel/familiar discrimination ratio compared to vehicle ($n=13$; $p \leq 0.001$) in an inverted U dose-response relationship. CDPBB demonstrated a significant increase from vehicle at 2.5 mg/kg ($n=9$; $p \leq 0.05$) and 10 mg/kg ($n=13$; $p \leq 0.001$), indicating that CDPPB improved SND compared to controls. In the pharmacological deficit protocol, administration of MK-801 ($n=3$–7) significantly decreased the discrimination ratio compared to vehicle ($n=7$; $p \leq 0.05$), indicating a SND deficit. When MK-801 and CDPPB were co-administered ($n=9$), CDPPB (10 mg/kg) significantly reversed the reduction of the discrimination ratio by MK-801 ($p \leq 0.05$). In the neonatal PCP model of schizophrenia, acute treatment with CDPPB significantly reversed the PCP-induced impairment in SND ($n=6$; $p \leq 0.05$). At both 8 and 13 weeks of age, the PCP-induced impairment was reversed in rats receiving chronic CDPPB treatment at 5 weeks of age ($n=9$; 8 weeks – $p \leq 0.001$; 13 weeks – $p \leq 0.05$). These results demonstrated that CDPPB mediated deficits of SND induced by a time delay, an NMDA antagonist, or a developmental model of schizophrenia, and that chronic, pre-symptomatic treatment of adolescent rats with CDPPB prevented the appearance of SND deficits in adults.

43.3.1.4 Set-Shifting

Set-shifting deficits measure cognitive flexibility and refer to the ability to modify ongoing behaviour in response to changing goals or environmental contingencies. One study utilised an automated operant chamber-based cognitive set-shift task [19]. Rats were first habituated to the testing chamber over the course of 7 days and then received daily training sessions on the set-shifting task. Rats were rewarded with food pellets for poking their noses into one of two available holes according to two distinct discrimination rules, which regularly changed and required the rats to shift their response patterns based on the rule that

was operant at the time. In the light discrimination rule, rats were required to poke their noses into the hole illuminated by a light; in the alternate discrimination rule, rats were required to poke their noses into the hole at a designated spatial location (left or right), regardless of illumination. When rats reached the performance criterion of ten consecutive correct responses, the rewarded dimension was immediately switched to the alternative dimension (extradimensional shift). The only signal that the dimension had changed was the absence of reward for previously correct responses. Rats were required to reach the performance criterion for four consecutive times (four sets) each testing day, resulting in three consecutive extradimensional shifts. The testing phase consisted of blocks of five consecutive days. The first 3 days of each block were used to establish a baseline level of performance for each individual rat. On the fourth day, rats were given a drug treatment before testing (either MK801 (0.1 mg/kg) + CDPPB (10 mg/kg), MK801 (0.1 mg/kg) + vehicle or vehicle + vehicle, all i.p.). The first drug was administered 40 min before testing, followed 20 min later by the second drug. On the fifth day, no injection was given to provide a measure of any long-term treatment effects. MK801 significantly impaired task performance compared to controls through increasing the number of trials required to complete the four sets, the number of errors made and the number of perseverative errors ($n=36$; $p=0.005$). CDPBB attenuated the deficits induced by MK801 on total trials to criterion and total errors, but had no effect on the number of perseverative errors induced by MK801.

A further study by the same group found similar results [108]. A set-shifting task utilising a plus maze was used, with rats given a week to habituate to the maze, followed by set-shifting testing. Testing was formed of two sets: in set 1, rats were trained on either a brightness (light vs. dark) or texture (rough vs. smooth) discrimination task to a criterion level; in set 2, rats were trained on the alternative discrimination strategy for 80 trials. The criterion level was set at eight consecutive entries into a reward arm of the maze. Drugs were administered via intraperitoneal injection (MK801 0.1 mg/kg; CDPPB 10 or 30 mg/kg). The first drug was injected 40 min before behavioural testing and the second drug 20 min later. Rats receiving MK801 took significantly more trials than controls to reach criterion ($p=0.008$). CDPPB administration at both doses significantly reversed the MK801-induced impairment in performance relative to control (CDPPB 10 mg/kg – $p=0.004$; CDPPB 30 mg/kg – $p=0.008$).

43.3.1.5 Spatial Learning

One study has, to date, examined the effect of CDPPB on spatial learning [3]. CDPPB (10 mg/kg) was suspended in a vehicle consisting of 20 % w/v 2-hydroxypropyl-b-cyclodextrin and administered by intraperitoneal injection in mice 20 min before testing. The Morris water maze was used to assess spatial learning: the maze consisted of a 90 cm diameter tub filled with opaque water at 23 °C (±1 °C), which contained a 6 cm diameter submerged platform. Over a 13-day testing period, four test trials were conducted each day (each separated by 5 min), with a randomised sequence of four separate starting points used each day. Latency to reach the platform was recorded for each trial, with a maximum swim time of 60 s per trial. Acquisition criteria were considered to be obtained when the latency to reach the platform was ≤15 s on each of the four consecutive trials. On the final day, a probe trial was conducted in which no drugs were administered. The platform was removed, and time spent in the quadrant in which the platform was previously located was recorded for each of the four trials. CDPPB enhanced performance in the Morris water maze: mice injected with CDPPB ($n=12$) took significantly fewer days to reach the acquisition criteria than controls ($n=13$; $p\leq0.05$); whilst in the probe trial, mice receiving CDPPB ($n=12$) spent longer in the target quadrant than controls ($n=13$; $p\leq0.05$), these results suggest that CDPPB can enhance hippocampus-dependent learning and memory.

43.3.1.6 Locomotor Activity

Two studies have investigated the effect of CDPPB on locomotor activity deficits. In the first study, rats were administered vehicle (60:40,

dimethyl sulfoxide/polyethylene glycol 400) or CDPPB (3–10 mg/kg s.c.) 20 min prior to subcutaneous administration of *d*-amphetamine sulphate (1 mg/kg base) [61]. Activity was assessed as mean distance travelled (in centimetres). There were no significant effects of treatment ($p > 0.87$) or treatment x time interaction ($p \geq 0.93$) when CDPPB was administered alone prior to amphetamine administration. However, following amphetamine administration, there was a significant dose x time interaction (F[69,759]=1.74, $p \leq 0.001$), which showed that CDPPB was more efficacious in reducing the distance travelled at earlier time points.

In the second study, rats were dosed with 30 mg/kg intraperitoneal CDPPB or vehicle (0.5 % methylcellulose) for 6 days [89]. On the seventh day, rats were given a final injection of CDPPB or vehicle – or a first injection of CDPPB for those in the acute treatment condition – and were immediately placed in activity monitors. After a 30-min habituation period, amphetamine (0.75 mg/kg s.c.) or vehicle (saline) was administered, and locomotor activity (expressed as centimetres travelled) was measured for 90 min. Amphetamine significantly increased distance travelled in all groups (F[4,36]=10.07, $p \leq 0.001$) compared to control. However, both acute and repeated treatment with CDPPB significantly reduced amphetamine-induced locomotor activity compared to control ($p \leq 0.05$). The effects of acute and repeated CDPPB treatment did not differ from each other.

43.3.1.7 Prepulse Inhibition

Kinney et al. explored the effect of CDPPB on sensorimotor gating by evaluation of prepulse inhibition (PPI) of the acoustic startle response [61]. Rats were administered subcutaneous amphetamine (2 mg/kg base) 20 min prior to administration of a vehicle or CDPPB (3–30 mg/kg s.c.). Testing began 10 min later, where the rats were exposed to 65-dB background noise for 5 min at the start of each session. Test sessions consisted of ten repetitions of six trial types, which consisted of a 10-ms prepulse at 70, 75, 80 or 85 dB, followed 100 ms later by a 118–120 dB 40 ms startle pulse (prepulse pulse conditions), the startle pulse alone (pulse alone) and a period where no stimulus was presented. CDPPB dose dependently reversed amphetamine-induced deficits, as demonstrated by a significant treatment x prepulse intensity interaction (F[9,132]=2.2, $p \leq 0.027$).

43.3.2 VU0360172

VU0360172 is a highly selective and orally active positive allosteric modulator of mGluR5 that also binds to the MPEP site [96]. It is rapidly and very significantly absorbed in vivo; the C_{max} of 7432.98 ng/ml (~21 μM) was achieved in systemic plasma within 1 h of oral administration of a 10 mg/kg dose, and a C_{max} of ~2 μM was achieved in the brain. One study has examined the effect of VU0360172 on amphetamine-induced hyperlocomotion in rats [96]. Amphetamine-induced hyperlocomotion was assessed using open-field chambers and measured according to the number of total beam breaks per 5-min interval. Rats were pretreated with 10, 30, 56.6 or 100 mg/kg VU0360172 or vehicle 30 min prior to injection with 1 mg/kg subcutaneous amphetamine. VU0360172 at doses of 30, 56.6 and 100 mg/kg was found to reduce amphetamine-induced hyperlocomotion relative to controls when administered intraperitoneally ($n = 7$–8 per condition; $p \leq 0.0001$). VU0360172 at doses of 56.6 and 100 mg/kg was also found to reduce amphetamine-induced hyperlocomotion relative to controls when administered orally ($n = 8$–9 per condition; $p \leq 0.0001$).

43.3.3 VU0364289

2-(4-(2-(Benzyloxy)acetyl)piperazin-1-yl)benzonitrile (VU0364289) is a novel N-aryl piperazine mGluR5 PAM which binds to the MPEP site. VU0364289 selectively modulates mGluR5 and has no significant modulatory activity at the other mGlu subtypes. The C_{max} of 1,280 ng/ml (~3.8 μM) was achieved in systemic plasma within 0.25 h of administration of a 10 mg/kg i.p. dose. Similarly to VU0360172, VU0364289 was found to reduce

amphetamine-induced hyperlocomotion in rats [41]. Rats were assessed in a SmartFrame Open-Field System, which measures locomotor activity by the number of breaks measured in 32 horizontal infrared photobeams, located 1 cm above the floor of the chamber. Rats were given a 30-min habituation period in the chamber before being given either VU0364289 (10–100 mg/kg i.p.) or vehicle. After a further 30 min, rats were dosed with either vehicle or amphetamine (1 mg/kg subcutaneously) and monitored for 60 min. VU0364289 significantly reduced amphetamine-induced hyperlocomotor activity in a dose-dependent manner compared to vehicle ($p \leq 0.05$).

43.3.4 DPFE

1-(4-(2,4-Difluorophenyl)piperazin-1-yl)-2-((4-fluorobenzyl)oxy)ethanone (DPFE) is a novel N-aryl piperazine highly selective mGlu5 PAM which binds at the MPEP site. It is rapidly absorbed, achieving the C_{max} of 1,093 ng/ml (~3.0 μM) in systemic plasma within 0.25 h of a 10 mg/kg i.p. dose. One study examined this compound, using several test conditions [41].

43.3.4.1 Locomotor Activity

Using the same procedure as described previously (see 'VU0364289' section), DPFE (10 mg/kg i.p.) significantly reversed amphetamine-induced hyperlocomotion compared to vehicle ($p \leq 0.05$), with approximately 60 % fewer ambulations observed over the time course of the study.

43.3.4.2 Sensorimotor Gating

Prepulse inhibition was assessed using startle chambers with a Plexiglas cylinder mounted on a piezoelectric accelerometer for detecting movement. Rats were pretreated with either vehicle, risperidone (3 mg/kg administered orally) or DPFE (30–100 mg/kg administered orally). After 20 min, apomorphine (1 mg/kg subcutaneously) was administered and 10 min later rats were placed into individual startle chambers. After a 5-min acclimatisation period, rats were presented with five presentations of the startle stimulus alone, followed by seven randomised presentations of the following trial types: no stimulus, startle pulse alone, prepulse noise alone and three prepulse plus startle pulse combinations. DPFE did not significantly reverse apomorphine-induced disruption of prepulse inhibition compared to control ($p \geq 0.05$). This is in contrast to the effects of other mGluR5 PAMs. It is possible that the oral route of administration accounts for this discrepancy.

43.3.4.3 Spatial Learning

Mice were pretreated with DPFE (10 mg/kg i.p.) or vehicle 30 min before testing. After this time had elapsed, mice were put in one arm of a standard Y-maze and allowed to navigate the maze for 8 min whilst being monitored by video-tracking software. Spontaneous alternations were recorded each time the mouse entered the three arms of the maze consecutively before re-entering an arm. The number of triplet entries (spontaneous alternation) was divided by the total number of arm entries to give the percentage of spontaneous alternation. DPFE had no effect on Y-maze performance compared to control ($p \geq 0.05$).

43.3.5 CPPZ

(1-(4-(2-Chloro-4-fluorophenyl)piperazin-1-yl)-2-(pyridin-4-ylmethoxy)ethanone) (CPPZ) is a positive allosteric modulator of mGluR5, which binds to the MPEP site. In one study of this compound, CPPZ (3, 10 or 30 μmol/kg, administered subcutaneously) has shown a reverse in hyperlocomotion triggered by the NMDA open channel blocker MK801 in CD1 mice [107].

43.3.6 ADX47273

Several studies have assessed the effects of ADX47273 on negative and cognitive deficits observed in rat models of drug-induced psychosis. Deficit behaviours such as conditioned avoidance and hyperlocomotion were attenuated by the administration of ADX47273 [67]. Additionally, novelty discrimination, spatial learning and sensorimotor gating difficulties have also been shown to be ameliorated by ADX47273 [16].

43.3.7 3,3′-Difluorobenzaldazine (DFB)

Two studies examined the effects of DFB on PPI deficits and locomotor activity in rats and mice displaying psychotomimetic symptoms after administration of ketamine or amphetamine. Whilst DFB did not affect PPI deficits [15], it significantly suppressed amphetamine-induced hyperlocomotion ($p < 0.05$) without influencing spontaneous motor activity [61].

43.3.8 LSN2463359

Two studies observed the effects of LSN2463359 on rat models of psychosis. One study reported that LSN2463359 reversed learning deficits induced by methylazoxymethanol acetate (MAM) in rats, but did not affect phencyclidine-induced deficits [31]. The other study reported that LSN2463359 had little to no impact on hyperlocomotion related to phencyclidine or SDZ 220-581 ((S)-α-amino-2-chloro-5-(phosphonomethyl)[1,1-biphenyl]-3-propanoic acid) and was only able to reverse learning deficits caused by SDZ 220-581, but not those caused by phencyclidine [32].

Conclusion

Despite decades of research by academia and the pharmaceutical industry, there has been very limited progress in the pharmacological treatment of schizophrenia [53], and the initially much heralded atypical antipsychotics have not clearly improved outcomes, albeit their side-effect profile differs from older first-generation drugs [114, 115]. This, combined with the enormous clinical and societal need this illness presents [99], has led to a push to explore novel domains, including compounds acting on the glutamatergic system. Historically, drugs acting on ionotropic glutamate receptors have not been the first choice for development due to side-effect profiles almost inevitable from modulating the primary excitatory neurotransmitter of the brain, which include psychotomimetic effects, cognitive impairment and excitotoxicity [58]. However, in an attempt to circumvent such potential toxicity, there has been a growing focus on metabotropic glutamatergic receptors and in particular how they modulate the NMDA receptor that is associated with cognitive function and dopamine release [43]. Thus mGluR binding is garnering interest as an indirect – and potentially safer – mechanism by which NMDA function, and clinical symptomatology, can be altered.

However, research findings are considerably modest at present. This is due to two main reasons. Firstly, there is a dearth of studies examining mGluR5 modulation in human patients of psychosis and in healthy controls. Whilst evidence from animal models is a useful indicator of the effects of mGluR5 PAMs, human studies are needed to aid in further developments. The poor solubility of PAMs of mGluR5 is a barrier to future clinical use, although progress is being made in overcoming this. Secondly, mGluR5 agonists may induce epileptic seizures and excitotoxicity. This limits their use as potential antipsychotic agents, suggesting that a greater focus should be on mGluR5 PAMs instead. The selectivity of allosteric modulators acting at mGluR5 increases their therapeutic appeal, given the reduction of the off-target actions that underlie side effects.

The current data are somewhat limited but would support the ongoing development of mGluR5 PAMs and agonists for the treatment of negative and cognitive symptoms of psychosis. mGluR5 PAMs and agonists present an advantage in that they may improve negative and cognitive symptoms of psychosis which are typically unaffected or worsened by commonly used antipsychotics. Although research is still in its infancy, mGluR5 appears to be a valid target for future pharmacotherapy through its links with structural proteins and ionotropic glutamate receptors and its ability to generate long-lasting change within the cell. These factors allow the mGluR5 to play a crucial role in learning and memory and therefore offer the potential for therapeutic intervention through modulation of mGluR5 activity.

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44 Corticotropin-Releasing Factor Receptors as a Potential Target in the Developments of Antidepressant Drugs

Glenn R. Valdez

Abbreviations

ACTH	Adrenocorticotropic hormone
ASVG-30	Antisauvagine-30
BNST	Bed nucleus of the stria terminalis
CRF	Corticotropin-releasing factor
FRL	Flinders resistant line
FSL	Flinders sensitive line
HAM-A	Hamilton anxiety scale
HAM-D	Hamilton depression scale
HPA axis	Hypothalamic-pituitary-adrenal axis
SSRI	Selective serotonin reuptake inhibitor
Ucn 1	Urocortin 1
Ucn 2	Urocortin 2
Ucn 3	Urocortin 3

44.1 Introduction

Depression is a highly prevalent form of mental illness that affects an estimated 350 million people [1]. In the coming decades, major depression is projected to become the second leading cause of disability worldwide, as well as the leading cause of disability in high-income nations [1]. Because stressful life events frequently trigger depressive symptoms, this illness is often characterized as a stress-related psychiatric disorder. A classic definition of stress is any response to demands, usually noxious, placed on the body [2]. An alternate definition describes stress as any alteration in the psychological homeostatic process [3]. Although body's response to acute stressors allows it to adapt to environmental demands, chronic exposure to stress has been hypothesized to lead to long-term alterations of the physiological systems that are thought to mediate the stress response. It is these long-term changes that may also underlie the symptoms of stress-related psychiatric disorders such as depression.

Corticotropin-releasing factor (CRF) is a 41-amino-acid neuropeptide that has long been considered to be one of the body's major regulators of the stress response. It is involved in mediating the neuroendocrine response to stress [4], as well as autonomic [5, 6] and behavioral responses to environmental demands [7, 8]. CRF is the main regulator of the hypothalamic-pituitary-adrenal (HPA) axis stress response, which has been traditionally used by biologists as a method of quantifying stress [4]. When the body is faced with a stressor, CRF neurons are activated in the paraventricular nucleus of the hypothalamus where they send axon terminals to the median eminence. From this area, CRF is released into the portal blood system and carried

G.R. Valdez
Department of Psychology,
Grand Valley State University,
1 Campus Drive, Allendale, MI 49401, USA
e-mail: valdezg@gvsu.edu

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to the anterior lobe of the pituitary gland where it stimulates the synthesis and release of adrenocorticotropic hormone (ACTH). ACTH subsequently stimulates the release of glucocorticoids by the outer shell of the adrenals. In addition, CRF in the medulla can activate the sympathetic nervous system, stimulating the release of adrenaline from the adrenal medulla and other parts of the sympathetic nervous system [5, 6].

Chronic elevations in CRF and the hormones involved in the endocrine stress response can result in various detrimental physiological effects. Increased CRF levels can lead to the decreased toxicity of natural killer cells in the immune system [9] and induce stress-like changes in gastrointestinal, cardiovascular, and metabolic functions [10–12] via activation of the HPA axis and sympathetic nervous system. ACTH secretion directly influences immune function and acts within the brain to regulate sleep [13, 14]. Hypersecretion of glucocorticoids can result in infection via suppressed immune function, whereas low levels of these hormones can result in inflammatory conditions in laboratory animals [14]. Although the neuroendocrine stress response is important in the regulation of physiological responses to stress, the behavioral response to stress appears to occur independent of HPA axis activation. Hypophysectomy and blockade of the HPA axis response via dexamethasone suppression do not alter the behavioral response to stress produced by central administration of CRF [15, 16]. Thus, it appears that a central site of action is responsible for coordinating stress-related behavior.

Two genes encoding distinct G-protein-coupled CRF receptors have been identified (Fig. 44.1). The CRF_1 receptor is found mainly in the pituitary, amygdala, hippocampus, cerebellum, and cortex and is generally associated with increases in stress-related behaviors [8]. CRF, which preferentially binds to the CRF_1 receptor [17], leads to increases in the behavioral stress response in animal models [18–22]. Clinical research has also shown that depressed individuals show increased levels of CRF in cerebrospinal fluid [23]. A number of nonpeptide CRF_1 receptor antagonists have been developed with the hope that these drugs may be of therapeutic value in the treatment of depression and other stress-related psychiatric illnesses [24–26], although none have demonstrated clinical utility to date.

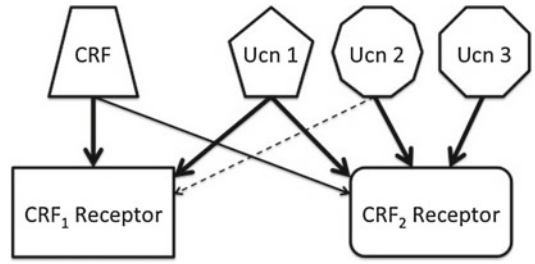

Fig. 44.1 Schematic representation of CRF-receptor subtypes and their associated ligands. Corticotropin-releasing factor (*CRF*) shows preferential binding affinity for the CRF_1 receptor. Urocortin 1 (*Ucn 1*) is equipotent in binding to the CRF_1 and CRF_2 receptor. Urocortin 2 (*Ucn 2*) and urocortin 3 (*Ucn 3*) are 1,000-fold more selective in binding to the CRF_2 receptor compared to Ucn 1. However, Ucn 2 induces low levels of activity at the CRF_1 receptor, whereas Ucn 3 does not induce CRF_1 receptor signaling

The CRF_2 receptor is found mainly in the lateral septum, ventromedial hypothalamus, and choroid plexus and exists in three splice variants: the CRF_{2a}, CRF_{2b}, and CRF_{2c} receptors [27, 28], and the discovery of additional neuropeptides belonging to the CRF family has aided in characterizing the role of the CRF_2 receptor in the stress response (Fig. 44.2). Urocortin 1 (Ucn 1) is a 40-amino-acid neuropeptide that is thought to be an endogenous ligand for the CRF_2 receptor, as it shows equally high binding affinity for both the CRF_1 and CRF_2 receptors [29] and produces a distinct behavioral profile compared to CRF. Central injections of this peptide more potently reduce feeding in food-deprived rats but only mildly increase locomotor activation compared to CRF [30]. Ucn 1 injections also lead to delayed increases in stress-related behaviors [31].

Urocortin 2 (Ucn 2) is another CRF-related neuropeptide composed of 38 amino acids [32]. Ucn 2 shares a similar sequence with stresscopin-related peptide, differing only in the cleavage site of the mature peptide [33]. A putative human sequence, originally identified as human urocortin-related peptide, shares 76% amino acid identity with the identified mouse sequence

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hCRF      SEEPPISLDLTFHLLREVLEMARAEQLAQQAHSNRKLMDII
oCRF      SQEPPISLDLTFHLLREVLEMTKADQLAQQAHSNRKLLDIA

hUcn 1     DNPSLSIDLTFHLLRTLLELARTQSQRERAEQNRIIFDSV
rUcn 1     DNPSLSIDLTFHLLRTLLELARTQSQRERAEQNRIIFDSV

hUcn 2       IVLSLDVPIGLLQILLEQARARAAREQATTNARILARV
mUcn 2       VILSLDVPIGLLRILLEQARYKAARNQAATNAQILAHV

hUcn 3       FTLSLDVPTNIMNLLFNIAKAKNLRAQAAANAHLMAQI
mUcn 3       FTLSLDVPTNIMNILFNIDKAKNLRAKAAANAQLMAQI
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Fig. 44.2 Amino acid sequence structure of mammalian neuropeptides within the corticotropin-releasing factor peptide family. Represented are the structures of human corticotropin-releasing factor (*hCRF*), ovine corticotropin-releasing factor (*oCRF*), human urocortin 1 (*hUcn 1*), rat urocortin 1 (*rUcn 1*), human urocortin 2 (*hUcn 2*), mouse urocortin 2 (*mUcn 2*), human urocortin 3 (*hUcn 3*), and mouse urocortin 3 (*mUcn 3*)

and is proposed to be the human ortholog to mouse Ucn 2 [34]. Although Ucn 2 is equipotent in its binding affinity at the CRF_2 receptor when compared to Ucn 1, it is 1,000-fold more selective in binding to the CRF_2 receptor [32]. A third CRF-related neuropeptide known as urocortin 3 (Ucn 3) has also been identified [34]. Ucn 3 is a 38-amino-acid neuropeptide that shares a similar sequence with stresscopin, differing only in the cleavage site of the mature peptide [33]. Compared to Ucn 2, Ucn 3 displays a similar potency but higher selectivity in binding to the CRF_2 receptor [34]. Doses of up to 3 nM of Ucn 3 do not induce adenylate cyclase in CRF_1 transfectants in vitro, a marker of CRF_1 receptor activity [34]. Ucn 3 immunoreactive projections have been found in the hypothalamus, lateral septum, bed nucleus of the stria terminalis, and amygdala [35], areas believed to mediate the behavioral stress response [36–38]. These areas also are known to express high levels of the CRF_2 receptor [39], supporting the notion that Ucn 3 is an endogenous ligand for the CRF_2 receptor. The role of Ucn 2 and Ucn 3 in regulating stress-related behaviors is still unclear. Some studies have shown that administration of Ucn 2 and Ucn 3 attenuates the behavioral response to stress [40–42], whereas others have found that these selective CRF_2 receptor ligands enhance stress-related behavior [43].

Despite the seemingly contradictory findings regarding CRF_2 receptors and stress-related behavior, which will be discussed more in depth later in this chapter, characterization of the CRF receptors suggests that these CRF_1 and CRF_2 receptors may have distinct roles in the regulation of depressive behaviors. Preclinical studies strongly suggest that activation of the CRF_1 receptor increases depression-related behaviors [44–46]. Although there is no general consensus regarding the role of CRF_2 receptors in the behavioral stress response, preclinical evidence suggests that underactivation of this receptor may be involved in the regulation of increased depression-like behavior in animals [47, 48]. The present chapter will review the role of CRF-related ligands and CRF receptors in depression and proposes targeting the CRF system as a potential pharmacotherapy for depressive disorders.

44.2 Corticotropin-Releasing Factor and the Behavioral Stress Response

Much of the data regarding CRF itself and nonselective CRF-receptor antagonists focus more on animal models of anxiety rather than depression. These results should be strongly considered when examining the role of CRF in depression, however, given the multifaceted complexity and high incidence of comorbidity between these two disorders [49–51]. CRF injected into unstressed animals under familiar conditions leads to

increased locomotor activation [18, 19], whereas in unfamiliar settings, centrally administered CRF can lead to behavioral suppression [18]. In addition, CRF administration can lead to even greater reductions in operant responding during the conflict test [20], a model of anxiety in which a reward is accompanied by presentation of an aversive stimulus in order to suppress responding for the reward. Other examples of stress-related behavior induced by central administration of CRF include an enhanced acoustic startle response [21, 52], increases in the conditioned fear response [22], and decreased appetite [53, 54]. Furthermore, transgenic mice overproducing CRF show decreased exploration of a novel environment compared to wild types, an effect potentiated by exposure to social defeat stress [55]. Overexpression of CRF in the amygdala also leads to increases in stress-related behaviors in rats [56].

Further evidence that brain CRF systems play an important role in the regulation of the behavioral response to stress comes from studies using nonselective peptide CRF-receptor antagonists. These antagonists attenuate both CRF and stress-induced behavioral changes. α-Helical $CRF_{(9–41)}$, a CRF-receptor antagonist, decreases the CRF-enhanced acoustic startle response when centrally injected in rats [57]. This antagonist also increases exploratory behavior of the open arms of the elevated plus maze following administration in CRF-overproducing mice [55]. Open-arm preference in the elevated plus maze, as indicated by the ratio of open-arm to total-arm time and entries, has been proposed to relate inversely to anxiety [58]. A second peptide CRF-receptor antagonist, D-Phe-$CRF_{(12–41)}$, reduces both CRF- and stress-induced increases in locomotor activation [59]. Astressin, a third CRF-receptor antagonist, decreases CRF-induced locomotor activation and increases open-arm exploration in the elevated plus maze [60].

CRF-receptor antagonists have also been shown to reduce stress-induced behavioral changes. In the elevated plus maze, administration of α-helical $CRF_{(9–41)}$ leads to increased exploration of the open arms of the elevated plus maze in rats subjected to restraint stress, swim stress, or social conflict stress [61]. Astressin also increases open-arm exploration in the elevated plus maze in rats subjected to social conflict stress [60]. D-Phe-$CRF_{(12-41)}$ attenuates stress-induced increases in locomotor activation and decreases in exploration of the open arms of the elevated plus maze [59].

With regard to animal models of depression, D-Phe-$CRF_{(12–41)}$ reverses increases in intracranial self-stimulation reward thresholds induced by central CRF administration [62]. Injections of CRF [63, 64] and CRF promoter-induced overexpression of CRF [56] have also shown to increase immobility in rats observed in the forced swim test. The forced swim test is an animal model of depression in which immobility is thought to model a depressive-like state. Treatment with standard antidepressant drugs can reverse immobility, an effect correlated with antidepressant efficacy in humans [65]. These results suggest that increased CRF activity may be a key component in regulating depressive-like behaviors in animals.

44.3 CRF_1 Receptors, Stress-Related Behavior, and Animal Models of Depression

Preclinical evidence strongly suggests that activation of the CRF_1 receptor increases stress-related behavior [8]. As described above, CRF, which preferentially binds to the CRF_1 receptor, leads to increases in the behavioral stress response independent of HPA axis activation. For example, ovine CRF, which shows an 80-fold higher affinity in binding to the CRF_1 receptor vs. the CRF_2 receptor [17], produces increased motor activation and decreases open-arm exploration in the elevated plus maze [40]. CRF_1 receptor knockout mice lacking CRF_1 receptors also show a decreased responsiveness to stressful stimuli [66–68] and less spontaneous motor activity [69]. These data demonstrating that activation of the CRF_1 receptor increases the behavioral response to stress suggests the CRF_1 receptor may be a key biological component in regulating stress-related psychiatric disorders.

A number of CRF_1 receptor antagonists have been examined in animal models of depression, yielding mixed results. CP-154,526, one of the earliest nonpeptide CRF_1 receptor antagonists to be developed [70], reversed escape deficit in rats exposed to inescapable shock without affecting controls in the learned helplessness task [71]. During this task, animals were exposed to a series of inescapable shocks and then given the opportunity to escape shock following a period of time. Decreased escape behavior has been proposed to indicate a depressive-like state [72]. When administered 60 min before a test session, acute and chronic injections of CP-154,526 and CRA1000, another nonpeptide CRF_1 receptor antagonist, reduced escape failure in rats in a manner comparable to chronic treatment with the tricyclic antidepressant imipramine [73, 74]. Acute and chronic treatment with the CRF_1 receptor antagonist R278995/CRA0450 also reduced escape failures [75]. However, the CRF_1 receptor antagonists DMP696 and DMP904 were not effective in learned helplessness task [76].

Data obtained using the forced swim test have also been equivocal regarding the effectiveness of CRF_1 antagonists in animal models of depression. For example, CP-154,526, R121919, and antalarmin, CRF_1 antagonists that are structurally similar to CP-154,526 [77], have been found to be ineffective in reversing swim stress-induced immobility in the rat [78]. Similar results have also been found using R278995/CRA0450 [75], DMP696, and DMP904 [76]. In mice, antalarmin, DMP696, DMP904, and R121919 were also unable to reverse immobility in the forced swim test [45]. In contrast, the CRF_1 receptor antagonist LWH234 reduced immobility, but did not affect stress-induced increases in ACTH [78]. Another study has found that SSR125543A and antalarmin can also attenuate immobility due to swim stress [79].

In the tail suspension test, subchronic dosing of R121919 and DMP696 has been shown to decrease immobility in mice within a similar manner to that of the selective serotonin reuptake inhibitors (SSRI) fluoxetine and paroxetine or the selective norepinephrine reuptake inhibitor reboxetine [45]. In contrast, antalarmin, DMP 904 [45], CP154,526 [80], and R278995/CRA0450 [75] were found to be ineffective in the tail suspension test.

These conflicting findings may be explained, in part, by the baseline behavior of the animals. Similar to nonselective peptide CRF-receptor antagonists, CRF1 receptor antagonists may only be effective in reversing stress-induced changes in behavior. The development of the Flinders sensitive line (FSL) rats, showing higher a baseline level of immobility compared to other rat strains [44], has led to support for this hypothesis. FSL rats receiving chronic treatment with SSR125543A show decreases in immobility in the forced swim test comparable to that observed following chronic injections of fluoxetine and the tricyclic antidepressant desipramine. These drugs did not affect immobility in Flinders resistant line (FRL) rats [81]. Similar results have also been observed in FSL and FRL rats receiving chronic treatment with CP-154,526, imipramine, and the SSRI citalopram [82]. Given these results, it appears that CRF_1 receptor antagonists are most effective in animal models of depression when tested in animals that show behaviors indicative of a depressive-like state.

More consistent results have also been seen in animals examined in the chronic mild stress model. BALB/c mice exposed to a series of mild stressors, including restraint, food restriction, and changes in housing, show a deteriorated physical state and decreased body weight. Chronic treatment with antalarmin or fluoxetine reverses the decrease in physical state [83]. Chronic treatment with SSR125543A or fluoxetine can also reverse deterioration of physical state in mice. In addition, both of these drugs attenuate the stress-induced reduction of cell proliferation in the dentate gyrus [84]. Interestingly, fluoxetine treatment led to an increase in cell proliferation in the dentate gyrus in nonstressed mice, whereas SSR125543A had no effects on cell proliferation in these animals [84], further supporting the hypothesis that CRF_1 antagonists may only be effective in altering depressive-like behaviors related to stressful conditions.

Recent data also suggest that CRF_1 antagonists may be a useful alternative to those resistant

to the therapeutic properties of traditional antidepressants [46]. In a series of experiments, mice exposed to chronic mild stress were examined in a number of stress-related behavioral tests following treatment with fluoxetine. Animals were then further divided into responder and nonresponder groups to fluoxetine treatment. Mice that did not respond to fluoxetine showed increases in nest-building behavior, an indication of increased motivation, and decreases in aggression when administered SSR125543A [46]. The findings of this study suggest that while traditional antidepressants may be beneficial to some, CRF_1 receptors may provide an alternative target for those who do not benefit from these medications.

44.4 CRF_1 Antagonists in Clinical Trials

The earliest clinical trials examining the effectiveness of nonpeptide CRF_1 antagonists in the treatment of depression were performed at the Max Planck Institute of Psychiatry in Munich, Germany, examining the CRF_1 antagonist NBI-30775. The initial open-label trial designed to assess the safety of NBI-30775 did not show significant alterations in liver enzymes or heart rate in patients with major depression. Moreover, consistent with the hypothesis that behavioral measures of stress occur independently of HPA axis activation, NBI-30775 did not alter the normal neuroendocrine response in response to intravenous administration of CRF. These patients also showed reduced scores in the Hamilton depression (HAM-D) and Hamilton anxiety (HAM-A) scales [85].

A subsequent trial showed that a 30-day treatment of low- or high-dosing regimens of NBI-30775 did not affect various endocrine measures, including HPA axis, hypothalamic-pituitary-gonadal axis and plasma renin activity, and aldosterone, human growth hormone, and insulin-like growth factor vasopressin and thyroid hormone levels. In addition, reports of adverse side effects such as headache, nausea and dizziness that were observed during the trial did not appear to be the direct result of the experimental compound [86]. NBI-30775 has also been shown to normalize electroencephalogram sleep patterns in patients diagnosed with major depression [87]. Finally, subsequent analysis of plasma leptin levels and body weight of the patients examined in the Zobel et al. study [85] showed that neither of these measures was affected by NBI-30775 treatment [88]. Although these data suggested that NBI-30775 is relatively safe and may be clinically useful, an unpublished study in the UK found elevated liver enzyme levels in two patients. This finding led to the termination of the development of NBI-30775, according to a media release from Janssen Pharmaceuticals.

Phase I clinical trials have been conducted to assess the effects of another CRF_1 receptor antagonist, NBI-34041, on neuroendocrine function [89]. For 14 days, 24 healthy male subjects received either NBI-34041 or placebo, and the HPA axis response to CRF and psychosocial stress was examined. NBI-34041 reversed the increases in ACTH and cortisol due to intravenous CRF or psychosocial stress but did not impair normal HPA axis function. Although these initial results demonstrate that NBI-34041 is effective in reversing physiological responses to stress without affecting basal hormone levels, further work is clearly needed to assess the safety and efficacy of this drug in the treatment of depression. Currently, there is no available literature concerning clinical trials for NBI-34041 in depression.

CP-316,311 is an additional CRF_1 receptor antagonist developed by Pfizer that has undergone clinical testing. Patients with recurring major depression were examined in a 6-week fixed dose double-blind placebo- and sertraline-controlled trial [90]. Although bioavailability data demonstrated adequate serum concentrations of CP-316,311 and urinary cortisol levels showed evidence of CRF_1 receptor blockade, patients administered CP-316,311 did not show any significant differences in HAM-D scores compared to those given placebo. Development of CP-316,311 was discontinued due to this negative result.

A multicenter clinical trial has also been conducted to determine the effectiveness of the CRF_1

antagonist pexacerfont in the treatment of generalized anxiety disorder [91]. Similar to CP-316,311, patients given pexacerfont had similar HAM-A scores compared to those given placebo, despite achieving efficacious serum concentrations of the drug. Clinical trials are also underway for CRF_1 antagonists in the treatment of post-traumatic stress disorder, substance use disorders, and stress-induced food cravings [92–95]. Although there remains interest in the field of developing CRF_1 antagonists with therapeutic value for depression and other stress-related psychiatric disorders, none have demonstrated clinical utility to date.

44.5 CRF_2 Receptors, Stress-Related Behavior, and Animal Models of Depression

Although the main focus in the development of antidepressants that target the CRF system has been on the CRF_1 receptor, CRF_2 receptors may present a compelling alternative given the lack of clinical success of CRF_1 receptor antagonists. The CRF_2 receptor is found mainly in the lateral septum, ventromedial hypothalamus, and choroid plexus [27, 28]. Activation of the CRF_2 receptor is most strongly associated with alterations in feeding behavior [30, 96]. Due to conflicting experimental findings, however, there is currently no general consensus regarding the role of CRF_2 receptors in the behavioral stress response.

CRF_2 receptor knockout mice show an anxiogenic-like phenotype when examined in the elevated plus maze, open-field, and light-dark emergence tests [97, 98]. Central infusion of CRF_2 receptor antisense decreases stress-coping behaviors and induces an anxiogenic-like response in rats [99]. Furthermore, rats examined in a model of post-traumatic stress disorder in which they were exposed to predator order over a 10-week period showed chronic upregulation of CRF_1 receptors and downregulation of CRF_2 receptors in the bed nucleus of the stria terminalis (BNST) [100]. This effect was attenuated by injections of *Lentivirus* overexpressing CRF_2 receptors [100]. As described previously, amidated 38-amino-acid synthetic peptides encoded by the Ucn 2 [32] and Ucn 3 [34] genes have been identified as selective CRF_2 receptor agonists. Central injections of Ucn 2 and Ucn 3 lead to suppressed motor activity in the locomotor activity test and an increase in open-arm exploration in the elevated plus maze [40, 41]. Ucn 3 also increases exploratory behavior in mice examined in the open-field test [42].

In contrast, there have also been findings that suggest an anxiogenic role for the CRF_2 receptor. Contrary to the findings discussed above, studies have found that central injections of antisauvagine 30 (ASVG-30) produced anxiolytic-like effects in the rat [101] and the mouse [43]. In addition, antisense inhibition of CRF_2 receptors in the lateral septum attenuates fear conditioning [102], and Ucn 2 has also been shown to produce a decrease in open-arm exploration in the elevated plus maze in mice [43]. However, further research has yielded additional insight to these conflicting results.

One possible explanation for these seemingly contradictory findings is that the doses used in these experiments may not be selective for the CRF_2 receptor. For example, ASVG-30 blocks footshock-induced freezing equally in CRF_2 receptor knockout mice and controls [103]. In contrast, the CRF_2 receptor antagonist astressin$_2$-B did not affect freezing in these mice [103]. ASVG-30 does have a moderate binding affinity for the CRF_1 receptor, whereas astressin$_2$-B is much more selective for the CRF_2 receptor [104, 105], and review of the previous literature shows that many of the studies that found anxiolytic-like effects following ASVG-30 administration used doses approximately threefold greater than the IC_{50} of this compound. Although Ucn 2 has been shown to induce an anxiogenic-like response when tested in the elevated plus maze [43], this ligand does have a low affinity for binding to the CRF_1 receptor [32]. In contrast, Ucn 3, which does not induce receptor signaling at the CRF_1 receptor, [34], appears to reduce behavioral measures of stress in the elevated plus maze on a more consistent basis [41, 106]. It is possible that the anxiolytic- and anxiogenic-like properties of

ASVG-30 and Ucn 3, respectively, may be due to low levels of binding activity at the CRF_1 receptor.

Alternatively, these findings may be the result of site-specific actions. Astressin, a nonselective CRF-receptor antagonist, but not antisauvagine-30, impairs CRF-enhanced fear conditioning when injected into the hippocampus. Both of these antagonists, however, attenuate fear conditioning when injected into the lateral septum [107]. Lesions of the lateral septum also produce anxiolytic-like effects in rat models of anxiety [108, 109]. Furthermore, mice exposed to high levels of stress show enhanced anxiety-like behavior when injected with Ucn 2 in the lateral septum [110].

Another possibility is that CRF_2 receptors may regulate specific aspects of the stress-coping response, such as sensory information. In C57BL/6J and 129S6/SvEvTac mice, both the CRF_1 receptor antagonist NBI-03775 and antisauvagine-30 attenuated enhancement of the acoustic startle response by CRF. In addition, Ucn 2 also increased the acoustic startle response, but with less efficacy than CRF [111]. Further investigation, however, showed that although NBI-30775 and ASVG-30 reduced CRF-induced increases in startle and CRF-induced deficits in prepulse inhibition, CRF_2 receptor activation via Ucn 2 and Ucn 3 injections enhanced prepulse inhibition of the acoustic startle response [112]. Also, rats injected with ASVG-30 in the anterolateral BNST show decreases in open-arm exploration similar to those seen in vehicle-injected controls following exposure to water-avoidance stress, whereas injections of the CRF_1 receptor antagonist CP376395 into the anterolateral BNST appears to reverse this effect [113]. However, both ASVG-30 and CP376395 decreased the acoustic startle reflex in this same study [113]. These data suggest that although it is possible that CRF_2 receptors may have some stress-inducing properties, they appear to be a critical component tempering the stress response.

With regard to depressive-like behaviors specifically, the role of CRF_2 receptors remains unclear. When observed in the forced swim test, CRF_2 receptor knockout mice display increased depression-like behavior as indicated by increased immobility [47]. In contrast, female, but not male, Ucn 2 knockout mice show less immobility time in the tail suspension and forced swim tests [114]. The difference in behavioral profiles between these strains of mice may be due to the body-wide absence of CRF_2 receptors and a different compensation in CRF_1 signaling in the CRF_2 receptor knockout mice. Ucn 2 knockout mice still have CRF_2 receptors available for Ucn 1 and Ucn 3 to bind and in fact show upregulated CRF_2 receptor expression in the dorsal raphe [114]. Conflicting results have also been found following administration of CRF_2 receptor-specific ligands. Ucn 2 injected into the dorsal raphe has been shown to potentiate learned helplessness behavior in response to inescapable shock, possibly due to interactions with the serotonergic system [115]. However, another study showed that Ucn 2 and Ucn 3 decreased immobility and increased climbing and swimming in mice examined in the forced swim test [48]. Clearly, further work is needed to fully understand the role of CRF_2 receptors in depression.

Conclusions

The data presented are clear evidence that the CRF receptors and CRF-related ligands are important in mediating heightened stress, which in turn may lead to stress-related psychiatric disorders. Although further study is needed, characterization of the CRF_1 and CRF_2 receptors suggests that these behavioral changes may be the result of an imbalance of CRF_1 and CRF_2 receptor activity, rather than simply CRF activation. The increases in CRF typically observed during the stress response appear to lead to an overactivation of the CRF_1 receptor given the binding profile of this neuropeptide [17]. This hypothesis is further supported by the data indicating an anti-stress function for CRF_2 receptor activation [40–42, 100, 112], leading to the hypothesis that the CRF_1 receptor is responsible for coordinating the activational components of stress, whereas the CRF_2 receptor acts as compensatory coping mechanism to oppose this action.

Currently, the effort with regard to medication development targeting the CRF system has focused on small molecule CRF_1 antagonists. Observations of both clinical and preclinical studies suggest that CRF_1 receptor antagonists are potentially effective candidate medications in the treatment of stress-related psychiatric illnesses. Patients diagnosed with affective disorders have shown increased CRF levels in cerebrospinal fluid and the hypothalamus, decreased CRF binding in the frontal cortex, and impaired HPA axis function [23, 116–118]. Preclinical evidence demonstrates that reducing brain CRF activity via antagonism of CRF_1 receptors can attenuate the behavioral changes observed in animal models of depression.

Although the evidence is compelling, there are still some issues that still need to be considered in the development of CRF_1 receptor antagonists. One highly encouraging characteristic of these drugs is their specificity in altering behavior only under stressed conditions, a feature that bodes well for side effect liability. Major side effects were not observed in clinical studies examining NBI-30775 [85]. However, animal studies have suggested that some CRF_1 antagonists may have slight sedative effects [75], endocrine disruptive actions [119], and transient abuse-related potential [120]. Another concern is that most structures have poor aqueous solubility and pharmacokinetic properties due to the fact that they are excessively lipophilic. Despite the efforts of the pharmaceutical industry, no CRF_1 receptor antagonists have demonstrated clinical utility to date. It is unclear if this lack of success is due to the lack of effectiveness of the compounds themselves, or the multifaceted complexity of the disorders they are designed to target, such as major depression. Nonetheless, the hope exists that further development of CRF_1 receptor antagonists and clinical trials that address these shortcomings may one day lead to new pharmacotherapies for the treatment of depression.

An alternative approach to targeting the CRF system in the development of new antidepressant medications may be to increase CRF_2 receptor activity. Although there is still no general consensus regarding the role of the CRF_2 receptor in the behavioral stress response, much of the data regarding the behavioral profiles of CRF-related neuropeptides and CRF-receptor knockout mice have led to a hypothesis that the CRF-receptor subtypes have an opposing role in the regulation of the stress-related behaviors. For example, CRF and Ucn 1, CRF-related neuropeptides with equal affinity for the CRF_1 and CRF_2 receptors [29], have been shown to increase overall behavioral activity in rats in a differential manner compared to Ucn 2 and Ucn 3. For example, de Groote et al. [121] found that CRF and Ucn 1 increase measures of exploratory behavior and grooming, whereas Ucn 2 and Ucn 3 only increase exploratory behavior. In addition, chronic exposure to stress leads to opposing profiles of CRF_1 and CRF_2 receptor expression [100]. In addition, it appears that some of the conflicting evidence may be due to the low-level binding activity at the CRF_1 receptor [103] and site-specific actions [107].

CRF_2 receptor-specific agonists have also been shown to reverse the effects of CRF. Ucn 2 and Ucn 3 decrease the stimulatory effect of CRF on locomotor activity in a familiar environment, and Ucn 2 injections attenuate CRF-induced decreases in exploratory behavior in the open field [122]. Although the precise mechanism of this action remains unclear, one hypothesis is that activation of the CRF_2 receptor may simply lead to a functional antagonism of the behaviors produced by CRF_1 receptor activation. Activation of the CRF_2 receptor may oppose the stress-inducing actions that result from CRF_1 receptor activation. Although this hypothesis must be further tested, the development of small molecule CRF_2 receptor agonists may also provide a novel approach for the treatment of depression.

The experiments discussed in this chapter suggest that the underlying mechanisms for increases in depressive behaviors may be the overactivation of CRF_1 receptors and possible underactivation of CRF_2 receptors. Studies describing the effects of CRF_1 receptor

antagonists imply that antagonizing the effects associated with activation of this receptor can attenuate the behavioral stress response. Although these compounds have also shown promising results when tested in preclinical models of depression, clinical trials have been unsuccessful thus far. Although data regarding CRF_2 receptor regulation of depressive behaviors is less clear, there is evidence that CRF_2 receptor ligands act opposing the effects of CRF_1 receptor activation. Thus, the development of small molecule CRF_2 receptor agonists may also be worth exploring as potential novel antidepressants. In conclusion, the data presented regarding CRF and its receptors and related ligands strongly suggest that targeting the CRF receptors has the potential to generate novel pharmacotherapies for depression.

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Nitric Oxide Signaling in Depression and Antidepressant Action

45

Gregers Wegener and Sâmia R.L. Joca

45.1 Introduction

Data from Europe [215, 216, 304] indicate that brain disorders account for 12 % of all direct costs in the Danish health system and 9 % of the total drug consumption was used for treatment of brain diseases. Expenses for brain diseases constituted 3–5 % of the gross national products and the total European expenses for all investigated brain diseases reaching almost 800 billion euros in 2010 [97, 214]. Among brain disorders, affective disorders were among the most costly diseases (110 billion euros) and anxiety disorders among the most prevalent.

The pathogenesis of mood disorders remains elusive, but it is evident that multiple factors, genetic and environmental, play a crucial role for adult psychopathology and neurobiology [43]. With regard to therapy, a significant proportion of affective disorder patients are partial or nonresponders, and there has been no major breakthrough in finding novel effective drug targets since the introduction of the currently marketed antidepressant drugs in the 1950s to the 1980s, which all are based on monoaminergic pharmacological effects. In addition, several of the major pharmaceutical companies developing CNS therapeutics, in these years, change from CNS drug development to other areas [296]. Consequently, there exists a pressing need to develop novel treatment strategies – and ultimately understand the etiology and pathophysiology of affective disorders.

Nitric oxide, originally termed endothelial-derived relaxing factor (EDRF) before it was discovered that NO and EDRF were the same substance, serves important roles in the cardiovascular system and macrophages [116, 224]. In addition, NO has been shown also to have an important role in the nervous system [34, 88], where NO serves as a messenger molecule in a number of physiological processes, including processes being linked to the major psychiatric diseases [142, 220, 238, 239]. This chapter will review general aspects of the NO system in major depressive disorder (MDD), as well as focus on inhibitors of NO production as putative therapeutic agents toward depression.

G. Wegener (✉)
Translational Neuropsychiatry Unit,
Department of Clinical Medicine,
Aarhus University, Aarhus, Denmark

Centre for Pharmaceutical Excellence,
School of Pharmacy, North West University,
Potchefstroom, South Africa
e-mail: wegener@dadlnet.dk

S.R.L. Joca
Department of Physics and Chemistry, School
of Pharmaceutical Sciences of Ribeirão Preto,
University of São Paulo, São Paulo, Brazil
e-mail: samia@usp.br

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45.2 General Aspects of Nitric Oxide

NO is a small molecule (MW 30 Da), which in vitro is a colorless gas and a product from the breakdown of N2. NO is degraded into nitrites and nitrates, and depending on the environmental conditions, the half-life lies from minutes to years [19]. In biological systems the half-life of NO is much shorter and estimated to be about 30s or less [19]. The molecule is uncharged and is therefore freely diffusible across cell membranes and other structures. NO is produced and released by many different cells in multicellular organisms and can thus act as a tool for intercellular communication [35, 87, 139, 171, 183, 192]. These properties are very important, as they form basis for the understanding of NO as a retrograde transmitter [7], which is proposed to play a major role in the induction of long-term potentiation and long-term depression (LTP and LTD) [25]. LTP and LTD have been observed to be impaired in experimental depression models and in depressive subjects [120, 204].

The combination of one atom of N and one atom of O results in the presence of an unpaired electron. However, NO is less reactive than many other free radicals and does not react with itself. Being a free radical, nitric oxide has both pro- and antioxidant properties [222, 236] (Fig. 45.1). NO can be protective against oxidative injury, depending on the specific conditions [236]. A nitric oxide radical can both stimulate lipid oxidation and mediate oxidant-protective reactions in membranes [235]. At high rates of NO production, the prooxidant versus antioxidant outcome depends critically on the relative concentrations of the individual reactive species [246]. The prooxidant reactions of NO occur with superoxide, whereas the antioxidant effects of NO are consequent to direct reactions with alkoyl and peroxyl radical intermediate during lipid peroxidation, terminating the propagation of lipid radical chain reactions [246].

NO may therefore limit injury to target molecules or tissues during events associated with excess production of reactive oxygen species. Based on these properties, it is not surprising that disturbance of NO production may greatly affect cell functioning (see below) and survival.

45.2.1 The Nitric Oxide Synthase Enzymes

The enzyme responsible for the synthesis of NO, nitric oxide synthase (NOS), appears in different isoforms, which are constitutive or inducible (see Table 45.1). The activity of the constitutive NOS depends on Ca^{2+} and calmodulin, whereas the

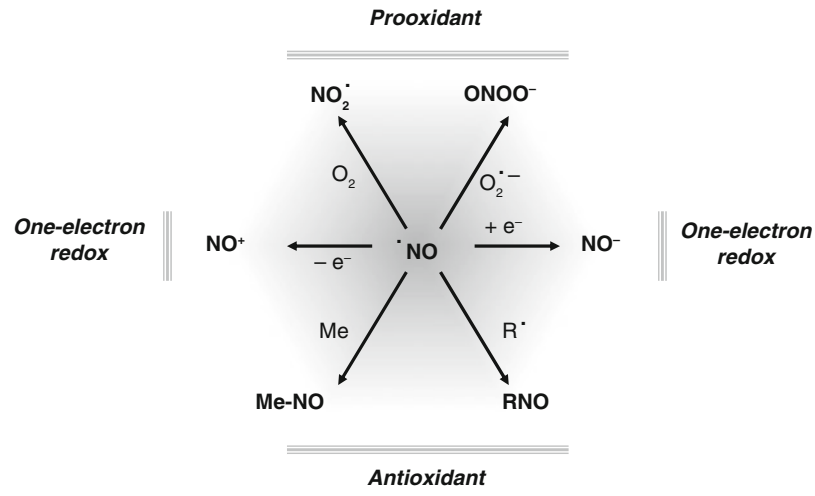

Fig. 45.1 Nitric oxide chemistry. One-electron redox (to NO^+ or NO^-), prooxidant (to NO_2 or $ONOO^-$), and antioxidant (to Me–NO or RNO) reactions are shown. *Me* metal center (e.g., iron or copper), *R* organic radical

Table 45.1 NOS isoforms

	Neural (nNOS, type I)	Inducible (iNOS, type II)	Endothelial (eNOS, type III)
First identified in	Neurons	Macrophages	Endothelium
Other cells expressing	Myocytes	Astrocytes	Neurons
	Astrocytes	Microglia	
Intracellular localization	Soluble or membrane bound	Soluble or membrane bound	Largely membrane bound
Ca^{2+} dependency	Activity depends on elevated Ca^{2+}	Activity is independent of elevated Ca^{2+}	Activity depends on elevated Ca^{2+}
Expression	Constitutive	Inducible	Constitutive
	Inducible under certain circumstances, e.g., trauma		
Amounts of NO released	Small, pulses	Large, continuous	Small, pulses
Proposed function	Regulation	Host defense	Regulation
Activators	Glutamate	Lipopolysaccharide	Acetylcholine
	Noradrenaline		

inducible NOSs are independent from both Ca^{2+} and calmodulin. A distinction of the isoforms is also made based on the tissue where the NOS was identified the first time and primarily located. Of the constitutive isoforms, NOS in endothelial cells is mainly located in the cell membrane and is termed eNOS. NOS in neuronal cells is located throughout the cell and termed nNOS. The inducible isoform, primarily expressed as a soluble and membrane bound isoform in a wide range of cells (especially macrophages and glia), is termed iNOS [79, 192]. However, exceptions from this rule exist, and nNOS has been found in a variety of nonneuronal cells and eNOS have been demonstrated in some neurons [78, 207]. Additionally, iNOS can be found constitutively expressed in certain brain regions under normal and pathological conditions [6].

45.2.2 Synthesis of NO

NO is synthesized in the brain by NOS from the amino acid L-arginine. In brief, L-arginine is converted to N^{ω}-hydroxy-L-arginine, which is further converted to NO and citrulline by NOS. The process is rather complex and further discussion lies beyond the scope of this text. Briefly, the process involves five electrons, three co-substrates, and five prosthetic groups [61, 143, 192].

45.2.3 Localization of NOS in the CNS

The NOS enzymes are widely distributed within the mammalian brain [26, 63]. The neuronal isoform accounts for the majority of the NOS activity in the brain [104], and NOS positive neurons are located in the hippocampal layers CA1–CA3, the medial amygdaloid nucleus, the olfactory bulb, the layers II–VI in the cerebral cortex, and the granular and deep molecular layers of the cerebellum and, with special interest regarding the serotonin system, in the dorsal and medial raphe nuclei [26]. Measurements of NOS activity in different brain regions have shown the highest activity in the cerebellum, the midbrain, the hypothalamus, the cortex, the striatum, and the hippocampus [17, 250]. Interestingly, NO has been shown to co-localize with several other known transmitters within the same neuron, e.g., serotonin (5-HT) in the medial and dorsal raphe nuclei [133], norepinephrine (NE) in the solitarian tract nucleus [265], γ-amino-butyric acid (GABA) in the cerebral cortex [288], and neuropeptide Y (NPY) and somatostatin in the striatum [144].

45.2.4 Regulation of NOS Activity

Regulation of the NOS enzyme expression has to be clarified in detail. Most of the studies performed have focused on the iNOS isoform. This isoform is usually not present in the cells under normal circumstances, but can be expressed following activation by different cytokines/endotoxins [193, 254]. In the CNS, iNOS is mainly expressed in glial cells and it acts in a Ca^{2+}-independent manner. It has been widely accepted that this isoform is expressed in very low levels in the healthy brain, being upregulated by immunostimulatory cytokines, thus with little participation in modulating neuronal activity under normal circumstances [3]. However, more recent evidence has identified a weak constitutive expression of iNOS in astrocytes of various brain regions, corresponding to approximately 10 % of total NOS activity, and raised the possibility that astrocytic-derived NO could interfere with physiological brain processes, such as synaptic neurotransmitter release and LTP [6, 40].

Less is known about the expression of nNOS and eNOS, but it has become evident that expression of nNOS in the brain and spinal cord during the embryonic and postnatal period can change markedly, which is in line with evidence indicating that NO is implicated in synaptic plasticity in the adult and in regulating neurite outgrowth, as exemplified by the finding that NO donors enhance neurotrophin-induced neurite outgrowth through a cGMP-dependent mechanism [53, 115, 117].

The cofactors and especially the NOS–Ca^{2+}–calmodulin interaction are primary regulators for NO production. Following an action potential, increases in the intracellular Ca^{2+} environment (around 500 nM [255]) trigger Ca^{2+}–calmodulin to bind to NOS, activating the NOS enzymatic activity. As the intracellular Ca^{2+} level can rapidly change, the catalytic activity can be turned on and off within a short time. iNOS binds calmodulin very tightly and continues to synthesize NO throughout the life of the enzyme, irrespectively of the intracellular Ca^{2+} concentration [192]. In addition to the cofactor and Ca^{2+} level regulations, phosphorylation is used to regulate the activity, as exemplified by the finding that nNOS phosphorylation by protein kinase C inhibits NO production [162]. NO itself has also been shown to regulate NOS activity [11, 38, 241]. The nature of this inhibition needs to be fully clarified, but can be hypothesized to involve nitrosylation [89].

Neuronal NOS is connected to NMDA receptors and sharply increases NO production following activation of this receptor [36], with the consequence that the level of endogenously produced NO around NMDA synapses reflects the activity of glutamate-mediated neurotransmission [4].

The NH2 terminus of nNOS contains a PDZ (postsynaptic density protein, disks large, ZO-1) domain, participating in the formation of active nNOS dimers and interacting with many other proteins in specific regions of the cell [47, 56, 153]. Proteins bearing PDZ domains typically localize to specialized cell compartments and are believed to be important in linking components of signal transduction pathways in multiple complexes [45, 243, 248].

By anchoring nNOS to membrane or cytosolic protein via direct PDZ–PDZ domain or C-terminal–PDZ interactions, NO signaling is altered. Postsynaptic density protein-95 (PSD95), a multivalent synaptic scaffolding protein and core component of the postsynaptic density, can link nNOS to N-methyl-d-aspartate receptor (NMDAR) [51, 187] and accounts for the efficient activation of nNOS by NMDAR stimulation by coupling NO synthesis by nNOS to the Ca^{2+} influx through NMDA receptors [253]. Binding of nNOS to PSD95 is a determinant of postsynaptic targeting of nNOS. nNOS has also been found capable of negatively regulating the NMDA receptor by S-nitrosylation [89].

However, there is also evidence showing that non-NMDA glutamate receptors (i.e., AMPA and type I metabotropic receptors) may contribute to NO generation [212]. Additionally, nNOS-mediated NO synthesis may also be induced as a consequence of the activation of muscarinic acetylcholine receptors (M1 and M3) and purinergic P2X7 and P2Y1 receptors, which are able to increase intracellular calcium concentration in response to receptor activation and can be found colocalized with nNOS in certain brain regions [33, 77, 227].

Besides regulation on the levels above, the synthesis of NO can be inhibited by direct inhibition of NOS (see below) or by limited availability of the substrate L-arginine.

45.2.5 Targets of NO

NO has multiple targets in the brain, with the soluble form of the guanylate cyclase (sGC) the most extensively characterized [64, 179, 254]. Activation of sGC subsequently increases the production of cGMP, and the level of cGMP in the cerebellum, striatum, and hippocampus has been shown to depend largely on the NOS activity [149, 164, 287]. In most brain regions, there is a widespread colocalization of nNOS and sGC, which may explain such effects [66].

Some physiological effects of NO are, however, independent of sGC activation, and it has been demonstrated that NO, induced by NMDA receptor stimulation, activates the p21 (ras) pathway of signal transduction with a cascade involving extracellular signal-regulated kinases and phosphoinositide 3-kinase [60, 312]. These pathways are known to be involved in transmission of signals to the cell nuclei and may therefore form a basis of a generation of long-lasting neuronal responses to NO. Other enzymes that constitute cellular targets for NO are cyclooxygenases, ribonucleotide reductase, some mitochondrial enzymes, and NOS itself [59, 86]. Finally, NO can nitrosylate proteins and damage the DNA [60, 270–272].

Besides its influence on glutamate, NO is known to have effects on the storage, uptake, and/or release of most other neurotransmitters in the CNS (see below), and since NO is a highly diffusible molecule, it may reach extrasynaptic receptors at some distance from the place of NO synthesis [141, 302]. NO is thus capable of mediating both synaptic and nonsynaptic communication processes.

45.3 NO and Depression

The role of the nitric oxide signaling pathway has been established in several different psychiatric disease entities, including schizophrenia, bipolar disorder, and major depressive disorder. A detailed overview of all these disorders is beyond the scope of this text. The present overview will therefore only relate to unipolar depression, where several lines of evidence support an association between abnormalities in NO and mood disorders.

45.3.1 Preclinical Studies

Preclinical studies of nitrergic signaling utilize methods from the concept of transgenic animals to stress models.

45.3.1.1 NO and Behavior

Mice with targeted disruption of the nNOS gene exhibit abnormal behaviors. In a recent published work, it was demonstrated that transgenic mice lacking nNOS showed hyperlocomotor activity in a novel environment, increased social interaction in their home cage, decreased depression-related behavior, and impaired spatial memory retention [282]. The hyperlocomotion has also been demonstrated in reports from other groups, although some variances in methodology exist [24, 202, 300]. In a study using adult nNOS-deficient and wild-type mice for their recognition memory abilities in a social olfactory discrimination paradigm, it was observed that short-term and intermediate-term recognition memory was normal in nNOS-deficient mice, but unlike wild-type mice, nNOS-deficient mice failed to consolidate an olfactory cued long-term recognition memory [134]. These data, together with proteomic observations, suggest that NO of nNOS origin is critically involved in the regulation of protein synthesis-dependent olfactory long-term memory consolidation within relevant brain structures including the olfactory bulb [134].

Interestingly, mice lacking the gene encoding the neuronal isoform of nitric oxide synthase (nNOS−/−) are more aggressive than wild-type (WT) mice in standard testing paradigms [203]. Testosterone is necessary, but not sufficient, to evoke the persistent aggression in these mutants. In male nNOS−/− mice, a dramatic loss of behavioral inhibition reflected by persistent fighting

and mounting behavior despite obvious signals of submissiveness or disinterest by their test partners was observed, a finding that was linked to blood testosterone levels [202].

Despite elevated corticosterone concentrations (see below), nNOS knockout mice seem to be less "anxious" or "fearful" than wild-type mice in some studies. Male nNOS−/− mice spend more time in the open field than WT mice [201] and display less anxiety in the elevated plus maze [316]. However, in other studies it has been reported that nNOS transgenic mice have an increased time spent in the closed arm of the elevated plus maze [300].

Since iNOS-mediated NO production is more prominent in response to inflammation and disease, a predominant phenotype of iNOS−/− is the increased susceptibility to some infections [313, 314]. However, significant changes in the level of neuroactive amino acids have been found in the brains of iNOS−/− mice, such as increased cortical glutamate levels, which were associated with decreased seizure response to PTZ administration [62]. This evidence also supports behavioral changes that have been found in iNOS−/− mice. In fact, iNOS null mice express increased reactivity to stress, such as increased anxiety in response to predator scent [1]. On the other hand, iNOS−/− mice also evidenced normal locomotor activity and increased resilience in the forced swimming test [184] (Fig. 45.2). However, these results are of difficult interpretation regarding the involvement of iNOS-mediated NO levels since these animals present compensatory increased NO levels in the neocortex while decreased NO levels in the hippocampus in response to stress [1, 39].

As a casual association exists between stressful life events and prior number of depressive episodes [138], a majority of the preclinical characterization of NOS involvement in disease has been carried out in various animal stress paradigms, ranging from models of unipolar depression to models of PTSD. Thus, several preclinical studies have confirmed excessive NOS activation and NO release in the cortex and hippocampus following a protracted stressful event [108, 109, 167, 168], as well as concomitant changes in hippocampal NMDA receptor density [109]. Moreover, in a recent study using a validated psychopathological animal model of depression, it was observed that environmental factors such as stress may augment a predisposed depressed individual to hyperactivity in the nitrergic signaling pathway, as measured by the expression of key genes involved in NMDA–NO response and increased nNOS expression and NOS activity [295].

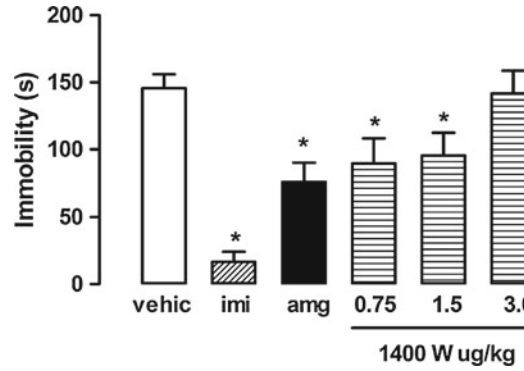

Fig. 45.2 Acute treatment with the iNOS inhibitor 1,400 W significantly reduced immobility time in the FST. Data are expressed as mean + SEM ($n = 5$–9/group). *Asterisks* indicate significant difference from the vehicle-treated group ($p < 0.05$, ANOVA followed by Dunnett's). *vehic* vehicle, *imi* imipramine, *amg* aminoguanidine, *NPA* n-propyl-L-arginine (Reprinted from Montezuma et al. [184] with permission. © Elsevier Ltd)

45.3.1.2 NO and the HPA Axis

Accumulating evidences suggest that NO is involved in the tonic control of the HPA axis function. nNOS is widely present in the HPA axis and in closely related anatomical structures. At the hypothalamic level, nNOS immunoreactivity can be found in parvocellular neurosecretory and medullary-projecting preautonomic neurones [8, 23, 44, 206], and at the lower level, nNOS mRNA and immunoreactivity have been detected in the adrenal cortex [286].

The involvement of NO in neuroendocrine regulation and specifically in the modulation of the release of hypothalamic neurohormones was based on the findings that the NO donor molsidomine was able to inhibit the interleukin-1-beta-induced CRH release from the hypothalamus [55]. Similar experimental evidence on the conclusions on an inhibitory role of NO on CRH

release was shown by augmentation of the IL-1-beta-stimulated ACTH release in the presence of the NOS inhibitor L-NAME (see below). Since the authors could not detect a direct influence of NO at the pituitary level, the ACTH release in response to IL-1-beta was considered to reflect CRH activity. Similarly, the stimulatory action of LPS on ACTH and corticosterone secretion was also augmented by inhibition of NO formation [244]. In an another study of activity of the HPA axis in intact and adrenalectomized rats, the effects of two NOS inhibitors were evaluated, with results suggesting that the central NO system exerts a tonic negative influence on the activity of the HPA axis in the presence or absence of circulating glucocorticoids [93].

This is confirmed in another study, where 7-NI and L-NAME reduced ACTH and corticosterone secretion induced by an alpha-1-adrenergic receptor agonist, and also diminished the HPA response to the nonselective beta-adrenergic receptor agonist isoprenaline, suggesting a complex functional relationship between NO and the regulation of HPA axis activity [82].

Comparing transgenic animals lacking nNOS and wild-type mice, the levels of CRH mRNA were similar, and plasma ACTH levels, under basal conditions and in response to forced swimming, were unchanged in mutant when compared to wild-type mice [221]. These findings suggest that nNOS gene disruption does not significantly impair basal CRH gene expression, but may be (although still debated) an important mediator of homeostatic adaptation, as confirmed by the finding that transgenic mice lacking nNOS, single housed at weaning, showed significantly higher basal corticosterone plasma levels [24].

Also larger animals have been studied. For example, in a study of the ACTH response in female Yucatan miniature swine, it was observed that chronic NOS inhibition with L-NAME (see below) increased the ACTH response to exercise, indicating that nitric oxide modulates the neuroendocrine component of the HPA axis during exercise, despite the possible change in blood flow following L-NAME [128].

Stress has also been found to influence the NO functioning. For example, swim stress increases the number of hypothalamic NOS-containing neurons, confirming the involvement of NOS neurons of the PVN in the response to different types of acute stressors [251].

45.3.1.3 NO and Other Transmitter Systems

Deletion of the nNOS gene not only eliminates nNOS protein but also, in common with many gene deletions, affects several "downstream" processes. For example, serotonin (5-HT) metabolism is altered in male nNOS−/− mice, where the ratio of the metabolite 5-HIAA and 5-HT was significantly reduced in several brain regions including the cortex, hypothalamus, midbrain, and cerebellum of nNOS−/− in comparison with wild type [48]. In the same study, norepinephrine, dopamine, and metabolites were generally unaffected [48].

Several in vivo studies have demonstrated that NO can modulate the extracellular level of various neurotransmitters in the central nervous system, e.g., 5-HT, DA, GABA, and glutamate [135, 161, 257–259, 274, 299] (Fig. 45.3). In addition, NO can inactivate the rate-limiting enzyme in the synthesis of 5-HT, tryptophan hydroxylase [146, 147], and it has been suggested to stimulate synaptic vesicle release from hippocampal synaptosomes [176, 177]. Furthermore, NO regulates 5-HT reuptake [230–232], inhibits uptake of [3H] DA by striatal synaptosomes [159, 160] and transforms 5-HT into an inactive form [80].

More recently, it was demonstrated that a physical interaction between the serotonin transporter and neuronal nitric oxide synthase, via PDZ–PDZ interactions, may underlie reciprocal modulation of their activity [45]. The connection between NO and 5-HT is substantiated by observations showing that NO as well as 5-HT is involved in the pathophysiology of migraine [150, 151, 283, 284], as well as the inverse relationship between NO and 5-HT in peripheral tissue.

45.3.1.4 NO and Neurogenesis

Neurogenesis has attracted significant interest in the past two decades, and it has been suggested that neurogenesis may be linked to recovery from clinical depression [67, 68] and that neurogenesis

may be a perquisite for antidepressant response [252]. However, it should be strongly emphasized that evidence for clinical relevant neurogenesis in humans does not exist. In the brain, neurogenesis has been observed in the subventricular zone and the subgranular zone of dentate gyrus (DG) [69]. Interestingly, it has been demonstrated that subventricular zone is surrounded by nNOS-positive neurons [245], and cells expressing nNOS also have been identified in neuronal precursors in DG [261], suggesting that nNOS could participate in the regulation of neurogenesis.

Moreover, it was demonstrated that inhibition of NO synthesis with 7-NI (see below) increases proliferation of neural precursors isolated from the postnatal mouse subventricular zone [172]. However, a recent report has also demonstrated that nNOS inhibition with 7-NI enhanced the proliferation of progenitor cells in the dentate gyrus [319]. The above results are in line with findings using a null mutant neuronal NO synthase knockout mouse line, where the number of new cells generated in neurogenic areas of the adult brain, the olfactory subependyma and the dentate gyrus, was strongly augmented, indicating that division

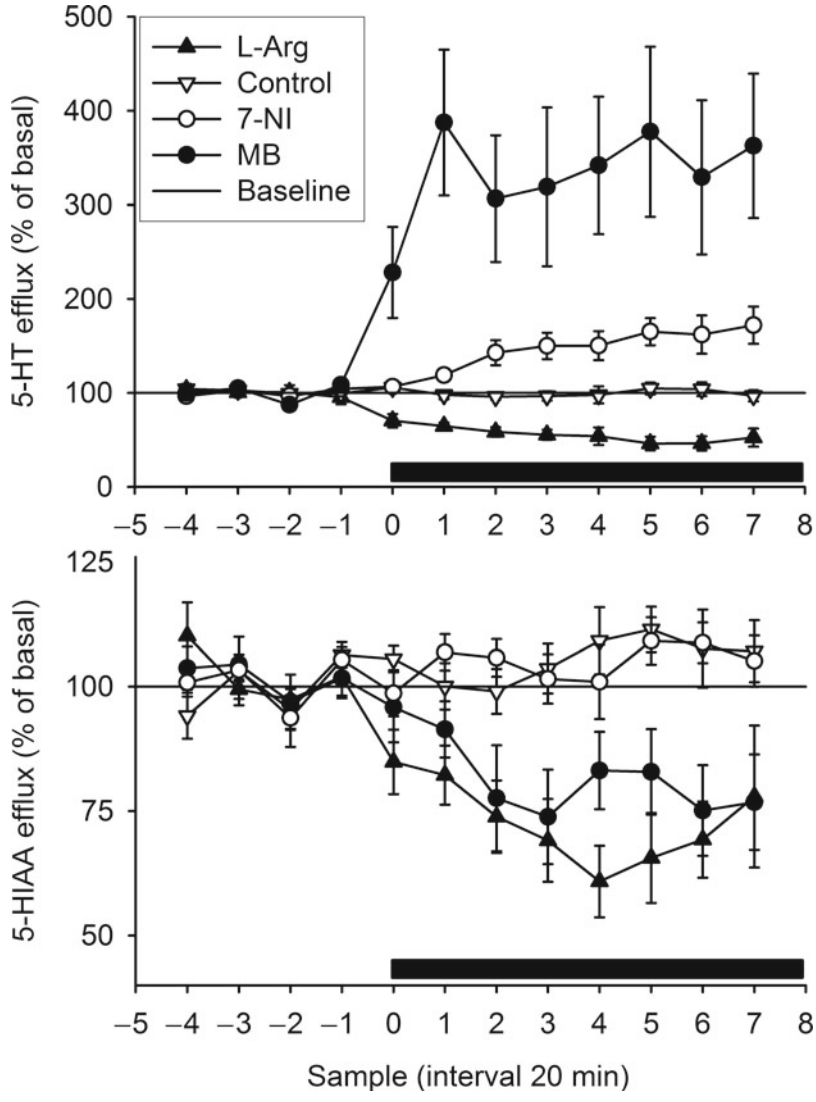

Fig. 45.3 Effect of local perfusion using microdialysis of L-arginine (2 mM), 7-NI (1 mM), MB (1 mM), or artificial CSF on 5-HT ($n=8$, $n=12$, $n=7$, and $n=13$, respectively) overflow in ventral hippocampus of rats. Drugs were infused into ventral hippocampus as indicated by *bar*. Results are expressed as % of basal efflux ± SEM (Reprinted from Wegener et al. [299] with permission. © Macmillan Publishers Ltd)

of neural stem cells in the adult brain is negatively controlled by NO [223].

In addition, inhibition of nNOS with 7-NI has been shown to reverse the neurogenetic impairment induced by a chronic mild stress regimen [318]. Interestingly, opposite of what may be expected based on the findings with the inhibitors, also administration of exogenous NO donors has been shown to stimulate cell proliferation [124, 317]. The neurobiology underlying this phenomenon remains to be established, but it should be mentioned here that several of the pharmacological tools available possess a U-shaped dose–effect pharmacology.

45.3.2 Clinical Studies

45.3.2.1 Postmortem Studies

Postmortem material from patients with major depression has demonstrated a reduced nNOS activity and protein content in various brain regions. In a study of eight patients (including two with schizoaffective diagnosis and two with bipolar depression) diagnosed according to DSM-IIIR, a reduced number of NOS-I containing neurons in the paraventricular hypothalamic nucleus was observed [22]. This finding was later expanded and confirmed in 11 patients and 11 matched controls [21]. In another study a strong trend ($p<0.06$) in decreased activity of the constitutive NOS in prefrontal cortex of 15 patients with unipolar depression diagnosed versus 15 nonpsychiatric controls from the Stanley Consortium was observed [305]. Also a study examining 12 depressed subjects and 12 psychiatrically normal control subjects, obtained at autopsy at the Coroner's Office of Cuyahoga County, Cleveland, OH, USA, found a significant lower amount of nNOS in locus coeruleus of depressed subjects [137]. However, no changes were observed in the cerebellum [137].

Since hippocampus is a crucial region in the pathophysiology of affective disorders, the possible hippocampal involvement is of great interest. Findings from the CA1 hippocampal area in brains from the Stanley Consortium have reported an increase in nNOS immunoreactivity in depression and bipolar disorder [217]. In the same study, no changes were observed in brains from schizophrenic patients.

45.3.2.2 Peripheral Markers

Several studies have examined peripheral NO metabolism in major depression, however with rather mixed results. In a study of suicide attempters, increased NO metabolites (NO_2 and NO_3) have been observed [140, 152], indicating a hyperfunction of the nitrergic system. The same finding was reported a few years earlier, where it was found that 17 drug-naïve patients suffering from depression, diagnosed according to DSM-IV, had elevated nitrite levels [277]. In the same study, treatment with antidepressant normalized the nitrite levels, correlating with clinical response [277]. Finally, in a study including 36 depressed patients, diagnosed according to DSM-IV, and 20 healthy subjects, there was no correlation between depressive symptoms and levels of nitrate, but a significant effect of antidepressant treatment, lowering the nitrate levels [112]. In addition, there are some studies demonstrating involvement of NO in some, but not all, forms of IFN-alpha-induced depression [278].

However, measurement of nitrate in serum will only detect the overall nitrate pool. Indeed, in a study by Srivastava and coworkers examining 66 cases of depression and 114 controls revealed a 73 % decrease in nitrite content in the polymorphonuclear leukocytes [269]. Since human polymorphonuclear leukocytes express nNOS-like neurons [292], this measure may be hypothesized to be more relevant than serum values.

This assumption is also reflected in a study where a decreased platelet NOS activity and plasma NO metabolites in depressed patients were found [49, 50].

Several human association studies have been published linking endogenous inhibitors of NOS with disease. The levels of the endogenous inhibitors, NG-monomethyl-L-arginine (SDMA) and NG-dimethyl-L-arginine (ADMA) [28–30], have been shown to be changed in depression, schizophrenia, and Alzheimer's disease [10, 58, 260]. However, it is not clear whether these associations are clinically important.

Taken together, although human studies have been predominantly carried out on peripheral tissue samples (e.g., plasma or serum), a support for the role of the NO system in psychiatric disease exists. Findings from the peripheral tissue seem to suggest an elevated NO metabolism in depressive states. However, it is worth mentioning the measures mentioned here were carried out on specific subcomponents in the blood compartment, and the generalization therefore may be limited.

45.3.2.3 Genetics

Recently, results from a few laboratories have implicated a role of NOS polymorphisms in the brain diseases such as bipolar disorders, schizophrenia, Alzheimer's disease, and autism [41, 84, 238, 240]. A few genetic studies have been carried out investigating nNOS in unipolar depression, and several genetic association studies are currently being carried out, and data will likely contribute to clarification of the NOS roles in these brain disorders.

In a population-based association study investigating nNOS in unipolar depression, it was tested whether the nNOS C276T polymorphism confers susceptibility to unipolar depression and treatment response to fluoxetine. No association with disease or SSRI treatment response was found in 108 Chinese patients [311], but due to the restricted design of the study, it is concluded that other variants of the nNOS gene may play a role.

Also, in a recent genetic association analysis of case–control samples (325 MDD patients, 154 BP patients, and 807 controls) in a Japanese population, using single nucleotide polymorphism (SNP, rs41279104, also called ex1c), no associations between one marker (rs41279104) in nNOS and Japanese mood disorder patients were detected, although the sample sizes were probably too small to allow a meaningful test [213]. Moreover, the paper did not perform an association analysis based on linkage disequilibrium and a mutation scan of nNOS [213].

In a large genome-wide association study of 435,291 SNPs genotyped in 1,738 MDD cases and 1,802 controls selected to be at low liability for MDD, it was reported that an association of nNOS with the disease was present, although the size of the NOS-I gene makes the authors cautious about the finding [276].

Interestingly, in a recent study carried out in a group of 181 depressed patients and 149 control subjects of Polish origin, it was examined whether a single nucleotide polymorphism (SNP) present in the genes encoding iNOS and nNOS could contribute to the risk of developing recurrent depressive disorder [83]. It was shown that both investigated polymorphisms are associated with depression and that the NOS2A and nNOS genes may confer an increased risk of recurrent depressive disorder [83].

In conclusion, although mixed findings directly related to the NOS-I gene exist, and despite the fact that several larger replication studies are needed, the available genetic studies support a role of NO in depressive disorders.

45.3.2.4 Pathways Interacting with NO and Depression

As mentioned above, the glutamatergic input constitutes the major activating transmitter to the neuronal NOS. A detailed review lies beyond the scope of this text, but some prominent findings will be highlighted here.

Several studies have demonstrated differences related to glutamate receptors in postmortem brain samples from individuals with major depression. A receptor-binding assay and a Western blot study revealed a reduction in [3H] L-689,560 (a potent antagonist at the glycine modulatory site on the NMDA receptor binding as well as a reduction in NR1 immunoreactivity in the superior temporal cortex in major depression) [205]. Moreover, receptor binding and autoradiography in patients with MDD showed increased binding of [3H]CGP39653 (glutamate site on the NMDA receptors) in the hippocampus, but not in the entorhinal or perirhinal cortex [20]. In the same study, in situ hybridization demonstrated that mRNA levels of the NR2A and NR2B subunits of the NMDA receptors and the GluR1, GluR3, and GluR5 subunits of the AMPA receptors in the perirhinal cortex, but not in the

hippocampus or entorhinal cortex, were significantly lower than those of a control group [20]. This is in line with reports suggesting that the levels of the NR2A and NR2B subunits of the NMDA receptors are decreased in the prefrontal cortex (Brodmann's area 10) of patients with MDD [75].

Stress is recognized as a main risk factor for major depression (see also below), and it has been demonstrated that acute stress is associated with increased glutamatergic neurotransmission in areas of the forebrain as measured by an in vivo microdialysis technique [14, 163, 242]. In this regard it is of significant interest that following chronic treatment with antidepressants, a reduction of glutamate release under basal conditions can be observed [32, 178, 285]. Using a different technique with direct measurement of glutamate release, it was recently reported that depolarization-dependent release of glutamate is selectively upregulated by acute stress relative to GABA release and that chronic treatment with antidepressants (desipramine, fluoxetine, or venlafaxine) completely abolished the stress-induced upregulation of glutamate release [190]. It can be speculated that this inhibition may be a relevant component of the therapeutic action of antidepressants [190]. Furthermore, it has been reported that 4-week administration of the SSRI fluoxetine caused upregulation of the subunits (e.g., NR2A, GluR1, GluR2) of glutamate receptors in the retrosplenial cortex of the rat brain and that these changes in the subunit levels were associated with an upregulation of dendritic spine.

Interestingly, the magnesium ion plays an important role in the NMDA receptor functioning and several studies suggest that other studies demonstrate that depletion of magnesium precipitates depressive symptomatology in both humans and animals [18, 114, 119, 189, 226, 233, 266, 279]. As activation of the NMDA receptor is known to increase the activity of NOS, these symptoms may arise due to nitrergic overactivity. Importantly, the depressive symptoms following magnesium depletion can be ameliorated following antidepressant treatment with either desipramine or *Hypericum* extract [266].

Finally, it is worth to mention the clinical studies carried out with the NMDA receptor antagonist, ketamine, clearly demonstrating a rapid sustained antidepressant effect [165, 169, 315]. However, although ketamine clearly affects the NMDA receptor, it may also have other important targets, such as the rapamycin pathway [155].

Considering these results together, it seems likely that glutamatergic neurotransmission is implicated in the action of antidepressants.

45.4 NO and Antidepressant Treatment

Over the past two decades, a number of preclinical studies have demonstrated that inhibition of NOS produces anxiolytic and antidepressant-like behavioral effects in a variety of animal paradigms, as well as studies showing involvement of NO in the action of classical antidepressants. These studies include systemic injections as well as targeted infusions into the brain. Only a few very limited clinical studies are available, and they are confounded by the nonselectivity of the drug used (see below, Sect. 45.4.1.4).

45.4.1 NOS Inhibition Produces Antidepressant-Like Effects

The typical NOS inhibitor is an amino acid, which associates with the substrate-binding site for L-arginine [94]. The inhibitor will compete with L-arginine, and usually extra arginine will reverse the NOS inhibition produced by the inhibitor.

45.4.1.1 Depression-Like Behavior

The best investigated inhibiting amino acids are L-NG-nitroarginine (L-NNA), its methyl ester L-NAME, L-NG-monomethyl-arginine (L-NMMA), and NG-propyl-L-arginine. L-NAME requires hydrolysis of the methyl ester by cellular esterases to become a fully functional inhibitor [94]. Acute antidepressant effects have been found in both rats and mice models. L-NNA and L-NAME have been reported to be effective in

both the forced swim test (FST) and tail suspension test (TST) in mice [57, 107] and in the FST in rats [106, 129]. The effect of the drugs seems to display a U-shaped pharmacology, where both low and high doses have no effect [57, 107, 136]. Pretreatment with L-arginine counteracts the behavioral effects of the L-NAME and L-NNA [57, 107, 125, 129], but has also been reported in some studies to have an antidepressant-like effect by itself [57]. Similar to the findings with the amino acids, antidepressant-like properties have also been demonstrated with the non-amino acid compounds. The primary benefit with the indazole and imidazole derivates is a potential superiority in selectivity among the different isoforms of the NOS enzymes. This was clear when 7-nitroindazole (7-NI) was discovered [13], as it did not have a profound effect on the blood pressure [185], which can be observed with most of the amino acid inhibitors. Studies suggest that 7-NI not only interacts competitively at the substrate-binding site in the NOS enzyme [13] but also acts competitively regarding the cofactor tetrahydrobiopterin (BH_4) [175]. 7-NI is also a potent inhibitor of bovine aortic endothelial eNOS in vitro, in spite of the lack of cardiovascular side effects of this compound in vivo [13] suggesting other factors to be involved. Other more selective isoform inhibitors have been screened. One such a compound is 1-(2-trifluoromethyphenyl)imidazole (TRIM), which is described as a potent and relatively selective inhibitor of nNOS both in vitro and in vivo [101, 103]. The selectivity of this compound seems to be centered around the cofactor BH_4 and the availability of BH_4 in the tissues [102].

In the FST, acute administration of 7-NI and TRIM has antidepressant-like effects, but does not affect locomotion [106, 111, 267, 289, 310]. Interestingly, the effects of 7-NI are centrally based, since intrahippocampal administration of 7-NI causes a dose-dependent antidepressant-like effect in the FST, an effect which could be prevented following intrahippocampal coadministration of L-arginine [132] (Fig. 45.4). Recently, it was demonstrated that the antidepressant action of ketamine, which has proven to be efficacious for the treatment of patient with severe treatment-resistant depression, could be attenuated with L-arginine, supporting the hypothesis that NO may play an important role in the mechanism of action of ketamine [157] (Fig. 45.5).

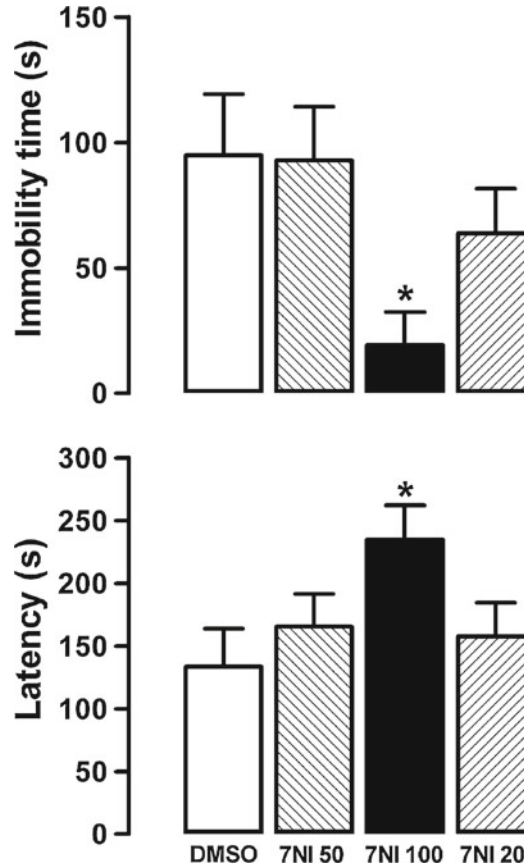

Fig. 45.4 Pretreatment with L-arginine (100 nmol/0.5 µl) prevents 7-NI (100 nmol/0.5 µl)-induced effects in the FST. Drugs were administered immediately after pretest. Data are expressed as mean ± SEM (n=6–9/group). *Asterisks* indicate significant difference from the vehicle-treated group ($p<0.05$, ANOVA followed by Duncan) (Reprinted from Joca and Guimarães [132] with permission. © Springer-Verlag)

45.4.1.2 Cognition and Memory

The clinically important features in depression, cognition and memory, have been extensively examined, and a major role for NO in the formation of memory and as a mediator in synaptic plasticity has been suggested [225, 234]. The majority of studies support a facilitatory role of NO in learning processes, and nNOS has been proposed to be the principal source of this retrograde messenger dur-

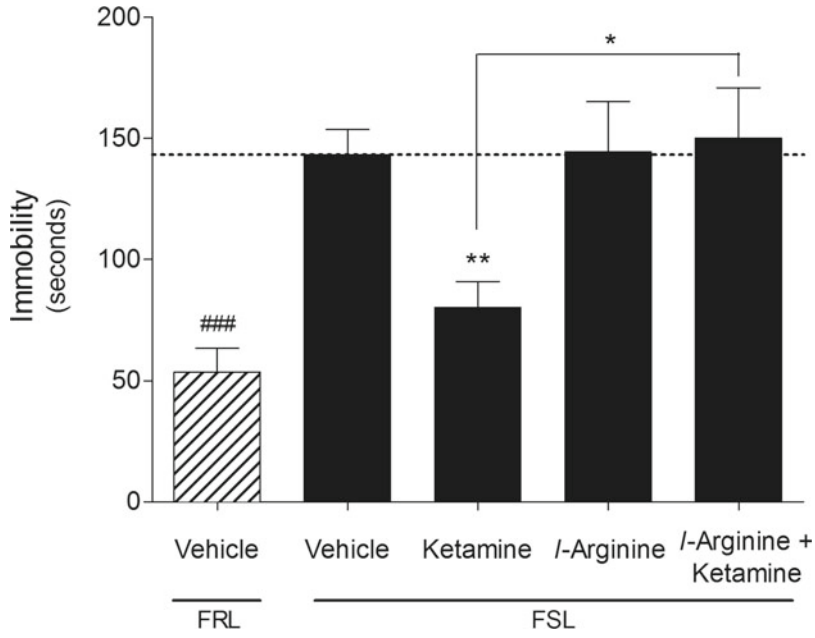

Fig. 45.5 Behavioral results obtained from the forced swim test (FST) in vehicle-treated Flinders-resistant line (FRL) and Flinders-sensitive line (FSL) rats and FSL rats treated with ketamine, L-arginine, or ketamine + L-arginine. Results are expressed as the mean ± SEM. ##$p<0.01$, ###$p<0.001$ (Student's *t*-test); *$p<0.05$, **$p<0.01$ (one-way ANOVA, Newman–Keuls test). Nitric oxide and ketamine's antidepressant mechanism (Reprinted from Liebenberg et al. 2015 [157] with permission. © Cambridge University Press)

ing long-term potentiation (LTP) [207, 256], a highly important process for memory formation [25, 166, 170]. However, some controversy about this finding exists, as LTP in the hippocampus and cerebellum was reported to be normal in nNOS transgenic mice [158, 208]. The involvement of NOS in memory has been confirmed in studies with NOS inhibitors. For example, it was shown that systemic administration L-NAME and L-NNA impairs acquisition but not retention of spatial learning in rats [31, 46, 73], and L-NA reduces hippocampal mediation of place learning in the rat [181, 182]. Similarly, intrahippocampal administration of L-NAME impairs working memory on a runway task without affecting reference memory [210, 211], and L-NAME has been shown to disrupt learning of an associative memory task, the conditioned eyeblink response in rabbits [46]. However, in a well-learned operant task – a delayed non-match to position, no effect of L-NAME was found [303], and similarly, L-NAME did not affect learning in a Morris water maze paradigm [15]. In agreement with these observations, central and systemic administration of the NO precursor, L-arginine has been found to significantly prolong the latency time in the passive avoidance test without inhibition of locomotor and exploratory activity [229]. The interpretation of the overall neurobiological consequences of these findings remains unclear. The early findings with NOS inhibitors do not seem to correspond well with the results published about other clinically relevant antidepressants, such as the SSRIs, where cognitive performance in patients has been shown to be unaffected [263] and independent from clinical recovery [113]. However, a recent rodent study found that acute administration of imipramine and paroxetine to rats impaired the discrimination of old from the recently presented objects [194]. Interestingly, following chronic administration, the imipramine-treated rats were unable to differentiate between the two objects, whereas paroxetine-treated rats spent more time exploring the old object [194]. Similarly, it is important to note that the studies with NOS inhibitors and cognitive testing predominantly have been carried out following

one acute dose. The relevance for this paradigm related to a clinical context is, as it is also the case with the other depression and anxiety tests, questionable. Only limited information is available concerning chronic administration of NOS inhibitors. However, it has been shown that L-NAME in the drinking water over 14 days impairs working memory in rats [52]. On the other hand, it has also been demonstrated that only acute, but not chronic, administration of L-NAME impairs LTP formation induced by a weak near-threshold tetanus [16] and that chronic L-NAME in the drinking water did not affect working memory in a water maze working task (Wohlert-Johannsen and Wegener, unpublished). Further studies must be carried out to elucidate the overall effects of NOS inhibition on cognition.

In the examination of the non-amino acid inhibitors, 7-NI induces amnesia in a passive avoidance task in the chick [121] and impairs learning and memory in different tasks such as the Morris water maze, radial maze, passive avoidance, and elevated plus maze tests [122, 180, 308, 309, 321]. 7-NI also produces taste aversions and enhances the lithium-based taste aversion learning in a conditioned taste aversion paradigm, an effect that was counteracted with simultaneous administration of L-arginine [297].

Some compounds, based on hydrazine structure, have been extensively studied in relation to cardiovascular [85, 126, 280, 294, 306] and endocrinological diseases [123, 247, 262, 275]. The compounds are predominantly inhibitors of iNOS, with much less activity on the other isoforms. Aminoguanidine (AG) is a hydrazine derivate and the best characterized compound [54, 96, 110], which selectively decreases cGMP levels produced by iNOS [95]. Furthermore, AG has been observed to protect against neurodegeneration produced by chronic stress in rats [218] and to prevent the impairment of learning behavior and hippocampal long-term potentiation following transient cerebral ischemia in rats [186]. Moreover, intracerebroventricular infusion of AG prevents the depression-like behavior following a chronic unpredictable stress paradigm [293]. Supporting these findings, a model of post-traumatic stress disorder (PTSD) seems to involve exclusively the iNOS isoform, as only aminoguanidine, but not 7-NI, was effective in attenuating neurobiological readouts [109]. Together, these findings highlight the possible involvement of inflammatory processes in depression and anxiety, which is plausible considering the significant involvement of stress of those disorders (see above). AG has also recently been demonstrated to display anxiolytic-like effects in EPM, open field test, light–dark test, and social interaction test in stressed mice [91]. In nonstressed mice, single i.p. injection of AG was able to induce antidepressant-like effect in the FST without concomitant locomotor changes. The administration of a more selective iNOS inhibitor (1,400 W) induced similar dose-related antidepressant-like effects [184] (Fig. 45.2). Since in mice FST the animals are not previously stressed, the results raised the possibility that the iNOS basally expressed in certain brain regions, such as the hippocampus, would have been affected by the drug treatment, thus indicating that it contributes to increase NO levels in response to stress exposure [184]. Whether these effects are present independently of the presence of stressors in different preclinical models remains to be established.

45.4.1.3 Endogenous Inhibitors

Some antagonists of NOS require special attention, as they may be considered as endogenous inhibitors. These include L-citrulline, agmatine, NG, ADMA, SDMA, and argininosuccinic acid. While L-citrulline is a very weak inhibitor, a derivate, L-thiocitrulline is much more powerful [81]. Agmatine, decarboxylated arginine [301], is potentially significant, as there is evidence of antidepressant effects in preclinical animal models of depression [9, 145, 156, 320], as well as in humans [98–100, 281]. It is, however, noteworthy to mention that agmatine also has been conceptualized as an endogenous clonidine-displacing substance on imidazoline receptors [154, 237] and to have affinity for several transmembrane receptors, such as α-2-adrenergic [219], imidazoline I1, and glutamatergic NMDA receptors [307]. Therefore, the effects observed in the preclinical studies may be mediated via these pathways and not linked to NOS.

No solid preclinical data exist for the other endogenous inhibitors, although there are reports of their presence in animals [209].

45.4.1.4 Clinically Tested Compounds

Methylene blue (MB), although not exclusively selective for the NOS, is potentially of special relevance since it is so far the only compound proven to be effective in patients [198–200]. MB oxidizes protein-bound heme and nonheme ferrous iron [249], inhibiting the stimulation of soluble guanylyl cyclase (sGC) by NO and nitrovasodilators [188]. MB potently inhibits NOS both in vitro [173, 174] and in vivo [291]. As early as 1899, MB was described to have a calming – probably antipsychotic – effect in patients [27]. However, more recent work has focused on the beneficial effects of MB in manic-depressive disorder, where a response of 63 % among 24 lithium refractory patients was found [191]. The studies were supplemented and expanded, confirming this action [198–200]. At the time of the study, the mechanistic hypotheses were based on changes in the vanadium ion [195–197]. Unfortunately, the studies cited above were not fully randomized, but better controlled trials are now being carried out [5].

Several preclinical studies confirm a positive effect of MB in the FST and EPM [72] although with a U-shaped dose–response efficacy curve. MB produces taste aversion in a conditioned taste aversion paradigm, an effect comparable to the effects of 7-NI, which also could be counteracted with simultaneous administration of L-arginine [297]. As indicated by the mode of action, MB is expected to be a very nonselective compound. Indeed, MB not only inhibits NOS and sGC but also several other heme-containing enzymes, like monoamine oxidase. Thus, MB is a potent inhibitor monoamine oxidase (MAO) [70, 92, 127] and various cytochromes. This effect probably accounts for the case reports suggesting a hyperserotonergic state following the use of MB [92, 273] and can also be an explanation for the clinical efficacy.

As MB affects the NO downstream signaling pathway, including sGC, a few compounds, which exclusively affect sGC, but not NOS, have been examined. Studies with selective (i.e., non-NOS) inhibitors of NO-dependent cGMP formation with [1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one] (ODQ) have shown antidepressant-like effects in the FST [111], as well as prevention of pro-depressant effect of L-arginine in the FST [71]. Similarly, ODQ has been shown to have anxiolytic-like properties, with an increase in the percent time spent on the open arm in EPM following administration of the drug [268]. These findings are in agreement with other studies showing that an increase in cGMP, following inhibition of phosphodiesterase type V, with sildenafil, can produce anxiogenic-like responses in the EPM [148, 290]. However, the mechanisms have not been fully elucidated, as sildenafil also has been shown to have antidepressant-like effects following central muscarinic receptor blockade [37].

45.4.2 Classical Antidepressants and NO

Direct interaction between clinically used antidepressants and nitrergic signaling has been shown in a few studies. In a study with patients with ischemic heart disease and depression, 17 received paroxetine and 14 patients nortriptyline, and it was observed that serum nitrite and nitrate levels were significantly decreased following paroxetine treatment but not nortriptyline [76]. In addition, paroxetine was also shown to be a significantly more potent inhibitor of the NOS enzyme activity than nortriptyline [76]. Similarly, several established antidepressants of distinct chemical classes, including imipramine, paroxetine, citalopram, and tianeptine, have all been shown to inhibit hippocampal NOS activity in vivo when applied locally in the brain in therapeutic relevant concentrations [298] (Fig. 45.6). This effect may involve a substantial pathway through the NMDA complex, and selective inhibition of NOS will produce pronounced behavioral effects.

It has also been reported that the precursor of NO, L-arginine, antagonizes the effects of the classic tricyclic antidepressant, imipramine [107]. This observation has led to hypotheses regarding the potential contribution of serotonergic–noradrenergic mechanisms in the observed

antidepressant-like effects of the NOS inhibitors (see below). Corroborating this hypothesis, a recent study reported that treatment with 7-NI, venlafaxine, and fluoxetine attenuates stress-induced neuronal activation in overlapping brain regions, thus suggesting that nNOS inhibitors and monoamine antidepressants may share common neurobiological substrates [264].

Subsequently, it has been demonstrated that low and ineffective doses of L-NAME were able to potentiate the behavioral effects of imipramine and fluoxetine but not reboxetine, a noradrenaline reuptake inhibitor, in the FST [105, 106]. In addition, it was shown that a serotonergic mediation of the antidepressant-like effects of L-NA and 7-NI was present, since serotonergic depletion abolished the antidepressant-like effect of the inhibitors [106]. In the hippocampus, the antidepressant-like effect induced by local administration of a selective nNOS inhibitor (N-propyl-L-arginine) could be prevented by coadministering a 5-HT1a antagonist, implicating endogenous serotonin in such effect [118]. However, not all inhibitors seem to display this profile, as it also was demonstrated that the effect of agmatine was independent of the 5-HT depletion [145]. However, as already discussed, agmatine likely has multiple effects on several receptor systems.

Finally, NO has also been implicated in the antidepressant role of several other substances, like tramadol [130], bupropion [65], and lithium [90].

Another important effect mediated by NO–cGMP pathway that is common to antidepressants is the modulation of BDNF levels, a neurotrophin important for cell proliferation, survival, and differentiation. Chronic treatment with antidepressants increases BDNF levels in the prefrontal cortex and in the hippocampus, and intact BDNF signaling in the brain is shown to be necessary for the behavioral effects of conventional antidepressants [2, 12]. NO seems to be also able to modulate BDNF levels, since it was demonstrated that NO donors (SNP, NOR3) decreased BDNF release in hippocampal cell culture, whereas the inhibition of NO production increased these levels [42]. Additionally, systemic treatment with L-NAME during 7 days increased BDNF mRNA levels in the dentate gyrus [228]. In another study, the antidepressant-like effect induced by 7-day treatment with 7-NI was associated with increased expression of BDNF protein levels in the hippocampus [131].

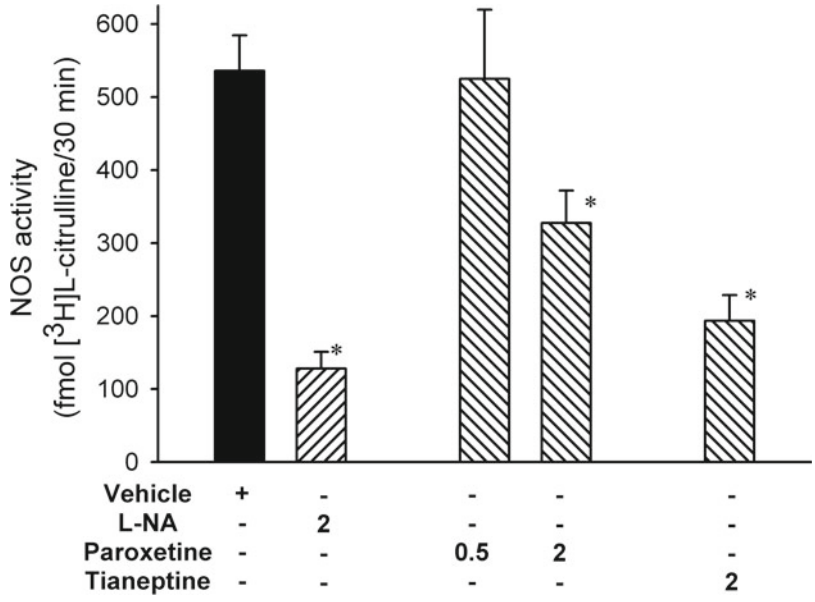

Fig. 45.6 NOS activity in vivo (microdialysis) after acquisition of steady state following local perfusion with vehicle ($n=19$), paroxetine (500 nM, =53 and 2 mM, $n=11$), and tianeptine (2 mM, $n=6$). An *asterisk* (*) indicates statistical difference from the control group ($p<0.05$). Values shown are means + SEM (Reprinted from Wegener et al. [298] with permission. © Elsevier BV)

Although these studies further corroborate the idea that common molecular mechanisms are shared by 7-NI and monoaminergic antidepressants, a recent study that compared the pattern of gene expression in rats treated with imipramine or 7-NI by serial analysis of gene expression (SAGE) found that there are also important differences in the genes regulated by such treatments [74]. Nevertheless, this study conformed the overlapping regulation of genes involved in oxidative stress and neuroplastic responses. Further studies are, however, still necessary to evaluate the contribution of such molecular changes for the antidepressant-like effects induced by nNOS inhibition.

Conclusion

Although the studies cited in the current chapter utilize different methodologies, from a preclinical genetic approach to postmortem human material, the physiological roles of NOS emerge relatively clear. Therefore, the conclusion of the current review is that despite significant challenges in developing compounds which may differentially inhibit the "right" NOS isoform at the right place, the NO system continues to be an interesting novel approach in the future development of antidepressants.

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The Role of Arrestins in the Neuroprotective Effects of Antidepressant Drugs

46

Sofia Avissar, Moran Golan, Valeria Feinshtein, Siyona Kolatkar, Doron Fux, and Gabriel Schreiber

Abbreviations

AP-2	Adaptor protein-2
BAD	Bcl-2-associated death promoter homologue
Bcl-2	B cell lymphoma 2 apoptosis regulator
BDNF	Brain-derived neurotrophic factor
CREB	cAMP response element-binding protein
ERK1/2	Extracellular signal-regulated kinase
GDNF	Glial-derived neurotrophic factor
GPCR	G protein-coupled receptor
GRK	G protein-coupled receptor kinase
MAPK	Mitogen-activated protein kinase
NT	Neurotrophin
Trk	Tyrosine kinase

S. Avissar (✉)
Department of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, Ben Gurion University of the Negev, Beersheba, Israel
e-mail: sofia@bgu.ac.il

M. Golan • V. Feinshtein • S. Kolatkar • D. Fux
Department of Clinical Biochemistry and Pharmacology, Ben Gurion University of the Negev, Beersheba, Israel

G. Schreiber
Division of Psychiatry, Ben Gurion University of the Negev and Barzilai Medical Center, Beersheba, Israel

46.1 Introduction

Beta-arrestins are multifunctional proteins that mediate receptor desensitization and serve as important signaling scaffolds involved in the pathophysiological processes related to mood disorders and in the molecular mechanism of action of antidepressant drugs. Neurotrophin hypotheses characterize major depressive disorder as related to anomalous neurogenesis in brain regions that regulate emotion and memory and suggest that antidepressant therapeutic efficacy is achieved through increased neurotrophin function, thus improving neuronal plasticity.

This overview will focus on the neuroprotective role played by beta-arrestins and by antidepressants hypothesizing antidepressant effects on beta-arrestins as a possible mechanistic explanation of antidepressant neuroprotective therapeutic roles.

46.2 Arrestins

The arrestins constitute a family of proteins that are capable of interacting with GPCRs following their activation by agonists and subsequent phosphorylation by GRKs [1]. Arrestins recognize both GRK phosphorylation sites on the receptor and the active conformation of the receptor following agonist binding, which together drive robust arrestin association with the receptors [2]. Arrestin binding leads to uncoupling of the receptor from its related G protein in such a way that,

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despite the continued activation of receptor by the agonist, it cannot exchange the GTP group on the G protein α-subunit for GDP [3], eventually causing desensitization of G protein signaling via downstream second messenger molecules [1, 2, 4, 5]. Although most of the research regarding the desensitization process has been carried out using the beta-2-adrenoceptor as a model, it is now clear that this process regulates the function of many GPCRs, including alpha- and beta-adrenoceptors and muscarinic cholinergic, serotonergic, and dopaminergic receptors [2, 6]. Beta-arrestins are involved in both receptor downregulation [7] and re-sensitization [8, 9].

To date, four members of the arrestin gene family have been cloned [10]: arrestin-1 and arrestin-4, also, respectively, known as visual arrestin and cone arrestin, are expressed almost exclusively in the retina [11], where they regulate photoreceptor function: rhodopsin and color opsins. By contrast, beta-arrestin-1 and beta-arrestin-2, also known as arrestin-2 and arrestin-3, respectively, are ubiquitously expressed proteins, which regulate GPCRs. Initially, both beta-arrestin-1 and beta-arrestin-2 were reported as preferentially expressed in the central nervous system with high beta-arrestin-1 protein and mRNA levels being reported also in peripheral blood leukocytes and in the lungs, whereas moderate to low mRNA levels were found in the heart, skeletal muscle, and the liver [12]. The abundant expression of beta-arrestin-1 in peripheral blood leukocytes supports the suggestion of a major role for the GRK/beta-arrestin system in regulating receptor-mediated immune functions [12].

Although initially known as negative regulators of GPCR-mediated signaling [1, 3–5], it was later discovered that beta-arrestins also interact with proteins of the endocytic machinery such as clathrin and the adaptor protein, AP-2, and thus promote internalization of receptors via clathrin-coated vesicles [13, 14]. Beta-arrestins were shown to play important roles as multi-protein scaffolds and adaptors in receptor endocytosis and signaling cascades in various MAP kinase modules, e.g., ERK, p38, and JNK, and to a wide variety of additional signal proteins such as c-Src, oncoprotein Mdm2, ARNO, NSF, etc. [2, 15, 16], independently of G protein activation [2, 6, 15–17].

In their inactive form, beta-arrestins are phosphorylated cytosolic proteins. Beta-arrestin-1 undergoes phosphorylation on serine-412 at its C-terminus [18] by ERK1/2. Following agonist stimulation, beta-arrestin-1 is translocated to the plasma membrane, where it undergoes dephosphorylation, a process that is not required for receptor binding and desensitization but is obligatory for receptor internalization. Subsequently, it is re-phosphorylated [18] and returns to the cytosol as a phosphoprotein. Similarly, beta-arrestin-2 also has phosphorylation sites at serine-361 [19] and threonine-383 [20]. Casein kinase II was found to be responsible for threonine-383 phosphorylation [20]. Like beta-arrestin-1, beta-arrestin-2 is also dephosphorylated at the plasma membrane, again, a process required for receptor internalization but not for its binding or desensitization [20].

46.3 Apoptosis

Programmed cell death (apoptosis) is a coordinated set of events eventually leading to the massive activation of specialized proteases (caspases) that cleave numerous substrates, orchestrating fairly uniform biochemical changes which culminate in cellular suicide. The biochemical activation of apoptosis occurs through two general pathways: the intrinsic pathway, originating from mitochondrial release of cytochrome C and associated activation of caspase-9; and the extrinsic pathway, originating from the activation of cell surface death receptors (DR), such as tumor necrosis factor receptor alpha-1 (TNF-R1), Fas (CD95), TNF-related apoptosis-inducing ligand receptor 1 (TRAILR1), and TRAILR2 [21], and resulting in the activation of caspase-8 [22]. Following TNF-R1-induced activation and Fas-Fas ligand-mediated activation in mammalian cells, a balance between proapoptotic (BAX, BID, BAK, or BAD) and antiapoptotic (Bcl-Xl and Bcl-2) members of the Bcl-2 family is established.

46.4 Neurotrophins

Neurotrophins (NTs) are a family of regulatory proteins known to mediate differentiation and survival of neurons. Particular spatial and temporal patterns of electrical activity, such as coincident activity in the pre- and postsynaptic neurons of a synapse, are widely believed to regulate the efficacy of synaptic transmission. Such use-dependent forms of synaptic plasticity encompass a wide range of structural and functional modifications. Beyond their role as permissive survival factors, neurotrophins also mediate modulation of synaptic transmission and synaptic plasticity [23].

The neurotrophins comprise a family of at least four structurally related proteins: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/neurotrophin-5 (NT-4/5) [24], which exert their effects through two classes of receptors which act in opposite ways: tyrosine kinase receptors (Trk receptors) and pan75 neurotrophin receptor (p75NTR), a member of the tumor necrosis factor α-receptor superfamily. The BDNF-TrkB pathway promotes cell survival and long-term potentiation, while the proBDNF-p75 NTR pathway promotes cell death and long-term depression [25, 26].

Trk receptor signaling activates several small G proteins including Ras, Rap-1, and the Cdc-42-Rac-Rho family, as well as pathways regulated by MAP kinase, PI 3-kinase, and phospholipase C-gamma (PLC-gamma). p75NTR activates a distinct set of signaling pathways within cells that are in some instances synergistic and in other instances antagonistic to those activated by Trk receptors. Several of these are proapoptotic but are suppressed by Trk receptor-initiated signaling [27].

46.5 Neurotrophins, Trk Receptor, and Beta-Arrestin Signaling

Beta-arrestins participate in cellular responses to growth factors in three ways: (1) By facilitating desensitization of GPCRs. GPCR stimulation followed by G protein activation can induce proapoptotic signaling, and beta-arrestins would counteract that signaling simply by virtue of desensitizing the offending receptors [28]. (2) By participating in the process of GPCR endocytosis as clathrin adaptor proteins [13]. The interaction of GPCR-bound arrestin with AP-2 in post-endocytic receptor trafficking has a critical role in the protection of GPCR apoptosis [29]. (3) By functioning as regulators of GPCR-dependent signaling. Indeed, the prevailing view is that beta-arrestins can signal to, for instance, ERK1/2 independently of G proteins [30]. Several studies also reported that beta-arrestin can associate with the PDGFR-β, TrkA, INSR, IGF-1R, and EGFR in either a ligand-dependent or ligand-independent manner [31–35], thereby suggesting either a unique interaction between Trk receptor and beta-arrestin or a potential role for beta-arrestin associated with a GPCR in a complex with the Trk receptor [36].

In case of alternative signaling, beta-arrestins could be involved in apoptosis-related signaling by various receptors. Whether the outcome of beta-arrestin-dependent signaling is pro-survival or proapoptotic depends on the specific configuration of the signaling system in which they act. When beta-arrestin-mediated signaling results in the activation of pro-survival pathways such as ERK and Akt, they provide cytoprotection [28].

Since their initial characterization, beta-arrestins have been thought of as cytoplasmic proteins that could be recruited to the plasma membrane and endocytic compartments following receptor activation. Kang et al. [37] revealed a novel unexpected function of beta-arrestin-1 as a cytoplasm-nucleus messenger in GPRC signaling, showing that stimulation of a member of the GPCR family, delta-opioid receptor, induced the nuclear translocation of beta-arrestin-1. Selectively enriched at specific promoters such as those of the genes encoding cyclin-dependent kinase inhibitor p27 and proto-oncogene protein c-Fos, beta-arrestin-1 facilitated the recruitment of histone acetyltransferase p300, resulting in enhanced local histone H4 acetylation and in transcription of these genes. The authors suggest that beta-arrestin-1 acts as a nuclear scaffold that recruits p300 to the transcription factor,

CREB, which leads to increased acetylation of histone H4 and the reorganization of chromatin, thereby increasing gene expression [37, 38]. CREB orchestrates diverse neurobiological processes including cell differentiation, survival, and plasticity.

46.6 Neurotrophins, Trk Receptor, and Antidepressants

Evidence accumulated over the last decade has laid the grounds for the formulation of new hypotheses that assign the pathophysiological basis of major depression to impaired neuroplasticity [39, 40], which is restored by antidepressant-increased neurogenesis and gliogenesis [41–43]. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) were found to play a critical role in multiple molecular mechanisms [42, 44, 45] and in epigenetic mechanisms that contribute to neuronal plasticity, in relation to the pathophysiology of major depression and to the mechanism of action of antidepressants [42, 46, 47].

Neurotrophic hypotheses of depression and antidepressant efficacy argue that stress and antidepressants chronically regulate mood via opposing actions on intracellular and transcription factors associated with cellular growth, motility, and survival, primarily in the hippocampus [48]. Consistent with this hypothesis, hippocampal CREB overexpression produces an antidepressant-like behavioral phenotype [49], while stress decreases BDNF expression and suppresses local CREB phosphorylation [50]. Enhancing cortical and hippocampal phospho-CREB has been proposed as a common antidepressant mechanism [51], based largely on studies of antidepressant action in rodents [52].

Antidepressants affect prominent signaling cascades involved in neuronal protection and survival: by activation of the MAPK/ERK and Wnt/GSK signaling cascades, they enhance neurotrophic and neuroprotective mechanisms in addition to neurogenesis [53].

Several studies suggest that BDNF may be involved in stress and depressive behavior [54–59] and that the beneficial effects of antidepressants are associated with an upregulation of BDNF expression [60–62].

GDNF, originally identified as a survival factor for midbrain dopaminergic neurons [63], is a member of the transforming growth factor-beta superfamily, essential for neuronal survival, plasticity, and development [64]. It is well documented that ADs increase GDNF levels [65–67], and recently it was found that depressed patients have lower levels of serum GDNF [68, 69] that were increased following antidepressant treatment [69].

CREB plays a major role in the transcription of neurotrophic factors in general [70–72] including BDNF [73] and GDNF [71] and also following antidepressant treatment [45, 74, 75].

46.7 B Cell Lymphoma Protein-2 (Bcl-2)

Bcl-2 is the prototype member of a protein family that functions to suppress apoptosis in a variety of cell systems. Bcl-2 is localized in the endoplasmic reticulum, nuclear envelope, and outer mitochondrial membrane.

Bcl-2 family proteins are known to determine the outcome of an intrinsic apoptotic process initiated by release of cytochrome *C* and apoptotic factors from the mitochondria [76]. Key regulatory proteins in apoptotic events are the Bcl-2 family of proteins, which can either promote cell survival (Bcl-2, Bcl-XL, A1, Mcl-1, Bcl-W) or promote cell death (Bax, Bak, Bcl-XS, Bok) [77]. The relative amount of equilibrium between these pro- and antiapoptotic proteins influences the susceptibility of cells to apoptosis [78]. Activation of the mitochondrial pathway is controlled by activation of Bcl-2 homology 3 (BH3)-only family members such as Bcl-2-associated death promoter homologue (BAD), Bim, Bmf, and Noxa. BAD proapoptotic activity is regulated primarily by phosphorylation at several sites [79, 80]. Survival factors induce BAD phosphorylation via several protein kinase signaling pathways [80] including activation of mitogen-activated protein kinase (MAPK)-ribosomal S6 kinase (RSK) and phosphatidylinositol 3-kinase (PI3K)-AKT [80–82].

Phosphorylated BAD associates with 14-3-3 proteins in the cytoplasm, preventing translocation of BAD to the mitochondria [83] and its interaction with the antiapoptotic proteins Bcl-xL and Bcl-2 [80, 84]. These proteins, freed from BAD, in turn associate with two other proapoptotic proteins, BAX and BAK. Such association prevents aggregation of these proapoptotic proteins on the mitochondrial membrane, stopping cytochrome *C* release and consequently inhibiting apoptosis [76, 84].

46.8 Beta-Arrestin-Mediated Effects on Bcl-2

Proteins of the Bcl-2 family are critical regulators of cytokine withdrawal-induced and stress-induced T cell apoptosis, as well as autonomous death of activated T cells [85–89]. In Bcl-2-deficient mice, T cells are much more susceptible to death, and leukopenia occurs as these mice grow old [90]. Conversely, in Bcl-2-transgenic mice, T cells are more resistant to many apoptotic stimuli, immune responses are prolonged [87, 88], and some autoimmune symptoms have been reported [91].

It has been shown that beta-arrestin-1 regulates transcription by associating with transcriptional cofactors at promoter regions of specific genes in the nucleus [37]. This model for the direct regulation of transcription by beta-arrestin-1 has been reported before for the genes encoding p27 and c-Fos and the associated regulation of growth inhibition in human neuroblastoma cells [37]. After stimulation of a G protein-coupled receptor, specifically the delta-opioid receptor (DOR), beta-arrestin-1 translocates to the nucleus and facilitates the recruitment of p300, a histone acetyltransferase, and subsequent transcription of specific genes [37]. Shi et al. [92] have found that beta-arrestin-1 positively regulated naive and activated CD4+ T cell survival. They described enhanced expression of the proto-oncogene Bcl2 through beta-arrestin-1-dependent regulation of acetylation of histone H4 at the Bcl2 promoter. Mice deficient in the gene encoding beta-arrestin-1 were much more resistant to experimental autoimmune encephalomyelitis, whereas overexpression of beta-arrestin-1 increased the susceptibility to this disease. CD4+ T cells from patients with multiple sclerosis had much higher beta-arrestin-1 expression, and "knockdown" of beta-arrestin-1 by RNA-mediated interference in those cells increased apoptosis induced by cytokine withdrawal. Thus it was demonstrated that beta-arrestin-1 is critical for CD4+ T cell survival and is a factor in susceptibility to autoimmunity.

Ahn et al. [93] have shown that beta-arrestin-2 mediates antiapoptotic signaling through regulation of BAD phosphorylation. Upon stimulation with angiotensin II, the proapoptotic protein BAD is phosphorylated through two signaling pathways, ERK-p90RSK and P13K-AKT. Activation of both pathways appears to be beta-arrestin-2-dependent. Beta-arrestin-2-dependent BAD phosphorylation leads to increases in BAD-14-3-3 association and decreases in the interaction of BAD with Bcl-xL. Such changes in the association of BAD with its partners lead to attenuation of its proapoptotic function.

46.9 Antidepressant Effects on B Cell Lymphoma Protein-2 (Bcl-2)

Mice hippocampal Bcl-2 expression is significantly increased following chronic but not acute treatment with two classes of antidepressants, selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) [94], confirming preliminary findings for SSRIs in the rat [95] and extending the findings that have been reported for mood stabilizers [96]. Chronic treatment with either amitriptyline or venlafaxine increased the intensity of Bcl-2 immunostaining in rat hippocampal mossy fibers [97]. Similarly, chronic administration of fluoxetine, reboxetine, tranylcypromine, and electroconvulsive seizures (ECS) results in upregulated Bcl-2 mRNA expression in rat central nucleus of the amygdala, cingulate, and frontal cortices [98]. Recent studies confirmed the effects of antidepressants of various classes on rat brain Bcl-2 and Bcl-xL [99, 100].

46.10 Antidepressants and Beta-Arrestins

Findings in various research models support a major role for beta-arrestins in the pathophysiology of mood disorders as well as in the mechanism of action of antidepressants [101–104]. Beta-arrestin-1 levels are significantly elevated by serotonin selective reuptake inhibitors (SSRIs), norepinephrine selective reuptake inhibitors (NSRIs), nonselective monoamine reuptake inhibitors, and MAO inhibitor antidepressants in rat cortex and hippocampus. This process becomes significant within 10 days and takes 2–3 weeks to reach maximal increase [105] in accordance with the timetable of clinical improvement. The above findings in animal brain were replicated in measurements in peripheral blood mononuclear cells isolated from anxious and depressed mice [106] and from women suffering from major depression [107]. In a mouse model of anxiety/depression induced by chronic corticosterone, fluoxetine reversed the decreased expression of beta-arrestin-1 and beta-arrestin-2 in the hypothalamus. Mice deficient in the gene for beta-arrestin-2 displayed a reduced response to fluoxetine, suggesting that beta-arrestin signaling is necessary for the antidepressant effects of fluoxetine [108].

Beta-arrestin-1 protein and mRNA levels in mononuclear leukocytes of untreated patients with major depression are significantly lower than those of healthy subjects. The reduction in beta-arrestin-1 measures is significantly correlated with the severity of depressive symptomatology [105, 109]. The low beta-arrestin-1 protein and mRNA levels are alleviated by antidepressant treatment. Normalization of beta-arrestin-1 measures precedes, and thus predicts clinical improvement [109].

Beta-arrestins undergo posttranslational modification by ubiquitination most often in response to 7TMR stimulation. Beta-arrestin ubiquitination plays major roles in receptor binding, endocytosis, and signaling. The time-course of beta-arrestin ubiquitination was found to correlate with the stability of receptor-beta-arrestin interaction: transient interaction is associated with transient ubiquitination (receptors of Class A), while persistent interaction is associated with sustained ubiquitination (receptors of Class B) [110]. Sustained ubiquitination of beta-arrestin is required both to form stable endocytic complexes, termed signalosomes, with the receptor, and to engage active ERK on the signalosomes [110, 111]. Such segregation of ERK activity in the endosomal domains may have at least two effects:

1. Depletion of nuclear phospho-ERK and attenuation of specific transcriptional responses
2. Phosphorylation of specific cytosolic proteins that may initiate alternate cellular responses such as apoptosis, chemotaxis, and cytoskeletal changes or in turn relay signals to the nucleus by phosphorylating other nucleocytoplasmic shuttling kinases

Immunoprecipitation experiments in C6 glioma cells showed that antidepressants were able to increase co-immunoprecipitation of ubiquitin with beta-arrestin-2. Antidepressant-induced increase in beta-arrestin-2 ubiquitination led to its degradation by the proteasomal pathway, as the proteasome inhibitor MG-132 prevented antidepressant-induced decreases in beta-arrestin-2 protein levels [112]. Antidepressants induced selective alterations in leucocyte beta-arrestin-1 and beta-arrestin-2 levels and ubiquitination. The levels of beta-arrestin-1 and beta-arrestin-2 and their ubiquitinated forms in leucocytes of yet untreated patients with depression were significantly decreased in a symptom severity correlated manner compared to control subjects [113]. Antidepressants normalized beta-arrestin-1 and beta-arrestin-2 levels and uncovered novel differences between the two isoforms: (a) while antidepressants normalized ubiquitination of beta-arrestin-1, ubiquitination of beta-arrestin-2 was unaffected and (b) while under antidepressants the ubiquitination extent of beta-arrestin-1 positively correlated with its level, an inverse picture of negative correlation was found between the ubiquitination extent of beta-arrestin-2 and its level [114]. It was concluded that antidepressants may serve as a tool to detect functional differences between the two beta-arrestin isoforms and that through these differential effects, antidepressants can induce specific alterations in alternative cellular signaling.

We hypothesize that the similar antiapoptotic, neurotrophic, and neuroprotective effects of either beta-arrestins or antidepressants in cellular, animal, and human systems may directly result from antidepressant enhancement effects on beta-arrestins.

Beta-arrestins uncouple G protein-coupled receptors [GPCRs] from G proteins and promote their internalization, leading to desensitization and downregulation and serving as negative regulators of GPCR signaling. Antidepressants, as well, uncouple GPCRs from G proteins and promote internalization and downregulation of GPCRs.

GPCR stimulation followed by G protein activation can induce proapoptotic signaling, and beta-arrestins, as well as antidepressants, would counteract that signaling simply by virtue of desensitizing and downregulating the offending receptors. It is thus hypothesized that antidepressants induce these therapeutic effects through their actions on beta-arrestins.

Beta-arrestins also function in alternative signaling cascades as scaffold proteins, interacting with several cytoplasmic proteins and linking GPCRs to intracellular signaling pathways such as the mitogen-activated protein kinase (MAPK) cascade, which includes (1) ERKs, stimulated by growth-related signals; (2) c-Jun *N*-terminal kinases (JNKs), activated by various stress stimuli; and (3) tumor suppressor protein p38, also activated by various stress stimuli.

Classically, activated MAPKs translocate to the nucleus where they phosphorylate and activate transcription factors that regulate programs of transcription leading to proliferation, differentiation, and other cellular processes. Figure 46.1 shows the MAP kinase cascade affected by beta-arrestins acting as scaffolds and as targets for the action of antidepressants. Thus, antiapoptotic, trophic, and protective effects of antidepressants and beta-arrestins may also stem from direct effects of antidepressants on beta-arrestins.

Beta-arrestins translocate from the cytoplasm to the nucleus and associate with transcription factors such as histone acetyltransferase p300 and CREB. These components also interact with regulators of transcription. CREB orchestrates diverse neurobiological processes including cell differentiation, survival, and plasticity. Enhancing cortical and hippocampal P-CREB has been proposed as a common antidepressant mechanism. In addition, antidepressants elevate neurotrophins expression in a beta-arrestin-1-dependent, CREB interactive pathway [114]. Thus, it is suggested that antidepressant effects on CREB can be explained by their interaction with beta-arrestins.

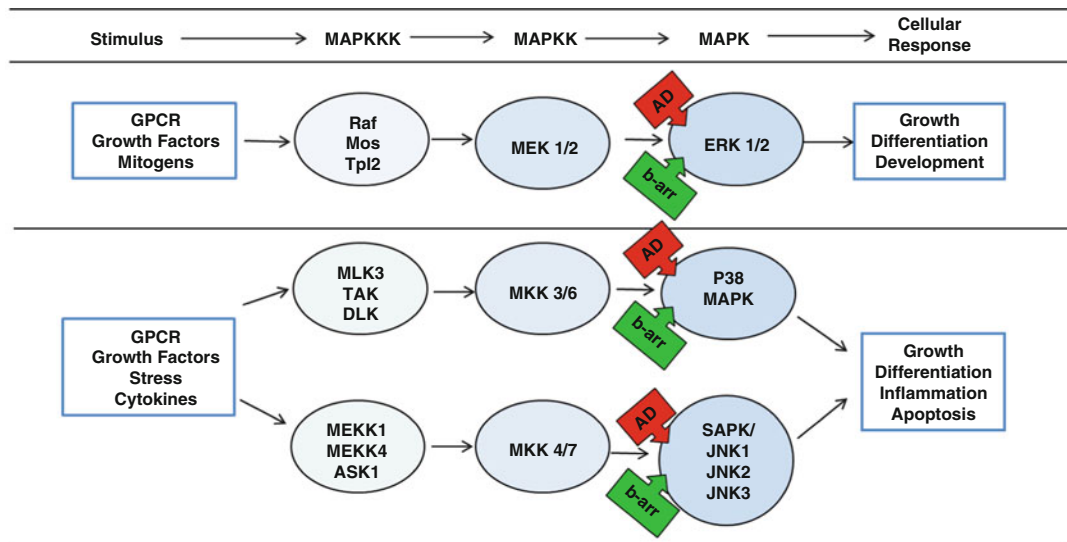

Fig. 46.1 The MAP kinase cascade: scaffolding roles for beta-arrestins and targets for antidepressants. *AD* antidepressants, *b-arr* beta-arrestin

It is currently unknown whether the effects of antidepressants on beta-arrestins described in the present review reflect a direct interaction of beta-arrestins as molecular targets for antidepressants or are secondary to antidepressant effects elsewhere in the intricate signaling pathways. Similarly, it is still not known whether the plethora of effects of antidepressants on signal transduction elements (whereby beta-arrestins serve as signaling scaffold proteins) and transcription factors and cofactors (whereby beta-arrestins mediate transcription regulation) is directly related to the antidepressant effects on beta-arrestins as their target sites. In any case, the emergence of G protein-independent signaling pathways, through beta-arrestins, changes the way in which GPCR signaling is evaluated, from a cell biological to a pharmaceutical perspective, and raises the possibility for the development of pathway-specific therapeutics, e.g., antidepressant medications targeting beta-arrestin regulatory and signaling proteins having antiapoptotic, neurotrophic, and neuroprotective therapeutic effects.

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Antidepressant Drugs and Phosphodiesterases

47

Zhuoyou Chen*, Xifei Yang*, Ying Xu, and Han-Ting Zhang

47.1 Background

Fluoxetine (Prozac) is the antidepressant drug that is considered to be the first-line medication in the early 1900s that could decrease depressive blues for good. However, an increasing number of studies have demonstrated that these drugs work for only a minority of people. More importantly, it has also been shown that these antidepressants may worsen the depressed patients' condition. The search of antidepressants focuses on various chemicals targeting serotonin. Fluoxetine is an antidepressant that is known as selective serotonin reuptake inhibitors (SSRIs). The general action of this drug is to increase the level of those so-called "feel-good" chemicals (such as BDNF) in the brain.

According to a recent research published in the journal *Frontiers in Evolutionary Psychology*, it has been suggested that serotonin likely acts as a chemical Swiss Army knife, playing a vital role in our brain and body. Therefore, alteration of serotonin levels may cause a massive and unwanted impact. It has been shown that changes in serotonin levels may lead to digestive problems or even early deaths in older people, according to the studies by the lead researcher Paul Andrews.

Previous research has shown that drugs affecting serotonin have little effects on most people with mild depression and are less effective for most of the people who are severely depressed. Psychologist Irving Kirsch has discovered that placebo pills have better effects than SSRIs for most of depressed patients. A research on Danish children in 2010 revealed a subtle but very important problem, i.e., babies whose mothers had taken SSRIs during early pregnancy showed an increase in the risk of developing heart diseases. According to the finding by Andrews, serotonin is vital to understanding these side effects and the cause for patients who have finished a course of

*Author contributed equally with all other contributors.

Z. Chen
Department of Neurology,
Changzhou Second People's Hospital,
Changzhou, Jiangsu Province 213003, China

X. Yang
Toxicology Laboratory,
Shenzhen Center for Disease Control and Prevention,
Shenzhen, Guangdong Province 518055, China

Y. Xu, MD, PhD (✉)
Department of Pharmaceutical Sciences,
School of Pharmacy and Pharmaceutical Sciences,
State University of New York at Buffalo,
Buffalo, NY 14214, USA
e-mail: yxu9@buffalo.edu

H.-T. Zhang, MD, PhD (✉)
Departments of Behavioral Medicine and Psychiatry,
West Virginia University Health Sciences Center,
Morgantown, WV 26506, USA

Department of Physiology and Pharmacology,
West Virginia University Health Sciences Center,
Morgantown, WV, USA
e-mail: hzhang@hsc.wvu.edu

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SSRI drugs and develop continuous and more severe depression. It has been suggested that the brain is disturbed by SSRI antidepressants, leaving the patient even greater depression than before. On the other hand, during the long-term usage, the brain lowers its levels of serotonin production than before when a patient stops taking SSRIs; however, it also changes the way receptors in the brain respond to serotonin, making the brain less sensitive to the chemical. Although these changes are believed to be temporary, its subsequent reflection may last for 2 years.

More interestingly, three recent studies referred by Andrews' review suggest that non-antidepressant users are more likely to live longer than elderly antidepressant users when other important variables are also taken into account. One recent study reported that the mortality rate of patients who were given SSRIs was significantly higher than those without medication. Andrews also mentioned that serotonin is an ancient chemical that regulates many different processes and destruction of these processes may cause adverse effects.

When it comes to the reason for why the drugs work only for some patients, scientists have now come up with an explanation. The depression is caused not only by the lack of feel-good serotonin but also by the failure of new cells in specific brain areas. Research shows that SSRIs are not beneficial for patients whose hippocampus has lost the ability to produce new cells as the hippocampus is responsible for regulating mood and memory.

47.2 Treating with Sertraline, Escitalopram, or Venlafaxine Could Change BDNF Serum Levels in Patients

47.2.1 Introduction of BDNF

It has been increasingly appreciated that neurotrophin plays an important role in the brain. Brain-derived neurotrophic factor (BDNF) emerges as a key neurotrophin that regulates neuronal function [1]. It is now well accepted that BDNF not only affects neuronal outgrowth, synaptic connectivity, and neuronal repair [2] but also is responsible for regulating activity-dependent synaptic plasticity [3]. Previous preclinical research has suggested that stress decreases the expression of BDNF in the hippocampus of rats [4, 5]; BDNF is also associated with the pathophysiology of psychiatric disorders in patients with major depression [6]. After a single direct infusion of BDNF into bilateral hippocampi, rats display an antidepressant-like effect on forced-swim behavior [7]. Serum BDNF levels are lower in patients with major depressive disorder (MDD), which is negatively correlated with depression rating scores [8, 9]. BDNF mRNA levels [10] are increased in rats following chronic administration of antidepressant drugs or electroconvulsive treatment. A growing body of evidence has suggested the clinical latency of antidepressant drugs [11] is associated with the development of neuroadaptive changes, involving neurotransmitter [12–14] receptors and their downstream signaling components such as transcription factors, neurotrophins, and other intracellular signaling molecules. Depending on the pharmacological mechanisms and length of therapy, preclinical studies have extensively investigated the effect of antidepressant treatment on BDNF regulation. For instance, BDNF mRNA is found to be increased in the hippocampus of rats after 14 days, instead of 7 days, after the treatment with fluoxetine. Moreover, fluoxetine treatment has been found to induce increases in BDNF protein levels in the pyramidal cell layer of hippocampal CA2 and CA3 subregions after 21 days of treatment of rats [15]. Venlafaxine is also found to cause a significant increase in BDNF mRNA in the granular cell layer of the hippocampus of rats after 7, 14, or 21 days of treatment compared to imipramine, which needs 14 and 21 days of treatment. Nonetheless, fluoxetine does not alter BDNF mRNA levels regardless of long- or short-term treatment. There are many influencing factors that might affect the serum BDNF levels; these include the specificity of brain regions and/or cell types, length of treatment, and pharmacological characteristics of drugs. Preclinical studies have shown that serum BDNF concentrations are positively related to

cortical BDNF levels. Therefore, it is likely that BDNF production and/or clearance in the brain is correlated to its serum form. In humans, several classes of antidepressant medications with different mechanisms of action have been found to cause increases in serum BDNF levels after short and intermediate duration of treatment in patients with MDD.

Recent studies have shown that BDNF also plays a role in the pathophysiology of depression and the activity of antidepressant drugs. Serum BDNF levels are lower in depressed patients, but increased in response to antidepressant treatment. However, it is still unknown how BDNF responds to different classes of antidepressant drugs. Serum BDNF levels were assessed in 21 patients with major depressive episode treated with sertraline, escitalopram, or venlafaxine and 20 healthy controls. Serum samples were collected between 10 a.m. and 12 p.m. at baseline after 5 weeks or 6 months of treatment. The BDNF levels were measured by immunoassay. The severity of symptoms and responses to treatment were assessed by the Hamilton rating scales for depression (HRSD). Baseline serum BDNF levels were significantly lower in depressed patients compared to healthy controls. While sertraline increased BDNF levels after 5 weeks and 6 months of treatment, venlafaxine increased BDNF levels only after 6 months. Escitalopram does not affect BDNF levels at either time point; however, a significant negative association was found between increased BDNF levels in percentage and decreased HRSD scores also in percentage after 6 months of treatment [12]. Taken together, these results suggest that different antidepressant drugs have variable effects on serum BDNF levels. This is true even though the three different drugs were equally effective in relieving symptoms of depression.

47.2.2 Sertraline, Venlafaxine, and Escitalopram Treatment

Studies reveal no distinct differences in the baselines among the three different antidepressant treatment groups with respect to gender distribution, BDNF serum levels, age, comorbidity, and HRSD scores when the demographic and clinical characteristics of the involved samples are taken into account. However, a significant difference of BDNF levels and age between the treatment and control groups was observed. After titration, the dose of antidepressants was maintained stable, and none of the study subjects was dropped out throughout the study.

47.2.3 Analysis of BDNF Changes After Treatment

Antidepressant treatment and duration of administration (5 weeks and 6 months) have been compared in terms of age, gender, baseline BDNF, and HRSD scores. Overall comparison showed significantly different BDNF serum levels between treatment groups after 5 weeks and 6 months of administration. For sertraline-treated patients, a significant increase in BDNF levels was observed after 5 weeks and 6 months of treatment. For venlafaxine-treated patient, a significant increase in BDNF levels was noticed only after 6 months of treatment. In contrast, no significant increase in BDNF levels was observed among individuals treated with escitalopram at any time point. The pairwise comparison for changes with baseline BDNF values, HRSD scores, gender, and age as covariates showed higher serum levels of BDNF for the sertraline-treated patients when compared to venlafaxine treatment group only after 5 weeks, whereas, the sertraline treatment group showed higher levels of serum BDNF when compared to escitalopram both at 5 weeks and 6 months. Moreover, significant differences between venlafaxine- and escitalopram-treated patients were observed only at 6 months, suggesting venlafaxine is to be with greater values for serum BDNF. A secondary analysis was performed to evaluate the persistence of a significant time effect, when the three treatments were grouped together. In fact, the results showed a significant time effect as a result of a significant increase in BDNF at 6 months when compared to baseline and at 6 months when compared to 5 weeks.

47.2.4 Conclusion

Patients with depressive episode show lower BDNF serum levels when compared to healthy controls. This result supports the findings of several previous studies that BDNF levels are significantly lower in depressed patients at baseline relative to normal controls [16–19]. In addition, we found that a significant increase in serum BDNF levels is observed after either 5 weeks of treatment with only sertraline or 6 months of co-treatment of sertraline and venlafaxine. Similar discovery was also reported by Gervasoni [16], who observed a significant increase in BDNF serum levels after 13 weeks (±6 weeks) on average of treatment with SSRI or tricyclic antidepressants in 26 patients with unipolar or bipolar depression. The study by Gonul et al. [17] also showed that the serum BDNF levels are significantly increased after 8 weeks of treatment with venlafaxine, sertraline, fluoxetine, or paroxetine in 33 patients with major depressive disorder. Finally, Huang et al. [20] have further confirmed that the levels of serum BDNF are increased when patients are treated with fluoxetine, paroxetine, venlafaxine, or mirtazapine after 4 weeks.

There are few studies showing that the level of BDNF in people affected by major depression has long-term effects on antidepressants [18]. Aydemir reported that the peripheral serum BDNF levels [19] are increased in ten patients with major depression after 3 months of treatment with venlafaxine. Our data also suggest that patients treated with SSRI or tricyclic antidepressants have increased peripheral BDNF levels. In a 12-month prospective study, patients were treated with sertraline and venlafaxine, which produce a long-term effect on peripheral BDNF levels. These findings suggest the possibility that BDNF plays an important role in the maintenance of antidepressant action. Nonetheless, the major finding of our study is how the BDNF serum changes among sertraline, venlafaxine, and escitalopram. The sertraline-treated patients showed an increase in serum BDNF levels at 5 weeks, the magnitude of which remains similar until 6 months. However, patients treated with venlafaxine showed an increase in BDNF serum levels only at 6 months but not at 5 weeks. The new finding in depressed patients shows really normalized serum BDNF levels after 5 weeks and 6 months of treatment of sertraline. Nevertheless, venlafaxine shows an impact on serum BDNF levels only after long-term treatment. These findings are consistent with the study carried out by Yoshimura [21], who showed that the serum levels of BDNF in 42 patients with major depression episode are significantly increased after 8 weeks of treatment with milnacipran. No significant increase in BDNF values was noted for escitalopram-treated patients at any time.

Aydemir has demonstrated an increase in serum BDNF levels in 20 depressed women after 6 weeks of treatment with escitalopram. Four females and three males were recruited in our escitalopram-treated group. It is interesting that different genders might have given rise to different outcomes; however, the relatively small sample size doesn't allow any further statistical analysis. Nevertheless, for the escitalopram-treated group, the mean baseline of BDNF levels was higher than any other groups, and the higher proportion of escitalopram-treated patients were mildly depressed.

These subtle differences are likely due to the relatively small sample size. It is notable, however, that the BDNF levels were lower than the other groups in the escitalopram-treated samples. Furthermore, the extent of the increased BDNF levels is different between sertraline and venlafaxine groups, despite the fact that different treatments seen in both groups might be related to the differential effect on a large number of molecular mechanisms that contribute to BDNF production. In fact, many preclinical studies have shown that different antidepressant medications may have various effects on BDNF transcription, release, activation of receptors, and secondary messengers. Recent studies have demonstrated that BDNF mRNA levels in different brain regions of rats were affected by duloxetine, another serotonergic/noradrenergic reuptake inhibitor depending on the length of treatment. For example, the increased levels of BDNF mRNA are seen in the prefrontal, entorhinal, and parietal cortex, but not in the hippocampus after chronic administration (3 weeks)

of duloxetine; in contrast, acute administration of this drug did not produce any significant change [22]. These results are confirmed by the study of Mannari et al., in which the authors found that chronic administration of duloxetine produced more BDNF in the prefrontal cortex of rats when compared to acute duloxetine. Only chronic administration of duloxetine at high dose produces more BDNF in cerebrospinal fluid of rodents; however, repeated administration of duloxetine at the highest concentration has no effect on the total BDNF levels either in serum or in plasma. TrkB is a high-affinity receptor for BDNF and its autophosphorylation results in BDNF activation and serves as an indirect signal of BDNF neuronal release. TrkB protein levels and TrkB autophosphorylation upon acute or chronic treatment with fluoxetine, citalopram, clomipramine, imipramine, reboxetine, or moclobemide have been tested in mice. All these antidepressants caused significant increase in TrkB autophosphorylation 30–60 min after a single injection in the anterior cingulate cortex and hippocampus of mice; however, none of the acute treatments influenced the total TrkB protein expression levels [23]. The finding that serum BDNF concentrations are variously subjected to an intricate and complex cascade of molecular mechanisms has been reported by a recent study. Sertraline affinity for r1-receptor might also explain the rapid action in increasing serum BDNF levels [24]. In a preclinical study, the interaction between venlafaxine and r1-receptors has also been demonstrated; the effects of venlafaxine on forced-swim behavior in rats were counteracted by an r1-antagonist [25]. Sigma receptors were first described as opiate receptors; however, this was ultimately disproven. Sigma agonists have been shown to possess antidepressant properties, which may occur via modulation of serotonergic transmission [26]. No r1-receptor affinity has been demonstrated for escitalopram [27], after 4 days of treatment with escitalopram, BDNF, and TrkB, and mRNA levels in the hippocampus and prefrontal cortex of juvenile rats are significantly increased [28]. This finding illustrates how a highly selective serotonin reuptake inhibitor is able to produce significant changes in BDNF.

In conclusion, these studies corroborate the existing literature on the positive increment of serum BDNF levels after long-term treatment with antidepressants. Although these findings are interesting, the current knowledge is still very limited regarding the different effects of three different antidepressants on serum BDNF levels.

47.3 Short-Term SSRI Treatment Normalizes Amygdala Hyperactivity in Depressed Patients

47.3.1 Introduction

Many studies show that in the processing of emotional information, patients with acute depression manifest a range of negative biases which likely contribute to the etiology and the maintenance of the depressed state [29]. For example, in contrast to healthy controls, negative or self-related emotional information in memory tasks [30] are selectively recalled by depressed patients who demonstrate negatively biased perception of key social signals such as emotional facial expressions [31, 32]. Such biases have been associated with aberrant responses across a network of neural areas involved in emotional processing, including an increased response of the amygdala to negative facial expressions in depressed patients compared to matched controls [33–35].

It has been recently proposed that the early reversal of these negative emotional biases [36] may mediate the therapeutic effect of antidepressant drugs. For instance, healthy volunteers may diminish the recognition of negative emotional faces after 7 days of treatment with the SSRI citalopram or the selective noradrenaline reuptake inhibitor reboxetine in addition to increased recall of positive self-referential words and attenuated amygdala response to fearful faces as measured by functional magnetic [37] resonance imaging. Similarly, during SSRI treatment [33, 38], depressed patients may also show attenuation of the amygdala response to sad and fearful facial expression. Nevertheless, in the present studies, amygdala responses were measured at baseline

and the therapeutic response had become established and subjective mood improved after 8 weeks of SSRI treatment. Therefore, it is conceivable that remediation of negative biases in depressed patients could be a consequence of depression resolution instead of an early effect of antidepressants during the treatment. Furthermore, it is difficult to exclude changes caused by order effects during repeated scanning or nonspecific effects of treatment without placebo controls.

In this latter study, we investigated the effects of short-term SSRI treatment in depressed patients on the neural response to fearful faces prior to clinical improvement in mood. Altogether, 42 depressed patients receiving SSRI treatment (10 mg escitalopram daily) or placebo were recruited in a randomized and parallel group design. The neural response to fearful and happy faces was measured on day 7 of treatment using functional magnetic resonance imaging. A group of healthy controls was imaged in the same way.

47.3.2 Demographic Data and Clinical Ratings and the Functional MRI Results

47.3.2.1 Demographic Data and Clinical Ratings

Clinical studies have shown that the patients randomized to receive escitalopram or placebo were well matched for age and gender; however, the depressed patients without any treatment indicated slightly higher proportion of female participants. Nonetheless, after 7 days of treatment with escitalopram and placebo, the patients were moderately depressed at baseline and a similar decline in HAMD scores. Mean (±S.E.M) scores of depressed patients were significantly lower in the placebo group. Similarly, there was no significant difference in terms of the decline in scores on the BDI or state anxiety ratings between the two groups.

47.3.2.2 Functional MRI Results

A two-way repeated-measures ANOVA model in FSL comparing hemodynamic differences across groups, such as Esc, Pla, and Con and conditions (fear and happy) revealed a significant interaction among groups and conditions in the right amygdala. To decompose this significant interaction, we compared drug-treated patients with Pla patients and drug-treated patients, Pla depressed patients, and controls within this amygdala region using FSL. These simple main effect analyses revealed greater BOLD response to fear in Pla vs. Esc and Pla vs. Con. By contrast, the BOLD response to happy faces was similar across groups. Adding resting perfusion maps as a voxelwise covariate has no effect on right amygdala BOLD response to differences between groups. Similarly, no significant differences were observed in resting brain perfusion either at the whole-brain level or using a ROI-based analysis of the right amygdala.

Consistent with the prediction that these changes in neural response do not relate to immediate changes in mood or anxiety with antidepressant treatment, there were no significant associations between change in HAMD, BDI, and state anxiety with the response in the amygdala to fear in the Esc group.

47.3.3 Attenuation in Amygdala Response Is Caused by SSRI Administration Rather than Changes in Clinical State

Many studies suggested that the attenuation in amygdala response was caused by SSRI administration rather than changes in clinical state. It is, therefore, difficult to distinguish placebo- and drug-treated groups at this point. Consistently, there was no association between the magnitude of the amygdala response to fearful facial expressions and the change in ratings during treatment. Finally, the differences in amygdala response between groups during SSRI treatment were not due to nonspecific placebo effects or an effect of repeated fMRI examination. Healthy volunteers in a similar study show similar results to SSRI-treated depressed patients, in which 7 days of treatment with the SSRI citalopram diminished

amygdala responses to fearful facial expressions [39]. The latter study used backward masking of emotional faces, which were presented below the threshold of conscious awareness. While the nature of the task could still be considered "implicit," however, the emotional stimulus presentations used in the present investigation were not masked, therefore, likely to activate limbic regions implicated in rapid automatic appraisal of emotional stimuli [40].

The consequences of antidepressant treatment in depressed patients are also consistent with the findings in our previous studies, in which fMRI was used to examine neural responses to sad and fearful faces during the periods of SSRI administration. For example, it has been reported that sertraline at doses of approximately 100 mg daily for 8 weeks lowered the amygdala responses to masked fearful faces [33]. With the similar treatment regimen, it was found in another study that sertraline treatment bilaterally in the amygdala reduced responses to masked fearful faces relative to baseline in patients treated with sertraline in the right amygdala. Fu et al. found that treatment with fluoxetine (20 mg daily) for 8 weeks in depressed patients decreases responses in the left amygdala to implicitly presented sad faces of increasing emotional intensity. In these studies, however, most patients had used the time of the second fMRI scan to improve clinically significant symptoms. The neurochemical mechanisms by which SSRIs lower amygdala responses to fear are not presently clear. In animals, application of serotonin to amygdala interneurons increases release of c-aminobutyric acid, by which the amygdala receives a substantial serotonergic input from the dorsal raphe. Thus, the changes in serotonin neurotransmission are well placed to alter the excitability of internal amygdala networks with the modified activity of glutamatergic projection neurons [41]. However, the location with regard to different neuronal types as well as serotonin receptor subtypes is not fully elucidated.

In our antidepressant studies in healthy volunteers, using the same or similar paradigms, changes in fear processing have been most apparent in the right amygdala [39, 42, 43]. Escitalopram treatment could lower fear responses relative to placebo in the right amygdala. Nevertheless, it is possible that a number of additional factors, such as the number of trials presented and the nature of the task, had significant influence on the detection of laterality. Moreover, the investigations of antidepressants in depressed patients outlined above do not apparently reveal a consistent pattern in this respect, which is the best replicated effect seen early and late in treatment, showing the specific effect of SSRI treatment [44] in response to negative facial expressions. However, some studies have also revealed increased amygdala responses to happy facial expressions after antidepressant treatment [45], which is not seen here. The reason for this discrepancy is unclear, but is likely due to the differences between drug treatments, patient or volunteer characteristics, and explicit or implicit emotional paradigms. Nonetheless, these findings are all in line with a diminishment in the relative processing of negative versus positive stimuli at the level of the amygdala.

47.4 Predicted Therapeutic Response to SSRI Antidepressants: Pre- and Posttreatment Findings

47.4.1 Introduction

There is an increasing number of evidence that differences in neuroimaging, electrophysiological (EEG), and neurocognitive measurements among depressed patients are predictive for therapeutic responses to antidepressant drugs. The recent studies replicated prior findings of pretreatment differences between nonresponders in EEG alpha power or asymmetry and SSRI responders and nonresponders, in which whether these differences normalize or are stable after treatment was examined.

There are a growing number of antidepressant drugs with distinct pharmacological profiles that are available for treating depression; however,

whether a patient benefits from a specific medication in clinic is still unknown. Studies have found that pretreatment differences among depressed patients identified using neuroimaging [46–49], neurocognitive [50–52], and electrophysiological [53–57] measures are related to subsequent clinical responses to antidepressant drugs. Several studies have suggested that the ability of quantitative electroencephalographical (qEEG) measures of "spontaneous" brain electrical activity in a resting state is an effective way to predict responses to SSRIs or other antidepressant drugs. Ulrich et al. [58] reported that increased posterior alpha in patients could eventually respond to amitriptyline, suggesting that there might be two subtypes of depression, which have different pathophysiology and antidepressant response. Knott et al. [60] found that depressed patients who showed response to imipramine have more alpha and less theta when compared to nonresponders. Similarly, Prichep et al. [59] confirmed that there are two subgroups of patients having an obsessive-compulsive disorder; one showed relative excess of alpha response to SSRIs, and the other showed relatively increased theta with little response to treatment. Cook et al. [61] found that there were no pretreatment differences between fluoxetine responders and nonresponders in theta; however, there was indeed a difference in "cordance," a measurement based on a form of surface Laplacian [62]. Recently, it has been found that fluoxetine nonresponders show greater alpha power (less activity) in the left hemisphere when compared to the right; however, fluoxetine responders exhibit an opposite asymmetry, showing the difference in alpha asymmetry between fluoxetine responders and nonresponders, which is predicted on the basis of dichotic listening findings [63]. There are two studies using tomographic (LORETA) analyses to infer theta current density in specific brain regions. Mulert et al. [64] reported that the increased pretreatment activity was located in rostral anterior cingulate cortex (ACC) in responders of depressed patients who were treated with citalopram or reboxetine. Similarly, Pizzagalli et al. localized pretreatment theta increases to rostral ACC in responders of nortriptyline. These findings suggest that pretreatment alpha or theta measurement might be of value as predictors of clinical response to SSRIs or other antidepressant drugs.

47.4.2 Results

47.4.2.1 Pretreatment Session in SSRI Responders, Nonresponders, and Control Subjects

There was a difference in overall alpha power among groups (SSRI responders, nonresponders, and control subjects); for instance, responders have significant difference when compared with control subjects. The enhanced alpha in responders was most evident at posterior sites where alpha is typically largest. Therefore, the significant difference of alpha could only be found at occipital sites among groups after separately examining at frontal, central, parietal, and occipital regions. It is well known that responders differ significantly from nonresponders in alpha asymmetry. More specifically, responders show greater alpha (less activity) over the right than the left occipital sites, and nonresponders tend to show the opposite asymmetry. Responders had significantly greater alpha at occipital sites and also tended to have greater alpha than nonresponders when compared with control subjects. There was also a trend for the predicted difference in alpha asymmetry among groups at occipital sites, although only approaching a conventional level of significance. The alpha difference between responders and nonresponders had a relatively large effective size. Healthy control subjects had essentially no alpha asymmetry and did not differ significantly from either patient group.

There was also a difference among the responder, nonresponder, and control groups in theta power at occipital sites. Responders had significantly greater theta power when compared with control subjects, whereas nonresponders did not differ significantly from the other groups. The difference in theta between responders and control subjects was also significant at the midline occipital site rather than the other midline

sites. Although there is a difference in theta across left and right occipital sites, no significant difference is observed among groups in the theta asymmetry.

47.4.2.2 Pretreatment Versus Fluoxetine Treatment Sessions in Responders and Nonresponders

Group differences in alpha were most evident at the occipital site, suggesting that the pre- and posttreatment session responders tended to have greater alpha power than nonresponders. Difference in alpha between responders and nonresponders had no change. Therefore, there was no significant change in alpha power across sessions. Many studies suggest that the left occipital site show less alpha relative to the right in responders and nonresponders show no significant asymmetry in the opposite direction. There was a significant group difference in occipital alpha asymmetry between the fluoxetine pretreatment and posttreatment sessions. Test-retest correlations showed that alpha power of fluoxetine pretreatment and treatment sessions is ranging from $r=0.92$ at frontal to $r=0.97$ at occipital sites. Test-retest correlations were lower for alpha asymmetry, being $r=0.63$ at frontal, $r=0.56$ at central, $r=0.73$ at parietal, and $r=0.86$ at occipital sites.

47.4.2.3 Prediction of Treatment Response

We examined the value of alpha power and asymmetry for predicting treatment response at occipital sites. In our prior studies, both alpha power and asymmetry showed reasonable positive predictive value but less negative predictive value. The small magnitude of this correlation suggests that each provides somewhat independent information for predicting treatment responses, which are positively correlated between the alpha power and asymmetry. Whether the predictive value may improve is worth of evaluation if the alpha power and asymmetry are combined. Therefore, there was agreement as to the prediction of treatment response across the alpha power and asymmetry measures in two-thirds of the patients. In these cases, the agreement across measures may improve the sensitivity and negative predictive values to 83.3 % and 80.0 %, respectively.

47.4.3 Discussion

Patients who responded to fluoxetine have more EEG alpha than healthy controls; in contrast, nonresponders have no difference compared to control subjects. As shown in our previous studies, an excess of alpha was observed in patients with affective disorders who had responses to antidepressant treatment or patients with an obsessive-compulsive disorder responsive to SSRIs [59] and secondary treatment with an anticonvulsant or lithium [58, 65]. Given the inverse relation of alpha power and cortical activity, our findings indicate that this increased alpha is evident in depressed patients [66–68]. Reduced cortical activity was viewed as evidence in a subgroup of depressed patients who were responsive to SSRIs; it is a predictor for those who benefit most from this treatment. In contrast, depressed patients who respond to tricyclic antidepressant drugs also show increased alpha [58, 60]. This, however, might not be specific to SSRI antidepressant drugs. Decreased alpha has been found to be predictive for improvement in mood after cognitive restructuring [69]. The increased occipital power in fluoxetine responders was found in the theta band, suggesting that this qEEG difference is included somewhat lower frequencies as well in addition to alpha. It is noteworthy that a distinct spectral component with a peak that spans both the alpha and theta bands is identifiable in the resting EEG with a reference-free approach [62]. In accordance with our previous findings, the SSRI responders also differ from nonresponders in their alpha asymmetry. At occipital sites, responders show an asymmetry indicative of greater activity over left than right hemisphere, where alpha is largest, whereas nonresponders have the opposite direction of asymmetry. The SSRI responders to left over right hemisphere activity are seen in asymmetry in

dichotic listening studies [60] for the patients with a "pure" MDD in EEG studies [58, 59]. Although the specificity of the posterior alpha asymmetry to SSRI responders is unknown, alpha asymmetry at the frontal but not posterior sites may predict mood improvement after cognitive restructuring [58]. In the present study, frontal alpha asymmetry shows no difference between fluoxetine responders and nonresponders. After 12 weeks of treatment with fluoxetine, there is no change in alpha power and asymmetry. It has been previously reported that the retest periods over 1 year in healthy adults are comparable to those with extremely high test-retest correlations for alpha power in depressed patients, in which alpha power is a stable and heritable trait [65]. Given that recovered depressed patients in a euthymic state show an elevated alpha power, Pollock and Schneider [66] hypothesize that depressed patients in a euthymic state elevation alpha power might probably reflect a trait difference in a subgroup of depressed patients. Our findings suggest that this trait is present in patients who show favorable response to SSRI.

47.5 Introduction of PDEs and Depression

Phosphodiesterases (PDEs) are a superfamily of enzymes that degrade cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP) [70–72]. There are 11 PDE families identified to date, many of which have different splice variants [73, 74]. The cGMP-specific PDEs include PDE5, PDE6, and PDE9, whereas PDE4, PDE7, and PDE8 are considered to be cAMP specific. The other PDEs including PDE1, PDE2, PDE3, PDE10, and PDE11 use both cyclic nucleotides [75]. There are a vast array of pharmacological processes that are influenced by PDEs, including ion channel function, pro-inflammatory mediator production and action, muscle contraction, glycogenolysis, gluconeogenesis [76], learning, differentiation, apoptosis, and lipogenesis. Given the essential regulators of cyclic nucleotide signaling with diverse physiological functions, PDEs are considered as the important drug targets for treatment of various diseases, such as heart failure, depression, asthma, inflammation, and erectile dysfunction [71, 75, 77, 78].

cAMP and cGMP, ubiquitous second messengers, have significant impact on various extracellular signals, such as hormones, light, and neurotransmitters. ATP and GTP under the catalytic reactions of adenylyl cyclase and guanylyl cyclase, respectively, are composed of cyclic nucleotides. Cell-surface receptors such as β-adrenoreceptor and prostaglandin E2 can also be activated indirectly [79]. Adenylyl cyclase can be directly activated by forskolin and guanylyl cyclase by nitric oxide (NO).

Increased intracellular signaling can activate its target enzymes, protein kinase G (PKG), and/or protein kinase A (PKA). Substrates such as ion channels, contractile proteins, and transcription factors are phosphorylated by these protein kinases, which play an important role in regulating key cellular functions. Phosphorylation not only alters the activity of these substrates but also changes cellular activity. Obviously, alteration of the rate of cyclic nucleotide formation or degradation may change the activation state of these signal pathways. The discussion will focus on PDE4 given that it is responsible for the majority of cAMP hydrolysis in neurons [80].

47.5.1 Overview of PDE4

Individual isoforms of PDE4 consist of four subtypes (PDE4-A, B, C and D), which are encoded by four distinct genes. These isoforms are believed to have an important influence on conferring isoform-specific targeting to signaling complexes [81] and distinct intracellular sites, underpinning compartmentalization of cAMP signaling. The use of distinct promoters for isoforms [82–85] together with control of mRNA stability [86] allows cells to exhibit specific patterns of PDE4 isoform expression.

PDE4 isoforms are divided into four groups. While short isoforms lack UCR1, super-short isoforms have a truncated UCR2 and lack UCR1; dead-short isoforms lack both UCR1 and UCR2

and have a truncated, inactive catalytic unit at both N- and C-termini [87]. For long isoforms, which locate between the isoform-specific N-terminal region and the catalytic unit, they have two unique regulatory domains, namely, upstream conserved region 1 (UCR1) and upstream conserved region 2 (UCR2). PKA and ERK phosphorylation plays the key functional roles in the regulation of PDE4 activity. Given that UCR1 contains a PKA phosphorylation [81] site, this allows long PDE4 isoforms to be activated [88], thus increasing the cellular capacity for cAMP degradation [88, 89] and providing an important part of the cellular desensitization machinery for cAMP signaling. The catalytic unit of all isoforms, except that of the PDE4A subfamily, can be phosphorylated by ERK, which plays a role in regulating PDE4 activity. More specifically, the UCR modules orchestrate the functional outcome of ERK [90–92] phosphorylation, resulting in activation of short isoforms and inhibition of long isoforms, but no change in super-short isoforms. Changes in the relative levels of long isoforms compared with short isoforms radically affect the consequences of ERK activation of cAMP signaling. This is enhanced in monocyte to macrophage differentiation. Short PDE4B predominates in macrophages, whereas long PDE4D isoforms predominate in monocytes. Therefore, pro-inflammatory mediators elicit an overall increase in PDE4 activity in macrophages, but they trigger an overall decrease in PDE4 activity in monocytes. Inhibition of long PDE4 isoforms by ERK phosphorylation is transient in response to an increase in cAMP, causing PKA to phosphorylate UCR1 that provides feedback regulation of PDE4 activity, thereby overcoming the inhibitory effect of ERK phosphorylation. The transient rise in cAMP is elicited by basic ERK activation as a result of its action on long PDE4 isoforms. The ability of PDE4 isoforms to bind ERK at specific docking sites, and to respond in very different ways to phosphorylation by ERK, clearly exemplifies the importance of PDE4 isoforms in transducing the actions of ERK on cAMP signaling.

In addition to their regulatory roles, the scaffolding protein myomegalin, the immunophilin XAP2, and phosphatidic acid interact through the interfaces of UCR1 and UCR2. Moreover, UCR1 and UCR2 appear capable of interacting with each other. They not only occur internally within a particular PDE4 molecule but also offer a means of facilitating homodimer formation in long isoforms. In the recent studies, it has been shown that the conformational changes in the PDE4 catalytic unit are generated by the interactions between UCR1 and UCR2, which are linked to activity changes seen upon phosphorylation by PKA and ERK, such as rolipram, resulting in consequential responses upon the binding of certain inhibitors.

47.5.2 Structural Biology of PDE4

47.5.2.1 Structure of Catalytic Domain

The crystal structure of the apo catalytic domain of PDE4B reveals a compact α-helical structure consisting of 16 helices, which are divided into three subdomains. The active site is lined with highly conserved residues and forms a deep pocket located at the junction of the three subdomains. Zn^{2+} is coordinated in a binuclear metal ion center, which contains the paired histidines and aspartates and two water molecules in the wider side of the active site. In addition to Zn^{2+}, Mg^{2+} is also located in this binuclear metal ion center, which is coordinated by the same aspartate that coordinates Zn^{2+} as well as five water molecules (one of which bridges Mg^{2+} and Zn^{2+}). This minimal interaction with protein residues indicates that Mg^{2+} makes no difference to structural integrity. Indeed, it seems that chelators, such as EDTA without affecting the proper folding of PDE4 [93, 94], could strip off Mg^{2+}. The active site pocket contains 12 of the 17 conserved residues from the 21 PDE gene family members. There are three clusters of residues in the active site with distinct functions: a hydrophobic clamp, which contains two highly conserved hydrophobic residues which sandwich the planar purine ring involved in substrate binding, and hydrolysis, where a cluster of conserved residues near the di-metal center are responsible for cyclic nucleotide hydrolysis [95]; and nucleotide recognition,

where a cluster of residues determines the orientation of the amide group of an invariant glutamine for selective binding to either cAMP or cGMP.

There are three pockets [96]: a solvent-filled side pocket (S-pocket), a metal-binding pocket (M-pocket), and a pocket containing the purine-selective glutamine and the hydrophobic clamp (Q-pocket) in the active site. The S-pocket mainly consists of hydrophilic amino acids and is filled with a network of water molecules in most of the inhibitor complexes. The M-pocket contains the di-metal ions and highly conserved hydrophobic, polar residues that coordinate the metal ions. The Q-pocket can be subdivided into three distinct areas: a "saddle" formed by the conserved glutamine and the P-clamp and two narrow, but deep, hydrophobic pockets that flank.

47.5.2.2 A "Glutamine Switch" Mechanism for Nucleotide Selectivity

In the PDE superfamily, the amide group of an invariant glutamine switches to another orientation by adopting one orientation to interact with cAMP in the active site upon flipping 180° to interact with cGMP. This so-called glutamine switch [97] is controlled by an intricate network of H-bonds. In PDE4, this glutamine forms a bidentate, which is then locked into the cAMP-specific configuration H-bond with the adenine moiety. This orientation is stabilized by H-bonding to the phenolic hydroxyl group of a nearby tyrosine. There are no supporting residues to anchor the orientation of the key glutamine residue in this PDE, thereby conferring dual specificity for cAMP and cGMP.

47.5.3 Mechanism of Cyclic Nucleotide Hydrolysis

It appears that an invariant aspartate activated by the coordinated Zn^{2+} and Mg^{2+} ions may serve as a general base to deprotonate the bridging water molecule. The resulting hydroxide can act as the nucleophile through an inline associative mechanism [98, 99] to attack phosphorus. The interactions of two phosphoryl oxygen atoms with the metal ions could stable the formation of a penta-coordinate intermediate (or transition state). The O3′ leaving group is found to be H-bonded to the O3′, which is protonated by general acid histidine. PDEs commit four amino acids to the donation of one proton, enhancing the protonation step that plays an important role in the evolution of these enzymes to catalyze the cyclic nucleotide hydrolysis. It has also been suggested that a dissociative mechanism [100] could proceed the hydrolysis, leading to the formation of a metaphosphate-like intermediate, and thus breaking the scissile bond that precedes the nucleophilic attack by the hydroxyl group on the phosphorus and the intermediate could be stabilized by the metal ions.

47.5.4 PDE4 Inhibitors

Theophylline belongs to a family of xanthine derivatives and was the first therapeutically used PDE inhibitor. Although it is considered to be only a weak nonselective PDE inhibitor, there are many different forms of theophylline, including 3-isobutyl-1-methylxanthine (IBMX), arofylline, doxofylline, and cipamfylline. Although a great number of xanthine derivatives have been developed and some of them are under clinical trials or underlaunched, xanthine is still considered to be the kind of PDE4 inhibitors that is relatively weak and generally nonselective. Rolipram, on the other hand, provides the paradigm for a selective PDE4 inhibitor. Many compounds have subsequently been developed, the dialkoxyphenyl (catechol) family of inhibitors being the largest and the best characterized. Some clinical trials of these inhibitors have been used to treat asthma; however, they were discontinued because of narrow therapeutic windows. These include rolipram, mesopram, zardaverine, IC-485, filaminast, and piclamilast. However, there are other PDE4 inhibitors currently in clinical trials, such as atizoram, tetomilast, CC-1088, and ONO-6126. Cilomilast and roflumilast are two most advanced inhibitors in this catechol class, which have completed Phase III clinical trials. While roflumilast has been approved, cilomilast is currently

awaiting for regulatory approval as treatment for asthma and COPD.

47.5.5 PDE4 and Depression

47.5.5.1 PDE4 Mediates the Effects of Several Antidepressants

PDE4 selectively hydrolyzes cAMP in the brain and results in the inactive monophosphate. It is an important component of the cAMP cascade. This role posits that PDE4 mediates the effects of several antidepressants. In a study [101], seeking to quantify the binding of ^{11}C-(R)-rolipram, a PDE4 inhibitor acts as an indirect measure method that mediates enzyme activity in the brain of individuals with major depressive disorder (MDD). MDD subjects showed a widespread, approximately 20 % reduction in ^{11}C-(R)-rolipram binding; however, it is not caused by different volumes of gray matter. Decreased rolipram binding of similar magnitudes was observed in most brain areas. Rolipram binding does not correlate with the severity of depressive or anxiety symptoms.

The second messenger cAMP has been found to play an increasing important role in both the pathophysiology and treatment of MDD. Many basic studies suggest that decreased signaling associated with depression [102–105] is through the cAMP cascade and that diverse antidepressant treatments serve as a common mechanism of action [106] to increase signal transduction of the cAMP cascade. PDE4 is an important component of the cAMP signaling cascade, which is selectively reduced by PDE4 in the brain by forming the inactive 5′-AMP from cAMP [107]. PDE4 can be imaged using positron emission tomography (PET). As a promising target of antidepressants, PDE4 may serve as a useful in vivo biomarker to assess the link between cAMP and MDD.

Several weeks of administration are typically necessary before antidepressants manifest their therapeutic effects. Human postmortem studies [101–105] have reported that the time lag is in line with decreased signaling through the cAMP cascade and with antidepressant-induced increases in gene expression that is downstream of the cAMP cascade in rodents [106], for instance, increases in genes of both BDNF and a transcription factor may regulate the expression of BDNF and cAMP response element-binding protein (CREB). Several postmortem brain studies have shown that multiple markers of the cAMP cascade were decreased in patients with MDD or depressed suicide victims, including cAMP-dependent protein kinase A (PKA) [103], CREB [104], BDNF [105], and adenylyl cyclase [101, 102]. Administration of various pharmacological classes of antidepressants, including monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, and tricyclic antidepressants, upregulates PDE4 [106], which has been consistently found to be chronic, not acute treatment. One postmortem brain study found that unmedicated patients with MDD had lower CREB levels, while medicated MDD patients had high CREB levels similar to those of control subjects [104].

PKA can mediate the enzymatic activity of PDE4 through a feedback mechanism, which is likely due to the high concentrations of cAMP, which stimulates PKA to phosphorylate PDE4, thus increasing the enzymatic activity and returning the concentration of cAMP to steady state [106]. Moreover, both in vitro and in vivo studies indicated that PDE4 levels or its activity parallel the activity of the cAMP cascade [107–109], which are consistent with this feedback mechanism. In particular, chronic administration could enhance or diminish noradrenergic neurotransmission and alter PDE4 levels to higher or lower levels, respectively, which is in the same direction as the corresponding activity of noradrenergic neurotransmission [107].

47.5.5.2 The Role of PDE4 in Depression

Impairment of signal transduction that regulates neuroplasticity and cell survival plays an important role in the treatment of MDD, which is thought to be an important mechanism contributing to major depressive disorders. PDE4 plays a key role in controlling intracellular cAMP concentrations and is considered to be a prime target for therapeutic intervention in a range of disorders

such as depression and impaired cognition. In particular, cAMP-mediated signaling appears to have a key role in the pathophysiology and pharmacotherapy of depression. Increases in intracellular cAMP via inhibition of PDE4 or stimulation of adrenergic receptors produce antidepressant-like effects in animal models. Consistent with this, inhibition of PDE4 by rolipram also produces memory-enhancing effects in animals [110, 111].

It appears that the distribution of various PDE4A, PDE4B, and PDE4D [112] varies among regions of the brain. This differential distribution suggests that PDE4 subtypes may play distinct roles; their roles in the central nervous system have only recently begun to be examined. Using a gene knockout technique, mice lacking a single PDE4 subtype such as PDE4D display antidepressant-like behavior [113]. It has been suggested that PDE4D is involved in the mediation of depressive symptomatology and antidepressant responsiveness. Given the potent antidepressant-like effect of rolipram [114] and the important role of PDE4D in the control of cAMP concentrations, PDE4D exhibits delayed growth, decreased fertility, and reduced responsiveness to the respiratory effect of a muscarinic agonist. Recently, the behavioral phenotype and pharmacological sensitivity of PDE4D knockout mice were investigated in models sensitive to antidepressant drugs. Immunoblot analysis showed that in the cerebral cortex without the PDE4D expression and hippocampus of PDE4D knockout (PDE4D−/−) mice, however, the expression of PDE4A and PDE4B have no change. PDE4D-regulated cAMP signaling may play a role in the pathophysiology and pharmacotherapy of depression. Compared to the wild-type (PDE4D+/+) and heterozygous knockout (PDE4D+/−) mice, PDE4D−/− mice exhibited decreased immobility in tail-suspension and forced-swim tests, which is indicative of an antidepressant-like effect on behavior.

Recent studies reported that BDNF [115] plays a key role in the survival of mature neurons as well as damaged neurons in the central nervous system. Recent studies revealed that activation of the cAMP signaling cascade is closely involved in the regulation of BDNF mRNA [116] expression, as well as the coupled β-adrenergic receptors. Studies were undertaken to examine the influence of acute or chronic administration of PDE4 [117] inhibitors with an antidepressant on the expression of BDNF mRNA. These findings demonstrated that administration of PDE4 inhibitors shortens the time required for the upregulation of BDNF mRNA, supporting the possibility that this treatment may provide an effective therapy for major depression. Further in-depth studies are definitely needed to unveil this puzzle.

47.6 Concluding Remarks

As an important component of the cAMP signaling cascade, PDE4 plays a critical role in controlling intracellular cAMP concentrations and is considered to be a prime target for therapeutic intervention in a range of disorders such as depression and impaired cognition. Inhibition of PDE4 by rolipram produces promising antidepressant-like and memory-enhancing effects through activation of intracellular cAMP signaling, which is also activated by stimulation of adrenergic receptors. While studies are still needed to identify the role of specific PDE4 subtypes, data to date support that PDE4D is involved in the mediation of depressive symptomatology and antidepressant responsiveness. This is consistent with the critical role of PDE4D in the control of cAMP concentrations in the brain and in the mediation of antidepressant activity of rolipram [114]. Given that a variety of antidepressants activate cAMP/CREB signaling and its downstream targets such as BDNF, the cAMP signaling cascade has been considered to be a common pathway by which antidepressants produce therapeutic interventions. This could make PDE4, cAMP, and BDNF useful biomarkers to assess the pathophysiology of MDD and even therapeutic efficacy of antidepressant drugs.

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Effects of Methylphenidate and Atomoxetine on Development of the Brain

48

Berrin Zuhal Altunkaynak, Mehmet Emin Onger, Aysin Pınar Turkmen, Kıymet Kubra Yurt, Gamze Altun, Murat Yuce, and Suleyman Kaplan

48.1 Introduction

The development of the neuronal system is a long and highly complex process. Similarly, brain development, while it is part of this process, entails more complex molecular mechanisms and hormonal changes that are independent of this process. Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder that is generally seen in children and adolescents. Psychostimulants such as methylphenidate (MPH) have been commonly used for the treatment of ADHD. However, atomoxetine (ATX), a non-stimulant noradrenaline (NA) reuptake blocker, is used as an alternative for the therapy of ADHD. Exposure to these drugs gives rise to behavioral, functional, and physiological effects on the brain during the postnatal developmental period. This chapter provides general information about the brain development process and the effects of MPH and ATX on neurodevelopment in the light of the actual findings and the literature.

B.Z. Altunkaynak • M.E. Onger • A.P. Turkmen
K.K. Yurt • G. Altun • S. Kaplan (✉)
Department of Histology and Embryology, Medical School, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey
e-mail: skaplan@omu.edu.tr

M. Yuce
Department of Child and Adolescent Psychiatry, Medical School, Ondokuz Mayıs University, Samsun, Turkey

Department of Histology and Embryology, Medical School, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

48.2 What are Methylphenidate and Atomoxetine?

48.2.1 Methylphenidate

ADHD, a neuropsychiatric disorder, shows attention deficit, hyperactivity, and impulsivity, with onset before the age of 7 years [13]. The neuropsychological model of this disorder has proposed that ADHD is caused by irregularities in the frontal subcortical circuits [63, 169]. In this regard, many studies concerning morphometric measurements, using magnetic resonance imaging (MRI) of different regions of the brain, have been carried out [53, 75, 112]. These studies have been conducted on the four major lobes of the brain (frontal, parietal, temporal, and occipital). But, according to the anatomical hypothesis, the frontal lobe is affected in ADHD more than the other lobes in the brain [53, 75]. Generally, the cause of several neurological and psychiatric diseases such as ADHD, Huntington's disease, and epilepsy is imbalance in the dopaminergic and noradrenergic systems [79]. Dopamine (DA) and NA play important roles in cognitive functions such as attention, concentration, and motivation-related wakefulness [151]. DA is primarily

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produced by mesencephalic and hypothalamic neurons [42]. Some clinical studies have shown that hyperactive and impulsive behavior is caused by defects of this catecholamine system [124].

Regulation of physiological functions occurs via multiple pathways. Dopaminergic receptor activities, DA transduction mechanisms, and the contribution of different elements to these processes have not yet been fully explained [25]. On the other hand, symptoms of Parkinson's disease such as bradykinesia, resting tremors, rigidity, and postural instability are associated with decreased concentrations of DA in the postsynaptic neurons. A deficiency of striatal DA leads to the degeneration of nigral neurons in Parkinson's disease.

Psychostimulants cause an increase in dopamine transport (DAT) by inhibiting the extracellular DA concentration. In this way, they provide an improvement in the symptoms of ADHD [118]. MPH–dl–threo–methyl–2–phenyl–2–(2–piperidyl) acetate – is a substitute for naturally occurring phenethylamine, and is used for the treatment of ADHD. MPH is generally well tolerated and is especially effective in decreasing the symptoms of ADHD and substance dependence, as well as preventing bulimia nervosa [1, 68, 80, 177]. MPH is safe and effective in the treatment of childhood neurodevelopmental disorders and it is prescribed frequently [1]. The etiology of these disorders is believed to be related to increased extracellular concentrations of DA and NA. These increased concentrations affect the cortex, medulla, and bulbus in the brain [137, 144]. The effect of MPH contributes to the treatment of hyperactivity in the brain via impulse control; therefore, it is known as a stimulant drug for the central nervous system [1, 80, 176]. The pathogenesis of ADHD is related to the DA and norepinephrine neurotransmitter systems.

MPH inhibits the transportation of DA via binding to the synaptic membrane with high affinity; hence, concentrations of DA and NA increase within the synaptic space [73, 135, 156, 171, 173]. Consistent with this, more recent neuroimaging studies in adults have also indicated an increase in extracellular DA following the oral administration of MPH [170, 171]. This increased neurotransmission of DA and NA leads to a prolonged intensified postsynaptic signal; MPH also binds to muscarinic and serotonin receptors in the brain, but the serotonin transporter has limited affinity in comparison with the neuronal NA transporter [31, 98]. In neuronal cells, DAT inhibition and changes in the signal timing of DA may be involved in the behavioral and locomotor effects of MPH [119, 135]. The effect of MPH on DAT in the human brain is illustrated in Fig. 48.1.

Physiological and biochemical changes are related to changes in dopaminergic neurons, such as increased activity in the striatum and nucleus accumbens [165]. DA plays a role in controlling the behavior of people, so the correct concentration of DA in the brain is very important. The reason for ADHD, in general, is an imbalance in the dopaminergic and noradrenergic systems [27, 58]. MPH is a central nervous system stimulant and is similar to amphetamines. However, MPH has a weaker effect than amphetamine. Stimulants reduce novelty seeking [74] and trigger neuronal activation from adolescence to adulthood [135]. Agitation, nausea, palpitations, vomiting, headache, loss of appetite, insomnia, nervousness, dizziness, increased heart rate, psychosis, increased blood pressure, and anxiety are general side effects of MPH. The long-term effects of MPH on brain development are not yet known. However, MPH slows children's growth or attenuates weight gain. MPH is used in children over age 6 years [72]. The effects of the drug depend on the exposure period (childhood/adolescence), time of assessment (immediately after use/a long period after use [in adults]), and gender [6]. Recent work has shown that brain-derived neurotrophic factor (BDNF) enhances sensitization to MPH in the brain [30]. MPH treatment alters circadian clock gene expression and N-methyl-D-aspartate (NMDA) receptor composition that is relevant in synaptic plasticity [10, 161]. MPH affects energy metabolism and oxidative stress [64, 125], and this effect causes the activation of apoptotic pathways and cell death. Brain plasticity in young and adult rats is affected by MPH exposure. MPH usage may lead to activation of the initial cascade of apoptosis by increasing Bax and reducing Bcl-2. At the same time, MPH inhibits apoptosis by reducing caspase-3 and cytochrome c [125].

Some symptoms of hyperactivity disorders are inhibited by the use of MPH, but a few studies have shown that MPH has a detrimental effect on the

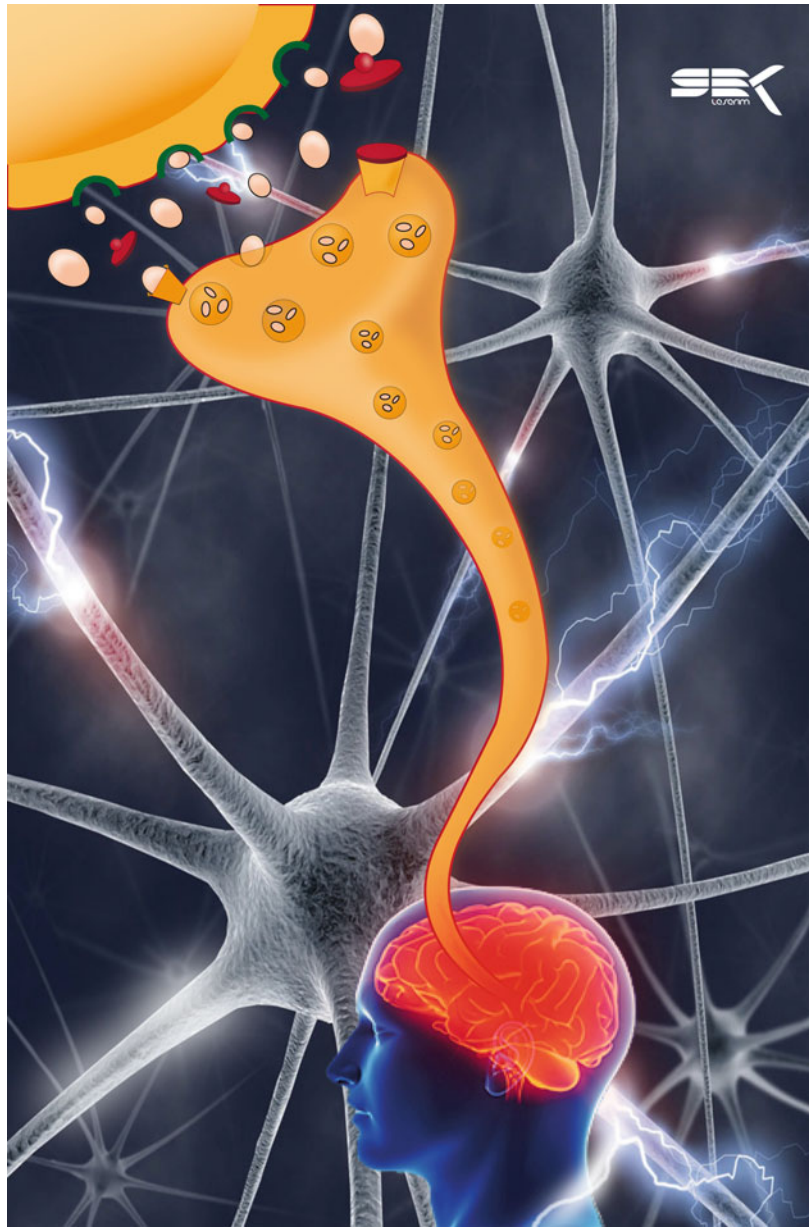

Fig. 48.1 The effect of *MPH* on *DA* transport in the human brain is illustrated. MPH blocks DA reuptake from the inter-synaptic cleft. It was redrawn from [177]

brain. MPH leads to an increase in the concentrations of superoxide particles in young rats. In addition, MPH treatment caused oxidative damage in the brains of young rats [99, 133]. Besides, MPH alters energy metabolism by changing the concentrations of creatine kinase, citrate synthase, and isocitrate dehydrogenase, as well as mitochondrial respiratory chain enzymes [56, 125, 130].

The main effect of MPH was demonstrated by measuring cerebral blood flow (CBF) with positron emission tomography (PET). Glucose was used as a PET marker (regional cerebral blood flow (rCBF)).

Relatively increased cerebral activity [103, 160, 171] and differential effects in the temporal lobe were observed [103, 160]. In these studies, localized effects of MPH in brain regions were demonstrated.

The excessive accumulation of DA in the synaptic space can lead to the production of reactive oxygen species (ROS), mitochondrial dysfunction, abnormal ATP production, increased

neurodegeneration, and neural cell death in the brain [19, 32, 116, 136, 168]. MPH (Ritalin; Novartis Pharmaceuticals, Basel, Switzerland) blocks DA and its transporters, leading to DA accumulation in the synaptic space; hence, increased extracellular concentrations of DA and NA. The conditions mentioned here occur primarily in the prefrontal cortex of the brain [8].

48.2.2 Atomoxetine

ATX is a non-stimulant medication and is commonly used in the treatment of ADHD [55, 88, 106, 108, 148, 150]; it is approved by the Food and Drug Administration of United States of America. The mechanism of action of ATX is exerted by inhibition of the presynaptic NA transporter, thrugh which NA uptake in the brain is blocked [48]. Thus, ATX causes an increase in brain cortical NA [31] and is classified as a non-stimulant. ATX shows minimal affinity for serotonin reuptake transporters [50] and glutamate receptors [97]. It is metabolized through cytochrome P450 in the liver. Its active metabolite, 4-hydroxyATX, is glucuronidated and excreted in the urine. ATX is regulated by the electrophysiological activities of the locus ceruleus (LC) [12]. It is effective for treating the symptoms of ADHD [174]. There is evidence that ATX has a selective role in the pathogenesis of the serotonergic, noradrenergic, and dopaminergic systems. In the data obtained so far, ATX is highly effective in the treatment of neuropsychological disorders, particularly comorbid anxiety and tics, alcohol and other substance abuse disorders [55].

In both human and animal studies with ATX, it was shown that this drug shows frequent receptor binding to noradrenergic neurons. For example, ATX application increases NA in the brain cortex, hippocampus, and cerebellum [154]. In a study of ATX, a statistically significant increase in blood pressure was reported, but it did not show clinical significance. In children and adolescents, ATX had no or only a little effect on growth, although some cases did show growth reduction [148]. ATX treatment is effective for attention deficit disorder, as proven by studies that showed its superiority to placebo. But ATX has been shown to be less effective for this disorder than MPH and amphetamine [107, 175].

ATX may be the first choice of pharmacotherapy for patients with substance use disorder, tic disorders, and anxiety disorders accompanying ADHD [55]. Imaging studies have suggested that the volumes of the prefrontal cortex and the caudate nucleus in children with ADHD are smaller than those of control groups. This is probably caused by neurodevelopmental suppression in the neuronal pathways and their link paths [38]. Although MPH is clinically more effective than ATX [57, 83, 107, 113, 149, 152], ATX offers several advantages compared with MPH. In pediatric patients with an absence of response to stimulant treatment, ATX has offered an alternative treatment facility, which has a reduced risk of motor side effects [21, 113]. When ATX is taken orally, it is rapidly and well absorbed because it is highly water soluble. The extent of absorption is unaffected by food. Thus, it may be taken with or without food [129]. However, ATX has serious potential side effects such as suicidal thoughts, hepatotoxicity, sedation, and weight loss or slowed growth [134]. ATX takes a longer time than stimulants to reach an effective concentration; maximal benefit may be delayed 1 or 2 months. ATX selectively inhibits NAT [24, 178], but MPH inhibits DAT and NAT (Fig. 48.2; [70, 136]).

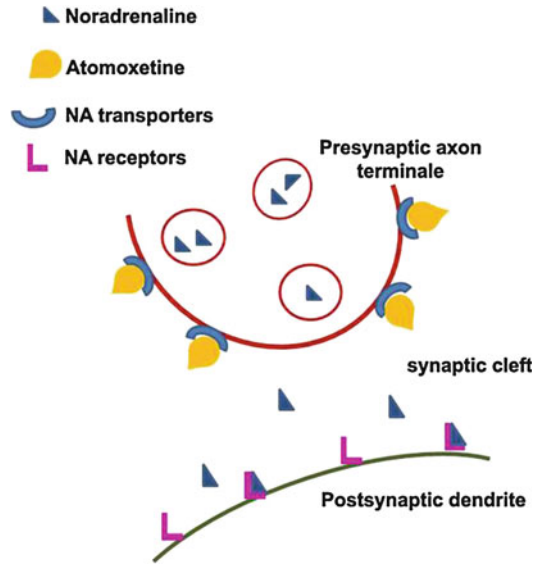

Fig. 48.2 Effect of ATX on DA transport in the human brain is illustrated. ATX blocks NAT in the presynaptic axon terminals. It was redrawn from [177]

48.3 A Long and Complex Process: Fetal Brain Development

Human brain development with neuronal differentiation occurs in the third gestational week and this development extends to late adolescence. The processes involved range from molecular mechanisms to essential physical and chemical environmental factors [111, 153]. The brain develops from the cranial portion of the neural tube, at the level of the fourth somites. When merging of the neuronal groove and closing of the rostral neuropore occur on the 25th day, the neural tube forms three primary brain vesicles in the cranial region—the prosencephalon, called the forebrain, in the anterior part; the mesencephalon, called the midbrain, in the middle part; and the rhombencephalon, called the hindbrain, in the posterior part (Fig. 48.3).

At the fifth week, the forebrain is divided into two secondary vesicles, termed the telencephalon and diencephalon. At the same time, the hindbrain is divided into two secondary vesicles, termed the metencephalon and myelencephalon. Thus, the neural tube at this stage has five vesicles. The neural tube is crimped by three flexures: the mesencephalic flexure, the cervical flexure (between the rhombencephalon and spinal cord), and the pontine flexure (Fig. 48.4; [52]). It is known that brain vesicles give rise to different structures. For instance, the cerebrum is formed by the forebrain. The anterior part of the third ventricle arises from the cavity of the telencephalon.

The prosencephalon has subdivisions that are known as the telencephalon and diencephalon during the sixth week [109]. The telencephalon consists of two lateral diverticula (cerebral vesicles) and an intermediate portion [110]. At the end of the fifth week, cerebral hemispheres appear

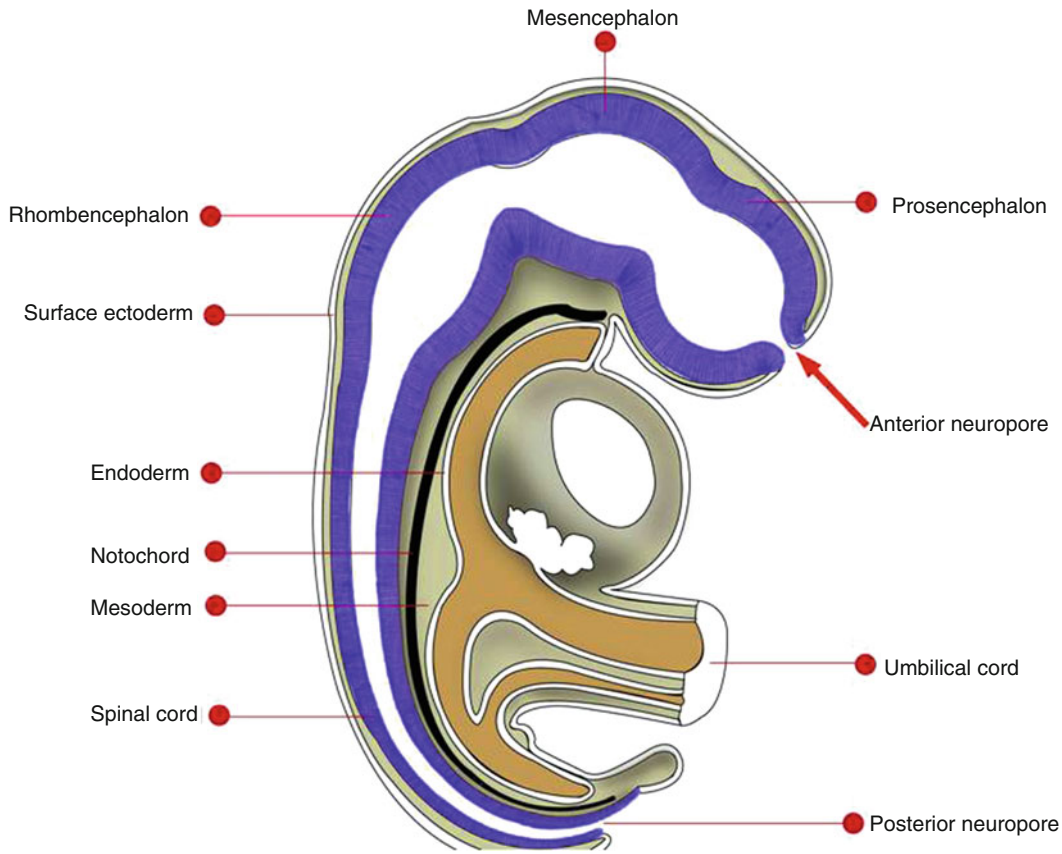

Fig. 48.3 Schematic representation of sagittal view of the end of neurulation (Adapted from Singh et al. [142])

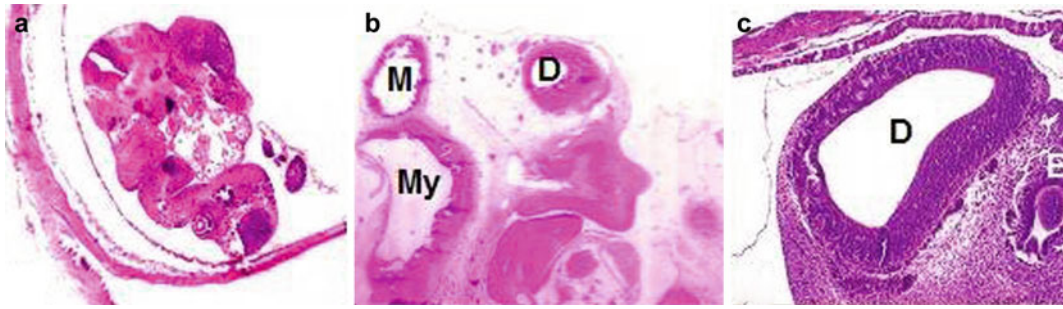

Fig. 48.4 (**a**–**c**) are light microscopic images of developing brain. Sagittal section of a 7-day-old rat embryo. The brain of the embryo consists of five brain vesicles. *D* Diencephalon, *M* metencephalon, and My myelencephalon are seen; *E* indicates developing eye (hematoxylin-eosin staining)

as diverticula near the prosencephalon [126]. The anterior portion of the third ventricle originates from the cavity of the middle telencephalon. The cerebral hemispheres enlarge over time and cover the hindbrain, midbrain, and diencephalon. These regions constitute the brain hemispheres, and the medial surfaces of the hemispheres merge on the center line. Later, the corpus striatum, rhinencephalon, and cerebral cortex are formed from the telencephalon. In the developing brain, an anterior portion of the prosencephalon merges with the right and left hemispheres. This area is termed the lamina terminalis and forms the anterior commissure, fornix, and corpus callosum [110]. The diencephalon has three tubercles at the 6th, 15th, and 21st weeks, and these tubercles differentiate into distinct components— the thalamus, the hypothalamus, and the epithalamus, respectively. The neurohypophysis develops from the infundibulum. Additionally, the ventral part of the diencephalon forms optic cups. The mesencephalon does not have any subdivision (Fig. 48.4). The pedunculus cerebri is formed from the basal lamina, and the corpora quadrigemina is formed from the alar lamina [126]. The neural canal in the mesencephalon forms the aqueductus cerebri, which merges with the third and fourth ventricles [110]. The cerebellum arises from the dorsal portion of the alar plaques. Neuroblasts in the intermediate zone of the alar plaque migrate to the marginal zone and differentiate into neurons within the cerebellar cortex. The other neuroblasts form the central nuclei that constitute the major part of the dentate nucleus, and the myelencephalon forms the medulla oblongata [52].

The neuronal migration to cortex is substantially completed during 24th week of pregnancy. Neuronal cells emerge from the germinal center from 7th week of gestation and migrate to brain surface in order to create the cortex from 22nd to 26th week. Neuronal migration continues into the subcortical areas until 32nd week [18]. While myelination of the dorsal areas of the brain (necessary for high-level cognitive functions) occurs later, myelination in the ventral and deep areas of the brain occurs early [159].

48.4 Development of the Neural System and Its Consequence: Neuronal Differentiation

Neurogenesis begins in the embryonic period in humans. The neural induction process that begins during gastrulation includes signaling molecules that are derived from the notochord. Following neural induction, the neuroectoderm differentiates into the neural plate and this leads to the folds of the neural precursor cells [147]. The meeting of these folds forms a tube, thus transforming into the neural system.

48.5 Highlights in the Postnatal Developmental Period

Human brain development begins in the third week of gestation; however, maturation of the human brain is a complex, life-long process, continuing in adulthood. This process can be ana-

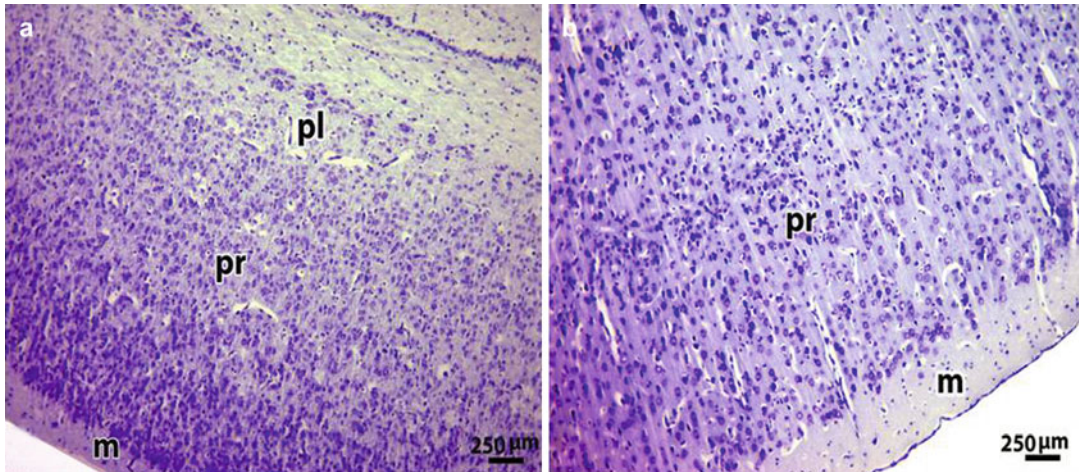

Fig. 48.5 (**a**, **b**) show histological sections of cerebral cortex from 1- and 8-week-old rat brains, respectively. *m* molecular layer, *pr* pyramidal layer, and *pl* polymorphic layer are seen (cresyl violet staining)

lyzed in detail by using current imaging methods [18]. The rate of brain development is very fast in both the late prenatal and early postnatal periods; for instance, the total volume of the brain that encloses the brainstem, cerebral hemispheres, and cerebellum is approximately 150 ml in infants, and in the course of time, it increases approximately threefold, reaching 400 ml during the early postnatal period [77]. There are changes in brain morphology during postnatal development, and significant changes are observed on MRI of the human brain during the first 2–3 years of life (Fig. 48.5).

However, comparisons of brain morphology in children and adults using magnetic resonance (MR) morphometric images showed that gray matter volumes in the subcortical nuclei and cerebral cortex were significantly larger in school-age children than in adults [81]. In many MRI studies in infants and children it was observed that gray and white matter volume increased dramatically in the perinatal period [77, 120]. Postnatal brain development is a long ongoing process, and MRI changes that are observed during this long process reflect the chemical results of oligodendrocyte proliferation and myelination [14]. The brain volume reaches approximately 90% of its adult volume during the preschool period [43, 78, 84]. In addition to all this information, mapping methods have suggested similarities between brain development and decreases in cortical volume, and accordingly, it was reported that local cortical thinning might be directly related to the myelination of nearby fiber tracts. Functional changes might result from the stimulation of cortical thinning by mature fiber tracts [146] (Fig. 48.6).

Studies carried out by a special cell counting method have shown that there are more than 100 billion neurons in the mature human brain [117]. In longitudinal studies in which brain development was analyzed during childhood and adolescence, quantitative MRI has clarified the complex structure of the brain [62, 145]. A fourfold increase is observed in the total volume of the brain from birth until childhood. According to knowledge obtained from current studies, the early years, especially from birth to 2 years, may be, in terms of energy expenditure, the most important period of postnatal brain development in humans. Furthermore, this period is important for the course of neurodevelopmental diseases such as autism and schizophrenia. It is observed that there is a significant increase in the whole brain volume during this neurodevelopmental period [121]. Brain development is an intense process that consists of the production of neurons, glial cells, and synapses. Of note, these volumetric changes occur postnatally, although neuronal production and migration mostly occur during prenatal development. The migration and proliferation of glial progenitor cells continue in the postnatal period, and differentiation and

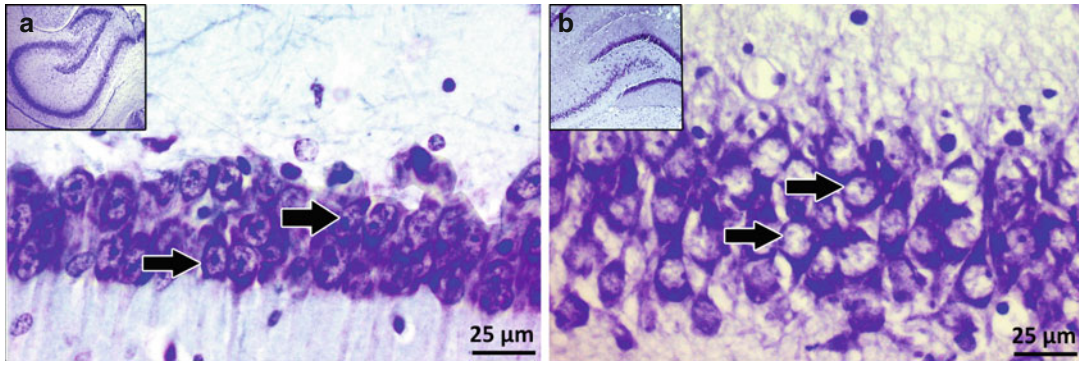

Fig. 48.6 (**a**, **b**) show histological sections of pyramidal neurons from 1- and 8-week-old rat hippocampus, respectively. *Arrows* indicate the structure of the pyramidal neurons (cresyl violet staining)

maturation also progress during childhood. But neuron-glial cell interface, which plays an important role in functional organization during postnatal development, has still not been exactly defined [40]. During the first 2 years of life, complex new synapses develop rapidly (Fig. 48.6).

Myelination of the white matter increases in the postnatal period, continuing until the end of the second year of life. Most myelination occurs at the end of the second postnatal year, but there is evidence that this process continues until the sixth decade of life [18]. Myelination begins in the caudal regions and continues toward the central cerebral regions, finally reaching the anterior and posterior poles. By the 6th week of the postnatal period, major myelination is complete in the posterior of the internal capsule. However, the anterior of the internal capsule is not entirely myelinated until the end of the first year of life. Myelination is seen in all the cortical lobes in the second half of the first year and is fully identified by the end of the second year of life. Cortical myelination in the frontal region is a prolonged process that occurs from childhood to adolescence [86]. Fetal and adult peripheral nerves are shown in Fig. 48.7; in the adult, more and larger myelinated axons are seen.

The differentiation of oligodendrocyte progenitor cells begins via myelin protein expression and occurs as the myelin membrane wraps around axons. Oligodendrocytes synthesize some growth factors; these factors contribute to axonal unity and neuronal survival time. Also, it has been shown that neuron-oligodendrocyte interactions affect neuron size and axon diameter [102]. Although neurogenesis is limited in the postnatal period, neurogenesis and neuronal migration continue in the subventricular zone. Additionally, neurons are produced in the dentate gyrus (DG) and these cells migrate through the subgranular layer toward the granular layer. Postnatal neurogenesis includes only a small percentage of the neuronal population. Conversely, the proliferation and migration of glial progenitor cells begins in the prenatal period, but the differentiation of oligodendrocytes and astrocytes continues postnatally. The proliferation of glial progenitor cells occurs in the subventricular zone of the forebrain, while these cells migrate into the white matter, cortex, striatum, and hippocampus [40]. Cell proliferation occurs in the subregions of the hippocampus, starting from the 24th gestational week and continuing until the end of the first postnatal year. While granule cells form until the end of pregnancy and formation continues in the postnatal period, hippocampal pyramidal neuron formation does not appear until the last trimester of pregnancy. Although the formation of granule cells continues after birth, the great majority of granule cells are produced by the 34th gestational week [139].

There are different classes of neurons in different layers of the cortex. Most studies suggest that specific kinds of neurons are produced by progenitor cells, and it has been found that, in corticogenesis, neural progenitor cells may produce any neuron type [60, 101]. The other

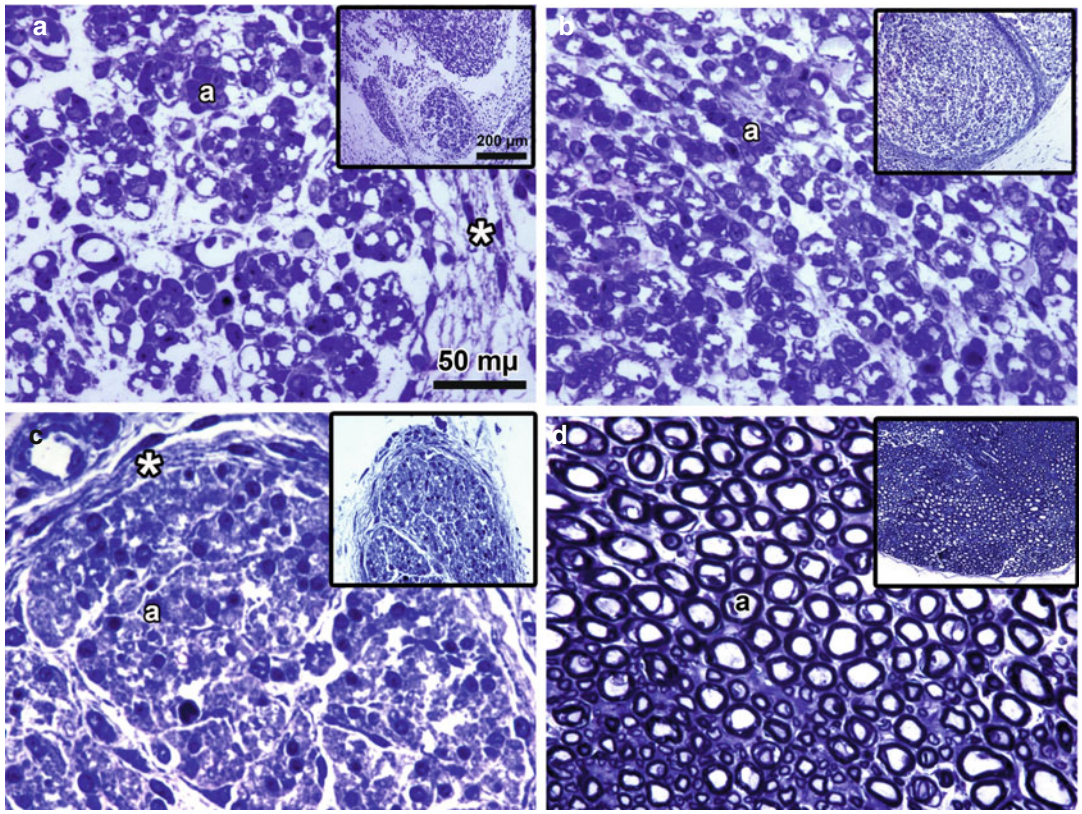

Fig. 48.7 Light microscopic images of peripheral nerves obtained from 11 day-old embryo (**a**), 20 day-old embryo (**b**), 7 week-old rat (**c**) and adult rat (**d**) are seen in **a**,**b**,**c** and **d** respectively. Asterisks (*) indicate perinerium. An axon (*a*). Toluidine blue staining. Scale bars: 200 µm and 50 µm

essential event in the neurodevelopmental process is the apoptosis of residual neurons [89]. Neuronal apoptosis plays an important role in the organization of neuronal development, especially in the embryonic development of the cerebral cortex, cerebellum, and brainstem. During prenatal life, neuronal apoptosis reaches its peak, whereas the apoptosis of glial cells occurs postnatally over a prolonged period [102]. While high concentrations of the Bax gene, which stimulates apoptosis by antagonizing Bcl-2 protein, are observed at birth, this gene is downregulated later in development [89, 166]. Caspase-3, caspase-9, Apaf1, Bax, Bcl-XL, and survivin, which are regulator proteins, also play a critical role in apoptosis. Survivin belongs to the apoptosis inhibitor family, and it plays a basically negative role in programmed cell death via inhibiting caspase activation [89]. Nevertheless, how survivin causes programmed cell death during the embryonic differentiation of neurons is not clear. It is known that neuronal loss in the neocortex occurs postnatally, but the molecular elements of this postnatal cortical apoptosis are not known [128]. During the initial myelination, oligodendrocytes undergo apoptosis, depending on signals from nearby axons, within a few days after neuronal differentiation [102].

48.6 Neurotoxic Effects of Methylphenidate and Atomoxetine on the Nervous System

MPH and ATX are pharmacological agents that alter catecholamine concentrations in neurons. The alteration in catecholamine concentrations is

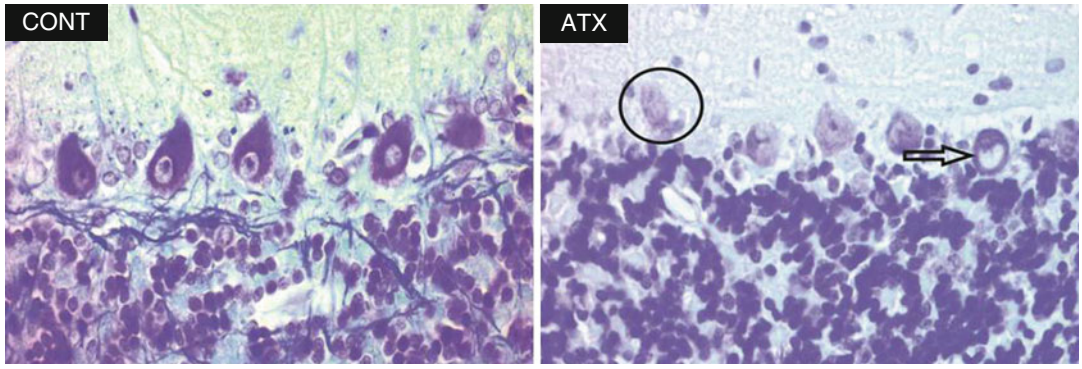

Fig. 48.8 This microscopic image shows that the ATX-treated group have degenerated Purkinje cells with large vacuoles (*arrow*), pyknotic nuclei, weakly stained cytoplasm, and irregular cell boundaries (seen in the *circled* area). The image on the *left* side shows healthy neurons in the control group (cresyl fast violet staining, under×40 objective)

one of the major causes of many neuronal diseases. The neurotoxic effects of DA, which is a substantial catecholamine, have been noted in many studies. There are four main DA pathways: the nigrostriatal, the mesocortical, the mesolimbic, and the tubero-infundibular system, which are involved in DA transmission in the hypothalamus and the pituitary gland. DA pathways are connected with several regions of the brain. Problems in these pathways lead to neurological, neuroendocrine, and psychiatric diseases. For instance, the primary cause of Parkinson's disease is decreased DA concentrations in the nigrostriatal pathway, which results from the loss of nigrostriatal cells [25].

Conversely, in ADHD and schizophrenia, DA activity is increased. Tourette's syndrome occurs as a result of increased DA activity in the basal ganglia region [141]. DA has toxic effects in cultured cells [66, 104]. Physiological concentrations of DA caused apoptosis in cultured post-mitotic sympathetic neurons [181]. The toxic effects of DA might be associated with its oxidative metabolites. Further in vitro studies have elucidated the molecular mechanisms of DA's toxic effects. It is known that increased concentrations of the p53 transcription factor [44, 123], and the activation of caspase 3 [76, 115] and nuclear factor (NF)-kB [17, 45] are responsible for apoptosis. So MPH and ATX might have neurotoxic effects exerted by these mechanisms. The neurodegenerative effects of ATX on the cerebellum are shown in Fig. 48.8.

48.7 Mechanism of Oxidative Stress

Oxygen is an essential element for the eukaryotic organism. It's including electrons is released from reduced substrates. Oxygen has a critical role in tissues and hypoxia is a mortal threat in the living organism; therefore, it may be that neurotoxicity is caused by hypoxia in the brain tissue [3, 46]. The electronic structure of oxygen is linked to the toxicity and chemical activity of [46, 69].

It has been reported that chronic MPH treatment in animals leads to increased lipid peroxidation in the prefrontal cortex, hippocampal region, cerebellum, and striatum [99]. Autooxidation of cytosolic free DA leads to ROS such as superoxide (O_2) and hydroxyl radicals (OH); as a result of this process, MPH-induced dopaminergic neuronal damage occurred [33]. The reactivation of oxygen species is highly toxic to tissues. This toxic effect occurs with elements of the normal cell structure such as lipids, proteins, and DNA, and it causes cell death. DA oxidation products were shown to be increased with direct intrastriatal DA application in rats [71].

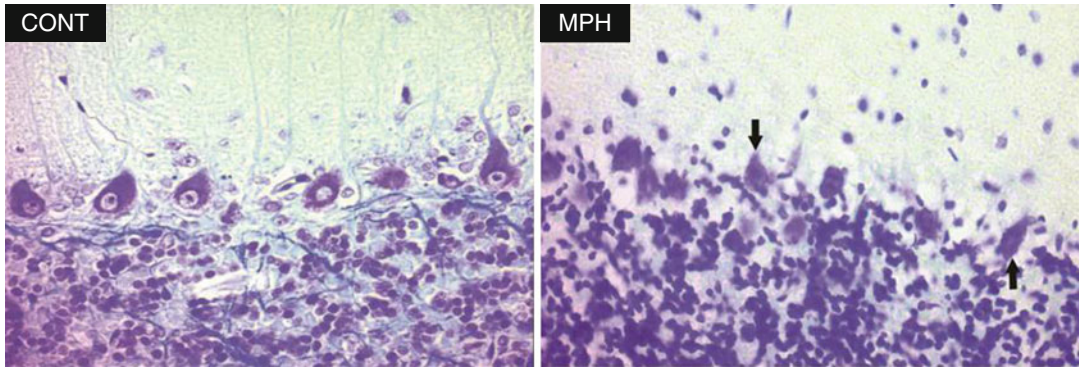

Fig. 48.9 Figures show histological sections of cerebellum in control and MPH-treated groups, respectively. *Arrows* indicate the degenerated Purkinje cells in the MPH-treated group (cresyl violet staining, under×40 objective)

In adolescent and adult rats, MPH treatment affected striatal volume and myelination; in particular, it was shown that MPH had a strong effect on the basal ganglia. MPH altered network strength in the anterior cingulate cortical network in adolescents and adults [164]. Urban et al. [162] administered 1 mg/kg MPH per day in rats and found reduced function in pyramidal neurons in the juvenile rat prefrontal cortex. Recent studies have shown that after long-term MPH administration, the drug may change brain function and cause persistent defects [94]. MPH or Levodopa, L-3, 4-dihydroxyphenylalanine (L-DOPA) treatment is used for Parkinson's disease, and after such treatment DA is metabolized via monoamine oxidase (MAO) or by auto-oxidation to produce cytotoxic ROS, and then neuromelanin formation occurs [155]. Also, according to unpublished data, MPH treatment could cause neurodegeneration in the rat cerebellum (Fig. 48.9).

48.8 Neuroprotective Effect

DA shows neuroprotective effects via receptor-dependent mechanisms [87]. For example, Amano et al. have demonstrated that D1 DA receptors are neuroprotective against the neuronal cell death induced by glutamate [4]. Further studies suggested that the D2 receptors of DA were more effective than D1 receptors in regard to neuroprotective mechanisms [41]. A protective effect of treatment with D2 receptor agonists was seen in rats with ischemic hippocampus [114]. The total volumes of white and gray matter in untreated ADHD patients were smaller than those in patients who had had treatment with a psychostimulant [36, 39]. After the treatment, it was observed, by using counting analysis, that the whole white matter volume had reached normal size [39]. Correlatively, ADHD related to reduced anterior cingulate cortex volume was normalized with psychostimulant medication, but caudate nuclear volumes were not normalized by drug treatment [138].

After psychostimulant treatment in ADHD patients, inward deformations are related to mood and psychology, and normal-shaped caudate nuclei were observed [143]. New neural progenitor cells occur in the DG of the adult mammalian brain. The neurons in the DG play a major role in learning and memory [35]. Neurogenesis is affected by multiple factors in the brain [2]. Regarding this, psychoactive drugs have been shown to induce neurogenesis. In addition, their roles in learning and memory are undeniable [96]. It has been shown that MPH at an effective dosage enhances learning and memory [51]. Its neuroprotective effectiveness is accepted as being related to DA and noradrenaline changes [90]. Lee et al. [95] reported that 10 mg/kg MPH treatment led to a significant increase in the neurons of the adolescent mouse DG.

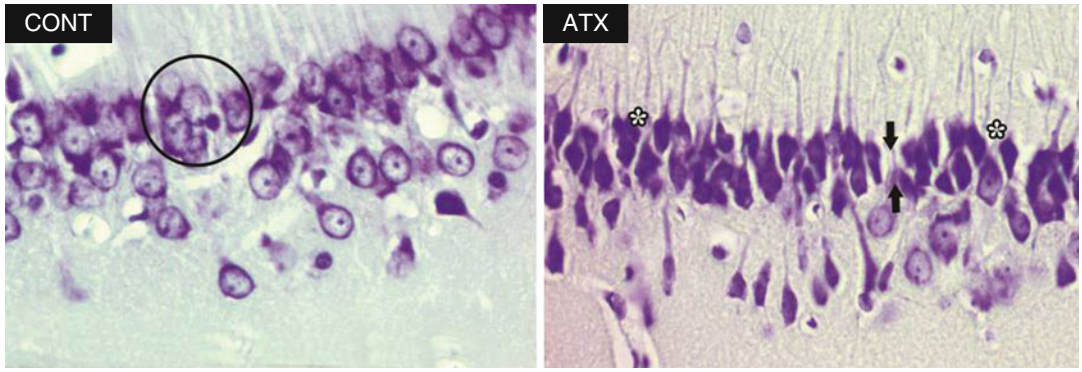

Fig. 48.10 The histopathological effects of ATX on hippocampal pyramidal neurons compared to control group. Figures from the control (*CONT*) and AXT-treated (*ATX*) rats brain are seen. These microscopic images show that the ATX-treated group have a decreased pyramidal cell layer (*arrows*) and there are many dead neurons that are darkly stained cytoplasm when compared with that of the control group and undefined nuclei (*asterisks*) can be seen. The image on the *left* side shows healthy control group neurons. Healthy cells are *circled* (cresyl fast violet staining, under × 40 objective)

In contrast, ATX treatment did not appear to have an effect on neurogenesis in the hippocampal region. These findings revealed that while cell proliferation and differentiation in the DG were significantly increased by MPH, ATX did not contribute to this differentiation and proliferation. Regarding this, MPH upregulated some neurotrophic factors that ensured differentiation and proliferation. Brain-derived neurotrophic factor (BDNF) was related to the administration of both ATX and MPH. Banerjee et al. [11] found that the BDNF mRNA concentration in the DG of the hippocampus was reduced 2 h after 2 mg/kg MPH administration. However, it was shown in another study that there was no change in BDNF concentrations in the hippocampus after the administration of 2 mg/kg MPH [132]. Additionally, Kim et al. [85] showed that BDNF concentrations in ADHD rats were reduced in the hippocampal region, while 1 mg/kg MPH administration for 28 days regulated the concentrations of BDNF in this group. Norepinephrine is the major hormone involved in the pathophysiology and treatment of ADHD [9, 22, 122, 180]. In Fig. 48.10, it can be seen that ATX treatment can cause neuronal loss in the hippocampus.

ATX functions as a norepineprine uptake inhibitor and at the same time as an inhibitor of the presynaptic norepineprine transporter [24, 178]. In light of this information, it appears that ATX as a potent inhibitor drug shows minimal affinity for 5-HT and DA uptake and also for neuronal receptors and neurotransmitters [61, 157, 178]. ATX neurotoxicity is thought to arise as the result of reactive oxygen particles forming due to DA oxidation, and this mechanism has also been reported to apply to MPH [64]. Studies of the neurodegenerative effects of ATX have not been reported. The structure of the child and adolescent brain is very plastic and sensitive. So, it may be easily vulnerable to drug treatment [91]. Pharmacological and environmental factors play a crucial role in the structure and functions of neurons. Behaviors and motivational and emotional functions are shaped during childhood [158].

MPH is commonly used in the treatment of some psychological disorders such as ADHD, and the treatment is very efficacious. Additionally, it is safe for children. Although MPH is commonly prescribed in childhood, during which the plastic and sensitive phases of brain development occur, treatment continues into adulthood, and there is limited information

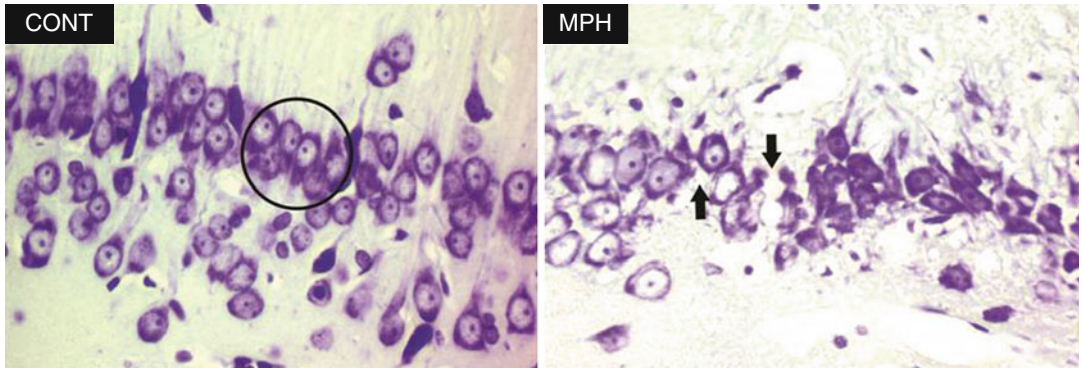

Fig. 48.11 The histopathological effects of ATX on hippocampal pyramidal neurons compared to the control group. These micrographs show histological sections of hippocampus in control (*CONT*) and MPH-treated (*MPH*) groups, respectively. *Arrows* indicate the decreased pyramidal cell layers in the MPH-treated group, and the *circled area* shows healthy cells in the control group (cresyl violet staining, ×40 objective)

about the long-term effects of MPH on brain structure and function [67].

There are no direct neurotoxic effects of MPH [172]. The application of neurotransmitters and their agonists is known to have strong morphogenetic effects on neurons and nerve tissue [93, 100] and small, long-term environmental factors can also affect cerebral morphology [26, 59, 82]. MPH and ATX show treatment activity by changing DA concentrations. In most psychological disorders toxic effects seen in the nerve cells are associated with changes in the cytosolic DA concentration. DA has both neurotoxic and neuroprotective effects and these depend on different pathological conditions [25].

Pharmaceutical applications that can change DA activity in the brain also cause some changes in the structure of the immature brain [105, 179]. For example, hippocampal neurogenesis in the DG of the rat is affected by antipsychotic drugs and psychostimulants [47, 54].

It's known that both MPH and ATX alter DA concentrations in presynaptic neurons. These pharmacological agents have both toxic and protective effects via changing DA concentrations in neurons. Figure 48.11 shows possible toxic effects of MPH on rat hippocampus. Degenerated hippocampal neurons and decreased pyramidal cell layers are seen in the MPH-treated group. These findings are seen in the micrographs in Fig. 48.11.

48.9 The Effects of Methylphenidate and Atomoxetine on Brain Development

48.9.1 Effects and Responses in the Hippocampal Region

ADHD, with symptoms such as inattention, impulsivity, and hyperactivity, is a complex condition and is widespread in children and adolescents. MPH is prescribed in the treatment of this disorder [1, 167]. MPH shows an effect by inhibiting the DAT. This inhibitor effect has high affinity, whereas the inhibitor effect of MPH on the NE transporter shows middle affinity [70]. Early recurrent exposure to MPH affects many behavioral, physiological, and functional events in the adult brain [23]. Especially, MPH effects are focused on the brain regions that are related to reward—the striatum and nucleus accumbens—following MPH exposure in the adolescent [3, 28, 37]. Each day, thousands of new neurons are produced and cell differentiation

and proliferation occur in the DG of the hippocampus, while half of the progenitor cells in the subgranular zone die between 6 and 22 days after generation. The newborn granule cells in the hippocampal region survive during this period of cell death by integration into this cycle [34, 65]. Juvenile MPH exposure gives rise to decreased survival of new neurons in the hippocampal region in the adult. However, this exposure does not affect the number of proliferating cells [49]. In support of the above approach, it has been reported that the reduced survival is related to increases in apoptosis [5, 92]. Another ongoing hypothesis is that, in juvenile rats, early MPH exposure enhances the apoptosis of newly formed neurons.

A different possibility in regard to the effect of MPH exposure is that this agent increases serum corticosterone concentrations in the juvenile period, and thus a response occurs as a stress response in the adult, and stress decreases neurogenesis [23]. The corticosterone response to stress increases in MPH exposed rats, whereas there is no great decrease in proliferating cells [49]. Corticosterone also has a crucial role in cell survival in the adult subgranular zone. Additionally, neurons that play a role as glucocorticoid receptors in maturation phases are targeted for cell death [49]. Subgranular cells form neurons in the DG and then integrate into the hippocampal region [71]. The addition of newly formed neurons to the DG occurs in the adult, and as a result of this positive effect, learning and memory occur. In contrast, inhibition of the production of new neurons leads to memory loss and the lack of old memories [2]. In brief, all the relevant data indicate that, after exposure to an appropriate dosage of MPH in the juvenile period, hippocampal alterations are seen [49].

48.9.2 Functional and Structural Features of Methylphenidate Treatment in Adolescents and Adults

Studies show that the effects of MPH on functional and structural features can change frequently depending on age. Following MPH treatment, decreased striatal myelinization occurred by virtue of a reduction in network connectivity in the anterior cingulate cortical region, whereas the morphologic features of cortical and subcortical areas were unaffected by MPH treatment. Additionally, a reduction in the striatal volume was observed after MPH treatment in adolescent rats, but not in the adults [164].

The thickness of the cortical region in humans reaches its highest level from 7 to 10. Five years [140]. This is related to the pruning of synaptogenesis in early adolescence, while myelinization continues during adulthood [29]. The effects of long-term MPH usage are expressed by the developing brain in adulthood [7].

48.9.3 Role of Methylphenidate and Atomoxetine in the Neurodevelopment Process and Neural Stem Cell Differentiation

MPH usage during brain development gives rise to adverse long-term effects on neurogenesis and neuronal development. The effects of treatment with a low dose influence cell proliferation and gene expression by supporting neuronal maturation [15, 163]. MPH can contribute to neuronal maturation, and it enhances neuronal production. It has been shown that maturation of the prefrontal cortex is delayed in children with ADHD [16].

ATX plays a role as a non-stimulant norepinephrine re-uptake blocker as an alternative in the therapy of ADHD. Several studies have observed that MPH and ATX changed gene expression processes and the efficiency of neurotrophic factors. However, MPH and ATX especially affect dopaminergic and noradrenergic pathways in the brain. Also, MPH, differently from ATX, regulates hippocampal physiological functions such as learning, memory, and the long-term potentiation [20]. It was reported that psychostimulants and antipsychotic drug administration played an important role in neurogenesis in the subgranular zone of the hippocampal region [54]. Several studies have suggested that

MPH usage did not affect neuron progenitor cell proliferation and neuronal differentiation in the hippocampal region during adolescence, early adulthood, and adulthood, despite decreases in newborn cells occurring during adulthood [131].

BDNF increases the proliferation and differentiation of neural progenitor cells [127]. Additionally, BDNF mRNA expression was reduced in the DG region following 2 mg/kg MPH treatment. However, mature hippocampal BDNF concentrations were not affected following the intraperitoneal injection of MPH. Further, high-dose treatment with MPH led to increased BDNF in the adolescent DG and, therefore, neural formation and differentiation increased [11, 132].

In conclusion, MPH and ATX are used for the treatment of ADHD. However, because these drugs have behavioral, functional, and physiological effects on the brain during its developmental period, treatment should be performed very carefully.

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S-Adenosyl-L-Methionine for Major Depressive Disorder

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Domenico de Berardis, Laura Orsolini, Felice Iasevoli, Carmine Tomasetti, Monica Mazza, Alessandro Valchera, Michele Fornaro, Giampaolo Perna, Monica Piersanti, Marco di Nicola, Giovanni Martinotti, Francisco López-Muñoz, and Massimo di Giannantonio

D. de Berardis (✉)
NHS, Department of Mental Health, Psychiatric Service of Diagnosis and Treatment, Hospital "G. Mazzini", ASL 4, Teramo, Italy

Department of Neuroscience, Imaging and Clinical Sciences, University "G. D'Annunzio", Chieti, Italy
e-mail: dodebera@aliceposta.it

L. Orsolini
Polyedra Clinical Group, Teramo, Italy

Academic Department of Experimental and Clinical Medicine, United Hospitals, Polytechnic University of Marche, Ancona, Italy

School of Life and Medical Sciences, University of Hertfordshire, Hatfield, Herts, UK

Villa S. Giuseppe Hospital, Hermanas Hospitalarias, Ancona, Italy

F. Iasevoli • C. Tomasetti
Polyedra Clinical Group, Teramo, Italy

Laboratory of Molecular Psychiatry and Psychopharmacotherapeutics, Section of Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Naples, Italy

M. Mazza
Polyedra Clinical Group, Teramo, Italy

Department of Health Science, University of L'Aquila, L'Aquila, Italy

A. Valchera
Polyedra Clinical Group, Teramo, Italy

Villa S. Giuseppe Hospital, Hermanas Hospitalarias, Ancona, Italy

Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine "Federico II", Naples, Italy

M. Fornaro
Polyedra Clinical Group, Teramo, Italy

Department of Education Science, University of Catania, Catania, Italy

G. Perna
Department of Clinical Neurosciences, Hermanas Hospitalarias, FoRiPsi, Villa San Benedetto Menni, Albese con Cassano, Como, Italy

Department of Psychiatry and Neuropsychology, University of Maastricht, Maastricht, The Netherlands

Department of Psychiatry and Behavioral Sciences, Leonard Miller School of Medicine, University of Miami, Coral Gables, FL, USA

M. Piersanti
NHS, Department of Mental Health, Psychiatric Service of Diagnosis and Treatment, Hospital "G. Mazzini", ASL 4, Teramo, Italy

M. di Nicola
Institute of Psychiatry and Psychology, Catholic University of Sacred Heart, Rome, Italy

G. Martinotti • M. di Giannantonio
Department of Neuroscience, Imaging and Clinical Science, University "G. D'Annunzio", Chieti, Italy

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F. López-Muñoz
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain

Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal

49.1 Introduction

S-Adenosyl-L-Methionine (commonly known as SAMe) is a naturally occurring molecule, located in several body tissues and fluids. SAMe was firstly discovered in Italy in 1952 [13]. It was introduced and sold as a dietary supplement in the US market in the late 1990s, even though it has been used as a prescription drug in Italy since 1979, in Spain since 1985 and in Germany since 1989 [28, 45]. It rapidly became one of the most widely used natural antidepressant [46, 52].

SAMe is synthetized from the amino-acid L-methionine and adenosine triphosphate (ATP) by methionine adenosyltransferase, through the one-carbon cycle. Its metabolic pathway depends on the dietary intake of vitamin B12, vitamin B6 and folate [22, 23, 30, 54, 64]. SAMe is a common substrate involved in methyl group transfers. The methyl group (CH_3) attached to its methionine sulphur atom is chemically reactive and participates into several transmethylation reactions. In fact, more than 40 metabolic reactions involve the transfer of a methyl group from SAMe to various substrates, such as nucleic acids, proteins, lipids and secondary metabolites. In particular, these synthetic reactions include the neurotransmitter synthesis (e.g. acetylcholine, serotonin, norepinephrine, melatonin and dopamine), methylation of phospholipids, glutathione synthesis, DNA transcription, and myelination and synthesis of carnitine, coenzyme Q10 and creatinine [3]. Moreover, it is also involved in various biological processes, including immune system, central nervous system (CNS) and anti-oxidative (radical scavenging, glutathione precursor) and anti-inflammatory processes [42].

SAMe may affect the regulation of various critical components of monoaminergic neurotransmission both by an indirect modulation of neurotransmitter synthesis (by promoting the synthesis of BH4 enzymatic cofactor) and a direct modulation of catabolic enzymes, monoamine transporters and neurotransmitter receptors via methylation. In addition, SAMe has been recognized to be one of the end-products of the 'one-carbon cycle', and several studies have suggested that major depressive disorder (MDD) is associated with a dysregulation in one-carbon metabolism [31, 53]. These findings have suggested that SAMe may have therapeutic potential in treating MDD [10, 11].

Several randomized clinical trials (RCTs) have supported that the antidepressant efficacy of SAMe in monotherapy is superior to placebo and tricyclic antidepressants [53, 54, 57]. Recent findings have also demonstrated its efficacy in patients nonresponsive to selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) [55, 66].

Pharmaceutical preparations of SAMe are available as intravenous (IV), intramuscular (IM) and oral (PO) forms. SAMe PO achieves peak plasma concentrations 3–5 h after ingestion of an enteric-coated tablet (400–1,000 mg). The plasma half-life is about 100 min. Oral formulation should be taken on an empty stomach. IM SAMe owns a bioavailability of around 96 %; it reaches peak plasma concentrations in around 45 min after injection. It is excreted in urine and faeces [32]. The dose range is typically 100–200 mg/day (or 200–400 mg/day) IM or 800–1,200 mg/daily PO, depending on severity and tolerance.

49.2 Efficacy of SAMe in the Treatment of MDD

Several clinical trials have shown that SAMe possesses an antidepressant activity [10, 12, 46, 56] both in monotherapy and as an adjunctive therapy in patients who failed or were partial responders to antidepressant drugs [2, 55]. Some studies focused on the evaluation of efficacy of

SAMe in the treatment of patients with stage II treatment-resistant MDD (TRD), i.e. patients who had failed to respond to at least 8 weeks of treatment with two adequate and stable dose of antidepressants of different classes (HAM-D≥16), according to the classification of Thase and Rush [65].

49.2.1 Studies of SAMe as Monotherapy in Patients with MDD

Parenteral SAMe. Some studies showed that parenteral preparation of SAMe, compared with a number of standard tricyclic antidepressants such as clomipramine, imipramine and amitriptyline, was generally equally effective in MDD [37, 39, 44, 48]. Bell et al. [6] compared antidepressant effects after 400 mg/day of intravenous (IV) SAMe with those after oral doses of imipramine following 2 weeks of treatment among 22 outpatients with MDD. SAMe determined a clinically significant improvement in depressive symptomatology, compared to imipramine. Other RCTs compared MDD patients treated with IM SAMe with placebo controls, reporting a marked improvement of depression compared to placebos [1, 49]. Several open studies [4, 5, 14, 25, 37, 40, 43, 60] showed that treatment with parenteral SAMe was followed by mood improvement in a substantial proportion of patients. In a double-blind placebo-controlled study carried out among patients with MDD, Carney et al. [15] demonstrated that 200 mg/day of IV SAMe ($n=16$) was equivalent to placebo group ($n=16$) in antidepressant efficacy. An Italian double-blind multicentre study demonstrated that 200 mg/day of IV SAMe was superior to placebo as a treatment for outpatients with MDD and co-morbid rheumatoid arthritis [18]. In an open and multicentre study conducted on 195 patients with MDD, Fava et al. [29] reported a clinically significant remission in depressive symptoms after both 7 and 15 days of treatment with 400 mg/day IM SAMe compared to placebo and no serious adverse events. A multicentre, double-blind, randomized parallel-group study, carried out to confirm both efficacy and safety of SAMe in the treatment of MDD, has demonstrated that there is no difference in efficacy between the group of patients treated with IM SAMe ($n=146$) at a dose of 400 mg/day and those treated with 150 mg/day of oral imipramine ($n=147$). Adverse events were significantly less reported in patients treated with SAMe compared to those treated with imipramine [51]. Two multicentre, double-blind studies have evaluated the efficacy and tolerability of oral and IM SAMe in patients with a diagnosis of MDD [26]. In one study, antidepressant effects after taking 400 mg/day of IM SAMe ($n=147$) were compared with those of 150 mg/day orally imipramine ($n=148$), for 4 weeks. The clinical effects of SAMe and imipramine treatment did not differ significantly for any efficacy measure.

Oral SAMe. Kagan et al. [38] carried out a randomized, double-blind, placebo-controlled trial in 15 inpatients affected with MDD. The results suggest that treatment with 1,600 mg/day of oral SAMe for 3 weeks is a safe and more effective antidepressant with a rapid onset of action, compared to placebo group. A double-blind, placebo-controlled study of SAMe in depressed postmenopausal women showed a significantly greater improvement in depressive symptomatology in the group treated with 1,600 mg/day oral SAMe compared to the placebo group after 1 month [59]. A double-blind randomized protocol compared oral SAMe with oral desipramine treatment for 4 weeks. A significant improvement in clinical response was observed in patients treated with SAMe compared with those treated with desipramine [7]. In the same multicentre, double-blind study by Delle Chiaie et al. [26], a second study was conducted in a sample of outpatients who orally took 1,600 mg/day SAMe ($n=143$) compared to those who took oral 150 mg/day of imipramine ($n=138$), for 6 weeks. The antidepressant efficacy of SAMe is comparable with that of imipramine, but SAMe is significantly better tolerated. An 8-week open-label study was conducted on 20 HIV-seropositive subjects diagnosed with MDD. Patients were treated with 200–800 mg of SAMe twice a day, adjusted according to the severity of symptoms and clinical treatment response, and a daily supplementation of 1,000 mcg vitamin B12 and 800 mcg folic acid.

A significant acute reduction in depressive symptomatology was observed, evident as soon as week 1 [63]. A randomized double-blind crossover placebo-controlled trial evaluated the effectiveness and safety of using SAMe (800 mg daily) to treat depressive disorder, attention deficit/hyperactivity disorder (ADHD) and cognitive deficits in individuals with 22q11.2 deletion syndrome. No significant differences in the safety were reported between SAMe and placebo. Individuals affected with 22q11.2 deletion syndrome with co-morbid depressive disorder (with/without psychotic symptoms) reported a good improvement on relevant clinical scales compared to placebo [33]. A double-blind, randomized, placebo-controlled clinical trial of SAMe versus escitalopram was conducted on a sample of 189 outpatients with MDD. Eligible patients were randomized for 12 weeks to SAMe (1,600–3,200 mg/daily), escitalopram (10–20 mg/daily) or placebo. No significant differences in response rates were observed in SAMe (36 %) vs. escitalopram (34 %) vs. placebo (30 %) groups. Remission rates were 28 % for SAMe, 28 % for escitalopram and 17 % for placebo. Significant differences were only found in the side-effect pattern between the escitalopram and SAMe group [47]. A recent RCT [61] assessed the antidepressant efficacy of SAMe versus escitalopram and a placebo control. Eligible patients were randomized to SAMe (1,600–3,200 mg/daily), escitalopram (10–20 mg/daily) or placebo group for 12 weeks of double-blind treatment. Response rates at endpoint were superior for escitalopram (45 %) vs. SAMe (31 %) vs. placebo (26 %), whilst remission rates were reported significant superior for SAMe (24 %; $p=0.003$) vs. escitalopram (23 %, $p=0.023$) vs. placebo (6 %).

49.2.2 Studies on SAMe as an Adjunct to Antidepressant Drugs for MDD

Studies assessing adjunctive SAMe with antidepressant drugs have evaluated an improvement in depressive symptomatology and cognitive functions in depressed patients [17]. A double-blind clinical trial reported an accelerated symptom improvement when SAMe (200 mg IM vs. placebo) is given in combination with a fixed dose of 150 mg/day oral imipramine [8]. Chinchilla et al. [20] reported that the supplementation with SAMe (100 mg IM vs. placebo) shortened the latency of response to fluoxetine (20 mg/day). A double-blind and randomized RCT [55] reported the results of an adjunctive trial of SAMe in 73 patients with MDD who had failed a prior SSRI/SNRI trial at an adequate dose for at least 6 weeks. Patients were randomized to SAMe (800 mg/twice daily) or placebo, both added to the ongoing antidepressant therapy and continued for 6 weeks. Group with adjunctive SAMe showed a significant improvement, compared to placebo group, both in response rate (36.1 % vs. 17.6 %) and in remission rates (25.8 % vs. 11.7 %). A secondary analysis of a single-centre, 6-week, randomized, double-blind clinical trial involving the use of adjunctive SAMe to SSRIs/SNRIs non-responders affected with MDD reported greater response and remission rates as well as an improvement in memory-related cognitive symptoms compared to placebo [41].

49.2.3 Studies on SAMe as an Adjunct for Treatment-Resistant Depression (TRD)

Rosenbaum et al. [58] conducted an open trial in 20 outpatients with MDD after oral intake of SAMe. A significant improvement was observed both in non-treatment-resistant and treatment-resistant group. A 6-week open trial was conducted on 30 antidepressant-treated adult outpatients with persisting MDD (i.e. partial or non-responders). Subjects were treated with 800–1,600 mg daily of SAMe. Following augmentation with SAMe to ongoing SSRI or venlafaxine, a response rate of 50 % and a remission rate of 43 % were observed on the HAM-D [2].

A randomized and double-blind, placebo-controlled adjunctive trial of SAMe (400 mg twice daily) vs. placebo added to an SSRI was conducted on a sample of patients with TRD. An

improvement in depressive symptomatology was reported for adjunctive SAMe (36.1 % of response and 25.8 % of remission) vs. placebo (25.8 % of response and 11.7 % of remission). SAMe also showed a good tolerability/safety as augmentation strategy in SSRIs non-responder patients with MDD [55]. An open-label, fixed-dose (800 mg/day), single-blind study was conducted on 25 outpatients with stage II TRD in order to evaluate the efficacy of SAMe as augmentation strategy to existent antidepressant (e.g. venlafaxine, agomelatine, sertraline, mirtazapine, escitalopram, duloxetine or bupropion). At 8 weeks, a significant decrease in Hamilton Rating Scale for Depression (HAM-D) score was observed (60 % of response; 36 % of remission) as well as in Snaith-Hamilton Pleasure Scale (SHAPS) and Sheehan Disability Scale (SDS) scores [24].

49.3 Side Effects and Pharmacological Interactions

Studies published to date suggest that SAMe is generally well tolerated and its side-effect profile is extremely favourable. The most frequently reported intolerable effects include mild gastrointestinal symptoms (e.g. nausea, stomach discomfort and diarrhoea), sweating, vertigo, dizziness, irritability, insomnia, tachycardia, restlessness and anxiety. Other side effects reported include anorgasmia, diminished mental acuity and hot flashes [46]. An increased risk of serotonin syndrome has been reported if combined with dextromethorphan, meperidine, tramadol and antidepressants with serotonergic activity. A case report reported the onset of altered mentation, fever, hyperreflexia and elevated creatine phosphokinase when SAMe (100 mg IM) was combined with clomipramine (75 mg) in a 71-year-old woman [36].

A study reported an increases risk of manic induction in vulnerable patients [16, 50]. A case report described a 61-year-old-woman with no previous history of suicidal ideations who self-prescribed SAMe for her depressive symptoms and attempted suicide 4 days later by burning herself [21].

49.4 Pregnancy and Breastfeeding

To date, there are no studies that have specifically evaluated the efficacy of SAMe in antenatal depression [27]. However, some studies evaluating efficacy of SAMe in the treatment of cholestasis during the pregnancy were conducted on a sample of pregnant women. These studies have not reported any adverse events for either mother or infant [35].

A placebo-controlled study reported a significant decrease in depressive symptomatology (75 % of reduction in 30 days) compared to placebo, in a sample of postnatal depressed women treated with doses of SAMe up to 1,600 mg daily [19]. There have been no reports of side effects or adverse events in infants who are breastfed during maternal use of SAMe.

However, limited evidence recommends a careful evaluation of its use during the perinatal period.

49.5 Concluding Considerations

These findings suggest interesting perspectives for the use of SAMe in MDD. Most clinical studies involve parenteral or IM injections of SAMe, rather than oral preparations. Mode of administration should be considered when interpreting findings of clinical studies (different pharmacokinetic profiles).

Several placebo-controlled RCTs and meta-analyses have demonstrated that SAMe (200–1,600 mg/daily) is more efficacious than placebo and superior or equivalent to tricyclic antidepressants, in the treatment of MDD. However, few studies were conducted on patients affected with TRD, especially as adjunctive strategy in stage II TRD patients [2, 34]. However, limited data have shown that SAMe augmentation at 800 mg/day may be an option for the treatment of stage II TRD patients, especially due to its few side

effects (i.e. constipation, nausea with decreased appetite) [24]. In addition, SAMe may be an effective complementary therapy in paediatric depressive disorders [9, 62].

However, SAMe should be used with caution in patients with a history of hypomania/mania, due to concerns over potential switching from unipolar depression to mania.

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The Role of Antiepileptic Drugs in Bipolar Depression

50

Juan D. Molina, Manuel Durán, Francisco López-Muñoz, Cecilio Álamo, and Francisco Toledo-Romero

Abbreviations

AA	Atypical antipsychotic"+": adjunctive therapy
AC	Anticonvulsant
BAP	British Association for Psychopharmacology
BMI	Body Mass Index
CANMAT/ ISBD	Canadian Network for Mood and Anxiety Treatments/International Society for Bipolar Disorders
CBZ	Carbamazepine
CVD	Cardiovascular disease
GBP	Gabapentin
Li	Lithium
LMT	Lamotrigine
LUR	Lurasidone
MDF	Modafinil
NICE	National Institute of Clinical Excellence
QTP	Quetiapine
RCT	Randomised clinical trial
SSRI	Selective serotonin reuptake inhibitors
TPR	Topiramate
VPA	Valproate
WFSB	World Federation of Societies of Biological Psychiatry

J.D. Molina, MD, PhD (✉)
Acute Inpatients Unit, Dr. R. Lafora Psychiatric Hospital, Carretera de Colmenar Viejo, Km. 13,800, Madrid 28049, Spain

Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain
e-mail: jmolinamar@hotmail.com

M. Durán
Acute Inpatients Unit, Dr. R. Lafora Psychiatric Hospital, Carretera de Colmenar Viejo, Km. 13,800, Madrid 28049, Spain

F. López-Muñoz
Faculty of Health Sciences, Camilo José Cela University, Villanueva de la Cañada, Madrid, Spain
Neuropsychopharmacology Unit, Hospital 12 de Octubre Research Institute (i+12), Madrid, Spain

Portucalense Institute of Neuropsychology and Cognitive and Behavioural Neurosciences, Portucalense University, Porto, Portugal

C. Álamo
Department of Biomedical Sciences (Pharmacology Area), Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain

F. Toledo-Romero
Psychiatry Service, "Virgen de la Arrixaca" Teaching Hospital, Murcia, Spain

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50.1 Introduction

In addition to epilepsy, with which bipolar disorder (BD) presents some common characteristics, such as an episodic nature, anticonvulsants are today used in neurology and psychology for the treatment of other central nervous system pathologies. The different mechanisms of action would explain their efficacy in the treatment of BD (modulation of GABAergic and glutamatergic neurotransmission, changes in voltage-gated ion channels or intracellular signalling pathways). The anticonvulsants approved for use in the treatment of BD, carbamazepine (CBZ), valproate (VPA) and lamotrigine (LTG), were the only ones to demonstrate clinical efficacy. One of the limitations to the development of drugs with a greater mood stabiliser effect is the lack of knowledge concerning the mechanism for treating bipolar disorder [1].

In patients with bipolar disorder, despite the fact that manic episodes are the most prevalent clinical symptom with diagnosis being based on the appearance of mania or hypomania, depression is presented for longer periods throughout the natural history of the disease.

In bipolar I disorder (BD-I), the symptoms and depressive episodes are three times more frequent than manic/hypomanic, with a ratio of 37:1 in BD-II [2–4].

In addition to depressive episodes, where family, social and occupational aspects are seen to be affected, the greatest dysfunction in patients is produced by subsyndromal symptoms. Both aspects determine an increased risk of relapse. The risk of suicide is also increased in the depressive phase, this probability being one of the highest among all mental diseases.

Patients in the depressive phase seek treatment two or three times more than in the manic phase [5]. In rapid cycling BD, there is a greater refractoriness to treatment in depressive than in manic/hypomanic phases. Many patients with bipolar depression are mistakenly diagnosed of major depression or their diagnosis is delayed [6, 7]. In addition, the efficacy of conventional antidepressants in bipolar depression is not well established [8]; as a result, there is a greater risk of disability, co-morbidity and suicide as a result of not receiving the most appropriate treatment [9].

Therefore, it is necessary to treat not only the acute episodes but also to prevent relapses, so greater effectiveness and tolerability should be sought while minimising any possible secondary effects in order to achieve correct adherence to treatment [10].

Despite the repercussions on the life of the patient and the prognosis of their disease, there has been scarce investigation into the treatment of bipolar depression in comparison to clinical studies of mania [11].

Among the treatments proposed for acute episodes of bipolar depression are mood stabilisers, antidepressants and anticonvulsants. During the last few years, atypical antipsychotics, especially quetiapine and olanzapine, have been demonstrated as superior to antidepressants due to their lower tendency to cause mood swings [12, 13].

With respect to maintenance therapy, prolonged use of antidepressants has not been recommended due to the risk of mood swings; however, they are widely used despite there being little evidence of efficacy. Only the combination of fluoxetine with olanzapine has been approved by the FDA. It is at this point that anticonvulsants, given their properties as mood stabilisers with less secondary effects and monitoring problems than lithium, which imply simplified treatment management, have appeared as an alternative that could be considered [14].

The concept of polarity has acquired special importance as patients tend to relapse with the same polarity as experienced during their first episode [15].

Distinct clinical guides place anticonvulsants as first- and second-line drugs for both acute episodes and maintenance therapy [16–20]; however, with the exception of fluoxetine, no other anticonvulsant has been approved by the FDA for treating acute episodes of bipolar depression.

This chapter examines the role of anticonvulsants both in the treatment of acute episodes as well as in maintenance therapy. It will discuss the place occupied by anticonvulsants in distinct clinical guides and consensuses and the correlation with use in clinical practice and make a

drug-by-drug analysis of the different clinical evidence in relation to their use.

50.2 Anticonvulsants

50.2.1 Valproate

50.2.1.1 Treatment of Acute Depression

Few studies have evaluated the efficacy of valproate in monotherapy for the treatment of acute bipolar depression [21].

In a 6-week, double-blind, randomised, placebo-controlled trial that included 25 patients with bipolar I depression, valproate was shown to be more effective than placebo in improving symptoms of depression and anxiety. New, larger multicentric trials will be needed to confirm these results. However, the antimanic properties, which are lacking in antidepressants and lamotrigine but are possessed by valproate [22], together with improved tolerability due to lower weight gain compared to antipsychotics, make it an interesting alternative treatment [23].

Another study of monotherapy with valproate, which endeavoured to determine the efficacy of extended-release divalproex to treat acute nonrefractory bipolar depression, demonstrated a significant reduction in Montgomery-Asberg Depression Rating Scale (MADRS) scores versus placebo. The analysis of MADRS items showed a greater improvement in core mood symptoms than those obtained for anxiety and insomnia. In addition, to a lesser although statistically significant degree, an association was observed between MADRS and the Mania Rating Scale scores compared to placebo. Some improvement could be seen in the depressive mixed state. As in the previous study, the small sample size ($n = 18$) means that new randomised clinical trials with a larger number of patients are required [24].

Another subsequent study, which in this case included 54 patients with depression and bipolar I or II disorder, suggested that extended-release divalproex sodium would be effective and well tolerated by patients with bipolar depression who had not been previously treated with mood stabilisers. This effect would be even more evident in rapid-cycling type I patients. The analysis of subgroups showed that there were no differences between valproate and placebo in patients with bipolar II disorder. The most common secondary effects were nausea, increased appetite, dry mouth and cramps. Again, as in the two previous studies, the small sample size means that larger studies are required in order to confirm the results [25].

In two recent reviews that analysed the efficacy and tolerability of valproate in the treatment of acute bipolar depression, and which included a meta-analysis of four double-blind, randomised clinical trials that included the three cited above, it was observed that this drug was effective and well tolerated. Valproate (39.33 %) was superior to placebo (17.5 %) with respect to response to treatment and remission (40.6 % valproate) [26]. There was no evidence of differences between the two groups relative to manic symptoms, discontinuation of treatment, lack of efficacy or adverse effects. Although not statistically significant, more cases of nausea were observed among those who took valproate [27]. However, once again, there is the setback of the small sample sizes included in the studies ($n = 142$); thereby, new studies that include larger numbers of patients are required to confirm these findings.

The combination of lithium with valproate in the treatment of bipolar depression displays similar results to the combination of lithium or valproate with antidepressants [28].

50.2.1.2 Maintenance Therapy

In one study of the efficacy of divalproex as maintenance therapy for the prevention of bipolar depression, valproate improved numerous symptomatic aspects of depression and reduced the probability of relapse, especially in patients who had previously responded to divalproex or those with more serious disease [29].

A controlled study with lithium versus placebo, which included 372 bipolar patients who were randomised to maintenance therapy with divalproex, lithium or placebo, did not demonstrate any improvement over placebo in relapse prevention in patients whose manic symptomatology had remitted for at least 3 months. The main

variable of the study was the time until first recurrence or any mood episode. Valproate did not demonstrate any superiority over placebo in preventing recurrences unlike lithium, which was superior. Valproate was superior to placebo in secondary analyses, with lower rates of discontinuation due to recurrent manic or depressive episodes and also in preventing relapses in severe patients. In addition, it was superior to lithium with a longer duration of successful prophylaxis and less deterioration in depressive symptoms and Global Assessment Scale Scores [30].

50.2.2 Carbamazepine

Carbamazepine is presented as an alternative or adjunct to lithium, which is inferior in efficacy except in atypical forms of the disease, BD-II, mood-incongruent psychotic symptoms, as well as in patients with bipolar disorder and concomitant somatic diseases [31].

In patients with severe depression prior to initiation of treatment, the response to carbamazepine seems best as it can enhance the effectiveness of other drugs, including lithium [32].

It is effective at preventing relapse [33]; however, among its limitations are the need for regular monitoring, its side effects, its interactions with many atypical antipsychotics and antidepressants which are often used in tandem and its teratogenicity.

50.2.2.1 Acute Bipolar Depression

In a double-blind, randomised placebo-controlled trial of carbamazepine to evaluate efficacy in the treatment of bipolar disorder, out of the whole sample ($n=24$), 5 of the 13 patients who presented clinical depression displayed a notable improvement in depression ratings [34].

Another double-blind, off-on-off trial to study the severe antidepressant effects of carbamazepine included 35 patients with DSM criteria for acute depression, of which 24 presented bipolar I or II disorder. Carbamazepine was administered after a period with placebo. A moderate improvement was displayed by 20 patients and a considerable improvement by 12. The highest rate of remission was among depressed patients diagnosed with bipolar disorder compared to those diagnosed with unipolar depression [35].

In a more recent study, monotherapy with carbamazepine displayed a great improvement in the Global Impressions-Severity of Depression (CGI-S) Scale with respect to placebo as well as higher rates of clinical response in bipolar depression (63.8 % vs. 34.8 %, $p=0.044$) [36].

A clinical trial was performed with levetiracetam in combined therapy; however, the results were not very encouraging as they were no better than the placebo [21, 37].

Faced with the lack of clinical evidence in the studies of carbamazepine for acute bipolar depression carried out up to now, its use as monotherapy cannot be recommended. A limited number of small, generally open studies have been performed in the majority with unfavourable results [36, 38], and only a few were moderately favourable [39].

Even so, the latest CANMAT guide update [16] suggests that there is new evidence supporting the use of carbamazepine as a third-line drug. This originates from a small random controlled trial ($n=44$) which demonstrated a similar efficacy with extended-release and instantaneous-release carbamazepine but with lesser secondary gastrointestinal and autonomic effects [40, 41]. This then could be a second-line drug in monotherapy as well as in combination with lithium, valproate or antidepressants + lamotrigine.

50.2.2.2 Maintenance Therapy

Due to tolerance and interaction problems, the most indicated use has been as a second-line drug in the treatment of patients who do not respond to lithium.

Carbamazepine is effective in the prevention of manic as well as depressive episodes as shown in a study of fourteen controlled or partially controlled clinical trials [42].

In one placebo-controlled study carried out in a small group of patients, carbamazepine was shown to be superior (60 % with valproate had a good response while only 22.2 % of the placebo group reported this improvement) [43].

A meta-analysis of fourteen randomised controlled trials ($n=464$) [44], which compared the

efficacies of carbamazepine and lithium, demonstrated that they were equally effective at preventing relapses, and in the case of carbamazepine, there were also less patient dropouts due to this drug producing less secondary effects than lithium.

One aspect to take into account in long-term treatment with carbamazepine is its lower antisuicidal effects compared to lithium or valproate [45].

50.2.3 Lamotrigine

Investigation into the use of lamotrigine in patients with bipolar depression began after the observation of clinical improvements in bipolar disorder patients treated with this drug [46, 47].

Lamotrigine is more effective in the depressive pole of bipolar disorder with greater clinical evidence in the prevention of recurrences [48], especially in patients with a large number of depressive episodes and in BD-II [49].

50.2.3.1 Treatment of Acute Bipolar Depression

Among the evidence regarding the efficacy of lamotrigine in the treatment of bipolar depression is a meta-analysis of five randomised clinical trials that included 1,072 patients diagnosed of this disorder and compared lamotrigine (100–400 mg/day) with placebo. An improvement of greater than or equal to 50 % of the depression evaluation scale baseline occurred more frequently in patients who received lamotrigine (44 %) than those who received placebo (35 %). Subsequent to the analysis, in one subgroup, it was found that lamotrigine was superior to placebo for severe primary depression, but not for moderate depression. Rates of treatment discontinuation were similar in the lamotrigine and placebo groups [50].

In one double-blind, randomised controlled trial comparing lamotrigine with citalopram in a sample of bipolar depression patients treated with a luteinizing hormone, each group experienced reduced MADS but without any significant differences between them [51].

One study demonstrated that adding lamotrigine to lithium is better than placebo in depressed patients [52].

In the combined results of five double-blind, randomised, placebo-controlled trials, four did not demonstrate any significant differences with respect to placebo in the main HAM-D (Hamilton Depression Scale) and MADRS (Montgomery-Asberg Depression Scale) variables [47].

In a study which evaluated the efficacy and safety of lamotrigine as a coadjuvant treatment with lithium in bipolar I and II depression, it was shown to be effective, superior to placebo and safe in combination with lithium [52].

50.2.3.2 Maintenance Therapy

Faced with the limitations of lithium, especially in long-term maintenance therapy, alternatives are required.

The efficacy of lamotrigine as maintenance therapy seems well established, especially for its efficacy in delaying relapse episodes and a predominantly antidepressive polarity index [53, 54].

A combined analysis of randomised clinical trials with lamotrigine versus placebo showed that over a period of 18 months lamotrigine-treated patients experienced a 36 % reduction in relapses [55].

Another review of clinical trials demonstrated its efficacy as maintenance therapy in BD-I, supporting the prophylactic effect of lamotrigine and its superiority to placebo, especially in preventing depressive episodes [56].

An 18-month double-blind, randomised, controlled clinical trial in BD-I patients with recent depression showed that lamotrigine and lithium were superior to placebo at avoiding any other type of mood episode. Lamotrigine was superior to lithium for depressive episodes, and lithium was superior to lamotrigine for manic/hypomanic episodes [57].

Combined Li+LMT therapy was demonstrated as superior to placebo for up to 68 weeks [58]. In a study of response to lamotrigine in a large group of patients with bipolar depression in whom seven HDRS-31 scale factors were identified (1-“depressive cognitions”, 2-“psychomotor retardation”, 3-“insomnia”, 4-“hypersomnia”, 5-“appetite and weight change”, 6-“anxiety” and 7-“anergia”), the results suggested that lamotrigine

provided benefits in depressive cognition and psychomotor slowness [59].

The risk of developing Stevens-Johnson syndrome and the latency period of the effects of lamotrigine, which can be up to 3 weeks, associated with its slow titration, imply a disadvantage that must be taken into account in its therapeutic management.

50.2.4 Other Anticonvulsants for the Treatment of Bipolar Depression

Up to now, there have been no randomised, controlled trials with oxcarbazepine, eslicarbazepine, retigabine, pregabalin, zonisamide, felbamate or vigabatrin in the treatment of acute bipolar depression.

50.2.4.1 Levetiracetam

In the latest update of the CANMAT 2013 guidelines, the use of levetiracetam as coadjuvant therapy in the treatment of bipolar depression has been added as "not recommended" [16].

One 6-week, double-blind, placebo-controlled clinical trial which included 32 patients with bipolar depression did not find any significant differences in depression scale changes between levetiracetam as coadjuvant and placebo. The patients in the group treated with levetiracetam as coadjuvant who were also receiving other drugs (antidepressants, antipsychotics, mood stabilisers and tranquilisers) did not demonstrate any differences with respect to placebo in the principal result (the change in HAM-D scores) [37].

50.2.4.2 Oxcarbazepine

This has been used, but not continuously, as an alternative to carbamazepine in bipolar patients in cases of intolerance to the latter, or when it has been necessary to administer other drugs that interact with the same. Even so, oxcarbazepine, the 10-keto analogue of carbamazepine, also interacts with other drugs and the risk of hyponatraemia can be higher than with carbamazepine [60].

In a double-blind, randomised controlled trial to study the effects and safety of oxcarbazepine and carbamazepine as adjuvant treatments with lithium in bipolar I and II patients, oxcarbazepine demonstrated better results with respect to tolerability and efficacy in reducing depression rating scale scores (YMRS, HDRS-21, MADRS, CGI-S and CGI-I) [61].

Another 12-month, double-blind, randomised controlled trial including 55 patients evaluated the long-term tolerability as adjuvant treatment with lithium as well as the prophylactic efficacy of oxcarbazepine in patients with bipolar I and II disorders. The first variable for determining the efficacy was the time in remission measured by YMRS and MADRS. The time to recurrence with oxcarbazepine was greater than with placebo.

Ten patients from the oxcarbazepine group ($n=26$) suffered relapse of some type, whereas this amounted to 17 in the placebo group ($n=29$). Depressive episodes occurred less frequently in the oxcarbazepine group which would suggest a greater influence on the depressive pole. The small study group could explain why the difference was not statistically significant [62].

A systematic review which evaluated the recurrence of episodes, patients' overall function, adverse events, tolerance and mortality, did not find sufficient evidence to be able to recommend the use of oxcarbazepine for maintenance therapy in bipolar disorder [63].

50.2.4.3 Pregabalin

Pregabalin is a structural analogue of GABA which is similar to but more potent than gabapentin. An open study in a group of ambulatory, treatment-resistant bipolar patients prospectively evaluated the efficacy of pregabalin as coadjuvant in acute treatment and maintenance therapy.

The study compared the baseline mood prior and subsequent to initiating treatment with pregabalin. The Clinical Global Impression-Bipolar Version Scale (CGI-BP) was used for patients who responded to initial treatment, which lasted for a minimum of 2 months and subsequently followed by a 3-year period of maintenance therapy.

It was observed that pregabalin produced mood stabilisation as well as antidepressant and antimanic effects. Double-blind, randomised trials will be required to confirm these findings [64].

50.2.4.4 Topiramate

With the inconvenience of having been a single-blind trial, a study of the tolerability of topiramate and bupropion coadjuvant to a mood stabiliser, which included 36 ambulatory patients, suggested that topiramate as adjuvant treatment could reduce the severity of acute depressive symptoms. The antidepressant efficacy therefore requires confirmation through double-blind, placebo-controlled clinical trials [65].

50.3 Anticonvulsants in the Clinical Guides for the Treatment of Bipolar Depression

Appropriate adherence to the clinical guides for bipolar disorder can be effective to improve the results in patients.

Two studies compared a group of patients who had been treated in accordance with clinical guides with another group whose treatment had not explicitly followed the same. The first group demonstrated significant improvements during the initial and subsequent stages of the treatment as well as in manic symptoms. Adherence to treatment proposed by the clinical guides was associated to greater reductions in depressive symptoms [66, 67].

A survey of patients treated as outpatients in a Canadian mental health centre observed that inadequate medication dose in bipolar II patients was one of the causes of non-conformity with the results in clinical guides [68].

Tables 50.1 and 50.2 show the recommendations given in various clinical guides for the treatment of acute bipolar depression and maintenance therapy with respect to anticonvulsants.

Clinical guides represent a useful support to the clinician's professional judgement which, on many occasions, will assist them when deciding which drugs not to prescribe.

The integration of clinical guide recommendations in daily clinical practice and the particular

Table 50.1 Anticonvulsants listed in clinical guides for the treatment of acute bipolar depression

Guidelines	BAP [17]	WFSBP [18]	CANMAT/ISBD [16]	ICG [19]	NICE [20]
First line	Moderate: VPA VPA+SSRI LMT+Li – Less severe: LMT	VPA LMT+Li LMT	LMT VPA+SSRI Li+VPA VPA+BPP	BD-I: LMT	AM+SSRI
Second line		CBZ MDF+VPA	VPA VPA+LMT[a] VPA + LUR[a]	BD-I: +LMT	
Third line/other		TPR+GBP	CBZ Li+CBZ VPA+VLF VPA+TCA VPA/ CBZ+SSRI[b]+LMT QTP+LMT[a]	BD-I: AC+QTP VPA CBZ	
Not recommended			GBP		LMT, VPA[c]

+=adjunctive therapy
[a]New or change to recommendation
[b]Except paroxetine
[c]In women of childbearing potential

Table 50.2 Anticonvulsants listed in clinical guides for maintenance therapy in acute bipolar depression

Guidelines	BAP [17]	WFSBP [18]	CANMAT/ISBD [16]	ICG [19]	NICE [20]
First line	LMT	LMT	LMT VPA VPA+QTP/RLAI/ARP/ZIP	BD-I: LMT	VPA
Second line	LMT+AA	VPA CBZ	CBZ Li+VPA Li+CBZ VPA+OLZ Li+LMT	BD-I: +LMT	
Third line/other			+TOP +OXC	BD-I: AC+QTP VPA CBZ	
Not recommended			GBP TOP		LMT[1], VPA[1]

+Adjunctive therapy
[1]In women of childbearing potential

characteristics of each patient are the perfect combination to carry out the most effective treatment [69].

The CANMAT/ISBD [16] and WFSPB [18] guides place lamotrigine as a first-line drug for the treatment of acute bipolar depression. Valproate is also included as first line in combination with antidepressants. The NICE [20] guides on their part recommend lamotrigine as an adjuvant. The BAP [17] guide includes lamotrigine and valproate in monotherapy, as well as the combination of antidepressants with valproate, as first-line drugs. However, there does not seem to be agreement in the time required to estimate whether a patient is a nonresponder or partial responder to treatment. The WFSFBP [18] guide considers that 4 months is needed to evaluate changes in treatment when there is no evidence of clinical improvement. Tables 50.1 and 50.2 list the indications contained in various clinical guides with respect to anticonvulsants for treatment of depression and maintenance therapy.

50.4 Secondary Effects of Anticonvulsants

Patients undergoing treatment for bipolar disorder and bipolar depression are usually polymedicated. Drugs that are not providing a real benefit could contribute to possible secondary effects. Approximately 40 % of patients take three or more drugs and 18 % four or more [70].

Table 50.3 lists the secondary effects of the drugs that are most commonly used in bipolar depression.

50.5 Recommendations for Monitoring Treatment with Anticonvulsants

Due to the co-morbidity present in patients with BD as well as the secondary effects of their medication, it is vitally important to follow-up and monitor those undergoing treatment.

Before initiating treatment, a clinical history must be taken which includes concomitant pathologies, without forgetting risk factors for cardiovascular disease (CVD), alcohol and/or tobacco consumption, family history of CVD risk factors and also to rule out any possible pregnancy. The abdominal circumference should be measured and/or body mass index (BMI) calculated and blood pressure measured. In addition, analyses must include haemogram, ions, urea, creatinine, hepatic function panel, renal profile, fasting blood glucose and lipid profile. Table 50.4 shows the steps to be followed to carry out periodic monitoring [71].

Table 50.3 Secondary effects of anticonvulsants

	Important side effects or that could affect therapeutic compliance	Serious or life-threatening secondary effects
VPA	Alopecia Drug interactions Tremor Weight gain	Evidence of risk in pregnancy Hepatotoxicity Hyperammonaemia Pancreatitis Stephens-Johnson syndrome
CBZ	Ataxia Drug interactions Rash	Agranulocytosis Aplastic anaemia AV block/bradycardia Evidence of risk in pregnancy SIADH/hyponatraemia Stephens-Johnson syndrome Thrombocytopaenia
LMT	Impaired vision Headache Cognitive impairment Peripheral oedema Drug interactions Rash	Risk in pregnancy cannot be ruled out Stephens-Johnson syndrome
OXC	Ataxia Drug interactions Rash	Agranulocytosis Aplastic anaemia AV block/bradycardia Evidence of risk in pregnancy SIADH/hyponatraemia Stephens-Johnson syndrome Thrombocytopaenia
TOP	Impaired vision Anorexia Cognitive impairment Nystagmus Weight loss Paresthesias	Decreased serum bicarbonate Leucopaenia Nephrolithiasis Thrombocytopaenic purpura
GBP	Peripheral oedema Requires adjustment for renal function	Decreased serum bicarbonate Leucopaenia

Modification of VA/DoD clinical practice guideline for management of bipolar disorder in adults 2010

Table 50.4 Monitoring recommendations

	VPA	CBZ	LMT
Initial	Haematologic/hepatic history	Haematologic/hepatic history	
Serum levels	Two titrations to establish therapeutic dose	Two titrations to establish therapeutic dose (4 weeks apart)	
Follow-up	Weight Complete haemogram Hepatic profile Evaluate menstrual cycle alterations Frequency: 1 year: tri-monthly Subsequently: annually Evaluate bone status	Complete haemogram Hepatic profile Ions, urea, creatinine Revise oral contraceptive effectiveness Frequency 3 months: monthly Subsequently: annually In case of rash, suspend medication and consult in the first 24 h Evaluate bone status	In case of rash, suspend medication and consult in the first 24 h

Modification of Felicity Ng et al. The ISBD consensus guidelines for the safety monitoring of bipolar disorder treatments

50.6 Recommendations in Relation to the Use of Anticonvulsants in the Treatment of Bipolar Depression During Pregnancy

Table 50.5 lists the risk assessment of the main anticonvulsants in pregnancy and lactation.

The NICE and AFBPN guides both recommend suspension of anticonvulsants (VPA, CBZ, LMT). The BAP mentions the teratogenic risk of anticonvulsants in descending order (VPA>CBZ>LMT) and suggests that VPA and CBZ should be avoided. The WFSBP guide recommends close monitoring and risk evaluation in every case. A review of available evidence in the ICG guide recommends that VPA should not be used as first-line treatment and advises strict monitoring as well as evaluation of the risk and benefit of continuing or suspending treatment.

50.7 Risk of Suicide

Depressive symptoms are responsible for most of the morbidity in BD and are associated with an elevated risk of suicide and relapses [72]. It is estimated that, throughout their life, between 50 and 75 % of bipolar patients attempt to inflict self-harm [73].

Table 50.5 Anticonvulsants in pregnancy and lactation

	Risk in pregnancy (FDA)	American Academy of Pediatrics classification	Classification of risk in lactation
VPA	D-positive evidence of risk	Compatible	L2= safer
CBZ	D-positive evidence of risk	Compatible	L2= safer
LMT	C-risk cannot be ruled out	Unknown	L3= moderately safe

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The risk of suicide is almost three times greater in patients treated with anticonvulsants (VPA, LMT, CBZ) than those treated with lithium as confirmed in a meta-analysis of six trials [74].

Unlike major depressive disorder where antidepressants have demonstrated their efficacy, the efficacy of conventional antidepressants in bipolar depression is not clear. Some treatment guidelines based on available clinical evidence include lamotrigine and the atypical antipsychotic quetiapine for the treatment of bipolar depression. The uncertainties about the treatment of bipolar depression mean that such treatment must be individualised and, on many occasions, empiric [8].

Conclusions

The antimanic properties of valproic with lower weight gain and better tolerability, would make this a drug to be considered for the treatment of acute bipolar depression, although small sample sizes of the studies that were analyzed would need larger studies. Some clinical guides already include valproate as monotherapy in acute bipolar depression or in combination with antidepressants.

In long-term treatment, valproate has been demonstrated to be effective at reducing some symptoms of depression, and in some clinical guides, it is the first-choice drug.

In the light of study results, carbamazepine, which has demonstrated efficacy in the prevention of relapse, especially in the case of patients with severe depression prior to commencing treatment, is not recommended as monotherapy in acute bipolar depression; however, in the CANMAT guide, it is included as a third-line drug.

Carbamazepine could be recommended as second line in maintenance therapy, being effective in preventing both manic and depressive episodes. Negative aspects that should be taken into account are the strict controls and monitoring required together with its lower antisuicidal efficacy compared to lithium.

Lamotrigine, either in monotherapy or in combination with lithium, constitutes one of the first-line drugs for the treatment of acute bipolar depression. The rate of eminently antidepressant polarity and capacity to prevent relapses suggest its great usefulness in long-term treatment, being superior to lithium at preventing relapse of depressive episodes. However, this is not the case in manic episodes.

Discouraging results were obtained in the studies with levetiracetam, and it is included among drugs that are "not recommended" by the CANMAT guide.

Oxcarbazepine was considered as an alternative to carbamazepine, although the former presents a greater risk of hyponatraemia; however, it was shown to be effective, compared to placebo, in preventing depressive recurrence but in small sample sizes. Therefore, larger studies will be required to evaluate these aspects more accurately.

With respect to other anticonvulsants, the lack of randomised controlled trials or negative results, such as with levetiracetam, means that their evaluation for inclusion as drugs of choice in the treatment of bipolar depression is still pending larger, double-blind randomised trials.

Clinical guides imply support for clinicians and, together with their daily clinical practice, the individualised evaluation of each patient. The role of lamotrigine is highlighted as a first-line treatment in either monotherapy or adjuvant therapy.

Anticonvulsants require monitoring and strict control due to their possible secondary effects that include, among others, anticonvulsant hypersensitivity syndrome and hepatic and haematologic manifestations. The teratogenicity of the same must also be taken into account. In general, suspension of anticonvulsants is recommended, this being the largest point of agreement between the different clinical guidelines in relation to VPA and CBZ.

The risk of suicide is greater with anticonvulsants compared to lithium, something which must be taken into account when prescribing and which requires patient monitoring.

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51 Melatonin and Other Neuroprotective Agents Target Molecular Mechanisms of Disease in Amyotrophic Lateral Sclerosis

Anastasios Fotinos*, Yongjin Zhu*, Lilly L.J. Mao, Nazem Atassi, Edward W. Zhou, Sarfraz Ahmad, Yingjun Guan, James D. Berry, Merit E. Cudkowicz, and Xin Wang

51.1 Introduction

Amyotrophic lateral sclerosis (ALS), was first described by French doctor Jean-Martin Charcot in 1869, also known as Lou Gehrig's disease, is a neurodegenerative disorder that involves the loss of upper and lower motor neurons. It causes progressive muscle weakness, atrophy and paralysis, weight loss, and eventually mortality. The prevalence of ALS is approximately 30,000 in the USA with a slight higher incidence in men. About 10 % of ALS cases are familial (fALS) [1]. The most common known mutation that causes fALS is called C9orf72, a hexanucleotide repeat expansion [2]. The second most common mutation is found in the copper/zinc superoxide dismutase (SOD1) gene and results in a toxic gain of function [1]. About 90 % of ALS cases are sporadic (sALS) and associated with no known genetic mutations. Though there are no reliable risk factors for developing ALS, a few environmental risk factors have been proposed, including pesticide exposure, cyanobacteria, and head trauma, with professional soccer players and US war veterans identified as high-risk groups [3].

Riluzole is the only FDA-approved treatment for improving survival in people with ALS [4]. Many potential treatments have been tested in the SOD1 rodent model and in humans with the disease. The goal of this chapter is to update the current state of knowledge in small-molecule therapies used in animal models; to summarize their effects on body weight loss, muscle damage, disease onset, and mortality; compare their structure-activity relationships; and further evaluate their potential success for clinical application.

*Authors Anastasios Fotinos and Yongjin Zhu have made equal contributions

A. Fotinos • Y. Zhu • E.W. Zhou • X. Wang, PhD (✉)
Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School,
Boston, MA 02115, USA
e-mail: xwang@rics.bwh.harvard.edu

L.L.J. Mao • S. Ahmad
Aimcan Pharma Research and Technologies Inc.,
398 Laird Rd, Guelph, ON N1G 3X7, Canada

N. Atassi • J.D. Berry • M.E. Cudkowicz
Neurological Clinical Research Institute,
Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Y. Guan
Department of Histology and Embryology, Weifang Medical University, Weifang, Shandong, China

F. López-Muñoz et al. (eds.), *Melatonin, Neuroprotective Agents and Antidepressant Therapy*,
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51.2 Clinical Manifestations and Experimental Models of ALS

ALS is characterized by muscle weakness and atrophy due to upper and lower motor neuron damage. Disease onset, duration, and survival vary considerably according to age [5], sex [6], race [7, 8], lesion location, and changes in body weight. Weight loss in ALS patients can arise from multiple mechanisms including loss of muscle mass, decreased food intake due to dysphagia, and increased energy expenditure [9–12]. Decreased body weight in the first 2 years after ALS diagnosis significantly correlates with shorter survival and faster rate of progression [13]. It is interesting that the effects of weight loss were found to be independent from dyslipidemia in people with ALS [14].

The contribution of inflammation, another important interactive factor of body weight in the pathogenesis of ALS, has drawn increasing interest [15–17]. Though inflammation in neurodegenerative diseases could have an indirect effect on body weight [18], body weight could be decreased through the failure of protein-energy regulatory mechanisms caused by pro-inflammatory mediators (C-reactive protein, TNF-α, IL-6, and leptin) [19–21].

The most remarkable function of skeletal muscle is the capacity to move freely through the contraction and dilatation of myofibers. Changes in muscular morphological, biomolecular, biochemical, and functional properties during the course of ALS could be considered potential biomarkers.

Skeletal muscle paralysis and muscle atrophy were found in ALS mice, especially in the hind limbs. Morphological methods and MRI showed significant reduction in myofiber diameter and muscular volume of hindlimb muscle in G93A-SOD1 mice. Muscle ultrasonography (MUS) is an easily accessible, inexpensive, and painless method to detect structural muscle changes in ALS. Affected muscles show a diminished thickness and appear whiter (i.e., increased echo intensity). In the diagnostic phase of ALS, muscle ultrasonography reveals marked abnormalities including diminished thickness and increases in echo intensity and fasciculations. Muscle thickness measured by MUS correlates with body weight, functional decline, and grip strength [22].

In some animal studies, histochemistry or immunohistochemistry methods were used to detect changes in enzymes or key proteins in skeletal muscles. The activities of elements of the mitochondrial respiratory chain in skeletal muscle were also decreased in ALS mice, including NADH-dehydrogenase, NADH-cytochrome *c* reductase, succinate-cytochrome *c* reductase, and cytochrome *c* oxidase.

Muscle strength is a clinically relevant measurement of disease progression for ALS and can be measured by a variety of methods. The most commonly used measures in clinical trials are maximum voluntary isometric contraction (MVIC) and manual muscle testing (MMT). In ALS mice, muscle strength, motor coordination, extension reflex, grip strength, stride length, balance beam performance, and tail suspension behavior were obviously weakened. Physical exercise increases muscle strength in animal models and improves functional measurements in people with ALS [23, 24].

Based on the associated and linked genes corresponding to fALS and sALS, a number of ALS animal models have been developed. The first was created in the mouse, encoding a mutation found in fALS that converts glycine residue 93 to an alanine (G93A) in the SOD1 protein [25]. The SOD1 mouse model exhibits all of the histopathological hallmarks observed in fALS and sALS. The mutant mice exhibit pathogenesis of muscle paralysis similar to that observed in clinical cases, including muscle loss and neuromuscular junction disruption. While mSOD1G93A has become the most widely used model for ALS, it accounts for less than 2 % of all clinical cases, all of the mutations in the Cu/Zn superoxide dismutase gene cause 20 % of fALS [26]. Other mutant SOD1 mouse models include G37R, G85R, G127X, D90A, and H46R.

Despite histopathological similarities, the timing of onset and progression vary among the animal models. The SOD1G93A model is reported to be more toxic than H46R, as evidenced by the

difference in onset and duration. In addition, differences in the course of the disease have been found among different congenic strains of the G93A mouse. For example, G93A/B6SJ has a more aggressive progression (around 17–18 weeks' survival) compared to G93A/C57 (about 20 weeks' survival). The number of copies of the mutant SOD1 gene and the particular strain of the transgenic ALS mouse used may also contribute to the discrepancies in observations.

Mainly based on disturbances in RNA metabolism and protein homeostasis, mutations in genes encoding TDP-43 (TAR DNA-binding protein 43), FUS/TLS, TAF-15, ubiquilin 2, and C9ORF72 have also been investigated and a number of animal disease models generated. Among these, transgenic mice carrying a human TDP-43 mutation (A315T) develop features similar to human ALS, while progressive motor neuron (pmn) neuronopathy and wobbler mice are two other animal models used. The pmn model resembles the SOD1 model, with hind limb paralysis and progressive motor neuron degeneration due to a defect in microtubule function for axonal transport [27]. Preclinical drug testing has primarily been performed in a high-copy-number SOD1G93A mouse model of ALS; therefore, new treatments should be tested on more than one animal model to better understand the multifaceted etiology of ALS disease, using the guidelines established by Ludolph [28].

51.3 Mechanism-Based Preclinical Drug Development

The mechanisms underlying ALS are very complex, including oxidant stress, mitochondrial dysfunction, apoptosis, excitotoxicity, SOD1 protein aggregation, and inflammation. Drug treatments have been classified according to which categories of pathogenic mechanisms they target. We will compare their effects on body weight loss, muscle damage, disease onset, progression, and survival.

The evaluation of disease onset and progression provides insight to the efficacy of a drug. It has been noted that the variety of test conditions administered in different experiments may make it difficult to reach definitive conclusions about the effectiveness of a drug. In animal experiments and clinical research, all ALS subjects experience body weight loss, due in large part to muscle atrophy. Body weight is not only the sign of disease onset, but it is positively correlated to prognosis in ALS, and has often been used to evaluate the therapeutic benefits of drugs. Besides body weight measurement, rotarod performance is an important behavioral test frequently used to diagnose disease onset in ALS and estimate muscle function in animal research. The following neurobehavioral tests are also commonly used: (1) Postural reflex test to examine the strength of the forelimbs [29]. (2) Balance beam test to assess body strength and equilibrium [30], with the beam walking test to measure motor coordination and balance in ALS animals [31]. (3) Screen test and paw grip endurance test, also termed hanging wire test [32] to indicate general muscle strength [32, 33]. (4) Tail suspension test or extension reflex test [34] in which the animal is suspended by its tail and the extension of both hind limbs assessed [35] (clinical scoring and endpoints are used to grade the onset of symptoms and improvement). (5) Foot print analysis to indicate whether stride and gait have been altered by ALS disease or treatment [36]. (6) Basso-Beattie-Bresnahan (BBB) locomotor rating used to estimate disease onset [37].

51.3.1 Antioxidant Agents

There is substantial evidence that oxidative damage is a key component in the pathogenesis of motor neuron degeneration in sALS and fALS [38]. This supports the possibility that antioxidant agents can prevent oxidative damage by scavenging free radicals. The fact that such agents minimally increase survival provides evidence that oxidative stress does contribute to disease pathogeneses [38].

Melatonin (*N*-acetyl-5-methoxytryptamine) is an indigenous antioxidant. Its possible role in neurological diseases including ALS has been widely investigated [39–43]. Rival et al. reported

that, like the administration of riluzole to *dEAAT1 RNAi Drosophila*, melatonin significantly enhanced performance in a *Drosophila* model with remarkable similarity to some ALS symptoms [44]. Our findings and those of other researchers in animal models support the results of the *Drosophila* model. In mSOD1G93A mice, melatonin (30 mg/kg) administered by intraperitoneal injection daily beginning at 6 weeks of age [43] elicited a 10 % delay in onset compared to vehicle control, and survival was extended by 7.4 %; additionally, melatonin produced a decrease in motor neuron loss but did not significantly affect body weight loss. At high oral doses [45] via drinking water, melatonin (0.5 mg/ml) starting postnatal day 28 in mSOD1G93A mice significantly delayed disease progression by 10 days (25 %). However, another report of different doses of melatonin in a small number of mice failed to find neuroprotection [46]. Interestingly, we demonstrated that ALS disease progression was associated with alterations in melatonin level in spinal cord samples from mSOD1G93A mice [43]. In contrast, administration after onset showed only a 2-day nonsignificant delay in disease progression. Evidence indicates that melatonin altered the expression of SOD1 in the lumbar spinal cord of neonatal rats [47].

Melatonin also prevented apoptosis in VSC4.1 motoneurons following exposure to the toxins H_2O_2, glutamate (LGA), or TNF-α [48]. The proposed protective mechanisms of melatonin are that it attenuates ROS production; the levels of intracellular Ca^{2+}; the phosphorylation of p38, MAPK, and JNK1; and calpain activity. Melatonin also inhibits cytochrome c and Smac release and subsequent caspase-1, caspase-9, and caspase-3 activation along with restoring melatonin receptor 1A levels within the nervous system, as well as reducing the expression of GFAP and RCA-1 in mSOD1G93A mice [43]. In addition, melatonin decreases receptor-interacting protein-2 upregulation [43].

Furthermore, in an ALS human trial of high-dose enteral melatonin, melatonin offered protection in human ALS and reduced oxidative damage [45, 49]. Chronic high-dose (300 mg/day) [45] melatonin (rectally administered) was well tolerated in sporadic ALS patients [45, 49]. The antioxidant effects of melatonin minimally reduce the pathology of ALS. Because melatonin is neuroprotective in animal models of ALS and has had some positive effects in clinical ALS patients with relatively low toxicity, it should be considered for larger clinical trials as a novel pharmacotherapeutic agent to treat ALS.

Vitamin E (alpha-tocopherol) is a fat-soluble vitamin that quenches reactive oxygen species (ROS). As vitamin E is a major membrane-bound antioxidant, supplementation delays onset and slows progression but has no effect on mortality [50, 51].

Peptide antioxidants target the inner mitochondrial membrane, where a major proportion of ROS are generated. A novel peptide SS-31 (D-Arg-2′, 6′-dimethyltyrosine-Lys-Phe-NH2) has been used in a G93A mouse model, starting at 30 days of age, via daily IP injections of 5 mg/kg. The treatment improved motor function, delayed onset by 7.9 % and progression by 12 %, and improved survival by 9 % [52], but SS-31 had no significant effects on body weight in mice. Another approach to reduce ROS is the use of metalloporphyrins, which catalytically scavenge reactive oxygen and reactive nitrogen species. FeTCPP, an iron porphyrin treatment, has been utilized in a G93A mouse model and shown modest neuroprotection, with an overall increase in survival of 9 days (5 %) [53]. Treatment slowed disease progression from 16 to 25 days, a difference of 54 %. At day 113, oxidative stress markers in both the gray and white matter of the lumbar spinal cord were substantially reduced, as assessed by malondialdehyde staining or by measuring total protein carbonyls. FeTCPP not only improved motor performance but also prevented loss of body weight.

The lipophilic metal chelators DP-109 (1,2-bis(2-aminophenyloxy)ethane-N,N,N′,N′-bis(2-octadecyloxyethyl)ester, N,N′-disodium salt) and DP-460 (N,N′-[1,2-ethanediylbis(oxy-2,1-phenylene)]bis[N-(carboxymethyl)-, 1,1′-bis [2-(dodecyloxy)ethyl] ester, which chelate calcium, copper, and zinc, were tested in the

G93A-transgenic ALS mouse model. DP-109 (5 mg/kg/day) and DP-460 (10 mg/kg/day) significantly delayed onset (by 7.5 % and 9.5 %, respectively) and extended survival (by 10 % and 9 %). Compared with untreated controls, DP109 and DP460 treatment delayed progression by 7 days and 3 days, respectively. There was also a reduction in ALS-related weight loss in both treatment groups, which is directly related to motor performance. However, the differences were not significant [54]. DP-109 or DP-460 also reduces markers of oxidative damage in the lumbar spinal cord of G93A mice [54].

The iron chelator M30 (5-(N-methyl-N-propargylaminomethyl)-8-hydroxyquinoline) exerts multiple pharmacological effects in a motor neuron hybrid cell line [55, 56]. As a radical scavenger and MAO inhibitor, M30 also confers neuroprotective effects by differentially inducing HIF-1α -dependent target genes, showing that it can upregulate a number of neuroprotective molecules and promote survival signaling pathways in the brain, such as increasing axonal growth-associated protein-43 (GAP-43) [57]. Oral gavage of M30 (1 mg/kg) 4 days a week to G93A high-copy transgenic mice beginning at 70 days of age delayed onset by 4.6 %, extended survival by 8 %, and attenuated body weight loss [56].

N-acetylcysteine (NAC) is a precursor to glutathione and is efficiently bioavailable as an antioxidant and liver-protecting agent. Administration of 1 % NAC in drinking water for 9 weeks significantly reduces lower motor neuron degeneration and increases muscle mass and fiber area, as well as the functional efficiency of the forelimbs in wobbler mice, an animal model of fALS [58]. In addition, 1 % NAC in drinking water starting from 4 to 5 weeks of age improves survival by 7 % and preserves motor performance in an SOD1G93A mouse model [59, 60].

EUK-8 and EUK-134 are synthetic, low-molecular-weight, salen-manganese complexes with both superoxide dismutase and catalase activities. In a low-copy-number SOD1G93A mouse model, both agents were given at a dose of (33 mg/kg) three times per week starting at 60 days of age. EUK-8 and EUK-134 did not delay onset but did delay progression (by 21 % or 68 %, respectively) and increased survival (by 7.6 % or 10.4 %). Results suggest that these agents act by slowing progression rather than through prevention [61].

A manganese porphyrin called AEOL 10150 (manganese [III] tetrakis[N-N′-diethylimidazolium-2yl] porphyrin), given after symptom onset, extends survival in ALS mice [62]. Three studies were performed using mSOD1G93A mice to assess combinatorial drug effects and optimal route of administration [62]. The first study gave AEOL 10150 via IP injection, with (5 mg/kg) loading dose on the first day of onset followed by daily IP with (2.5 mg/kg). Treatment delayed progression 2.96-fold and increased survival 1.26-fold [62]. Similarly, another study gave IP injection of AEOL 10150, 2.5 mg/kg/day, started at disease onset which led to a significant increase of survival by 11% [63]. The second study gave intraperitoneal (IP) AEOL 10150 (2.5 mg/kg), along with dietary supplementation of creatine 2 % and rofecoxib 0.005 %, started at disease onset. Treatment delayed progression 2.87-fold and increased survival 1.24-fold, while treatment with only creatine and rofecoxib had no significant effect [62]. The third study administered AEOL 10150 (2.5 mg/kg/day) via subcutaneous (SC) injection, eliciting a 2.43-fold delay in progression and 1.22-fold increase in survival. Histology in all groups showed motor neurons with reduced levels of nitrotyrosine and malondialdehyde relative to vehicle-treated mice [62].

We assessed published data by narrowing the window (Fig. 51.1) during which the drug is most effective and gives the greatest benefit, either in delaying onset or slowing progression, ultimately extending survival. All antioxidant agents were successful in improving survival and slowing the progression of the disease, making them reasonable candidates for use throughout the course of the disease (Fig. 51.1). The greatest increase in survival was 26 %, produced by manganese porphyrin administered after onset. Interestingly, all the agents except manganese porphyrin were given before onset and had a positive but modest effect in delaying onset symptoms. The greatest onset decreases were 13.7 % produced by vitamin E [50] and 9.5 % produced by DP460 [54]. However, these are only modest effects compared

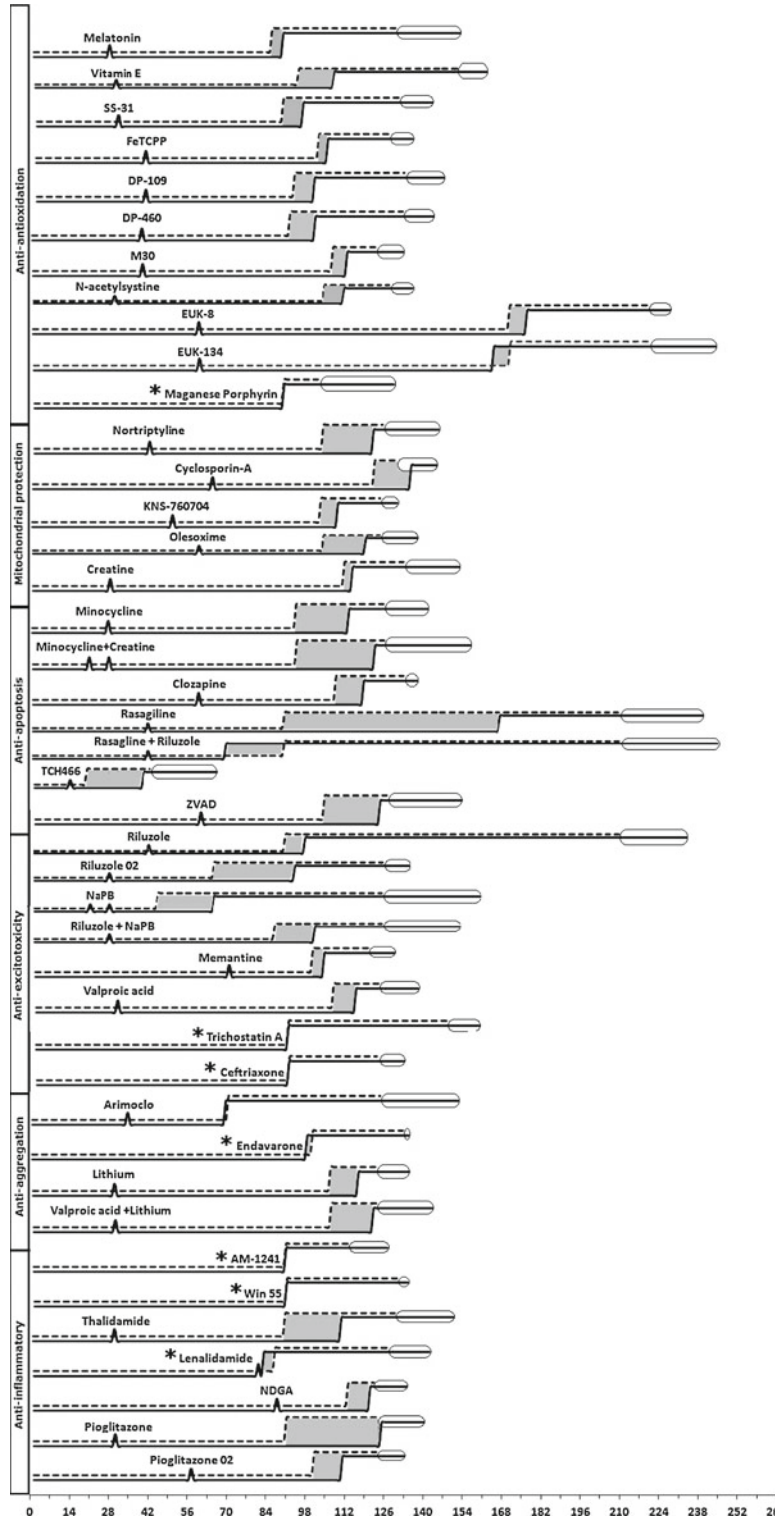


Fig. 51.1 Comparison of effects of neuroprotective agents on disease onset and mortality in ALS animals. Onset and mortality changes produced by neuroprotective agents are displayed graphically based on their action mechanisms. ^: Age of animals for administration of neuroprotective agent. Only available data were shown. ■ and □: Onset and mortality changes as window frames between neuroprotective agent and vehicle control treated animals

to other agent groups, such as zVAD-fmk's 19.5 % [64].

51.3.2 Mitochondrial Protective Agents

Mitochondria are power houses for all the metabolic needs of the cell. However, many ROS are generated during the process of ATP formation in the mitochondria. The mitochondrial permeability transition pore (mPTP) is a state during which proton motive force is disrupted [65, 66]. Among the causes of mitochondrial abnormalities and disruption of the mitochondrial mPTP are oxidative damage and peroxynitrite [67]. When ROS are unmanageable within the mitochondria, this can lead to cell death through the release of pro-death molecules, causing neurodegeneration in the case of ALS.

Nortriptyline is a mitochondrial protective agent that delays disease onset by inhibiting mPTP. In a G93A mouse model, nortriptyline (administered 10 mg/kg or 20 mg/kg) delayed disease onset (by 7 % and 12.5 %, respectively) and increased survival (by 6.9 % and 5.8 %). Disease progression was delayed only in the 10 mg/kg group: 24 days, a 7 % increase compared to the vehicle-treated group. Though 30 mg/kg was also administered, it only slightly delayed onset (3.5 %) and negatively affected survival [68]. Treatment with nortriptyline in mice showed no change for body weight but did improve muscle strength and motor coordination [68].

Cyclosporin A (CsA) prevents mitochondrial transition pore formation and is neuroprotective. Weekly intracerebroventricular (ICV) injections of 20 μg CsA starting 65 days before onset attenuated the decline of motor performance in G93A mice, helped maintain physical performance, preserved body weight, and extended survival by 12 % [69]. A similar study began administration at 3 months, which was chosen as the diagnostic point of late-stage onset. CsA treatment extended survival from the diagnosis point to mortality by 24.2 days, compared to an 11.8-day change with vehicle alone [70].

The dopamine agonist pramipexole (PPX) and its nondopaminergic enantiomer KNS-760704 ((6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine dihydrochloride monohydrate) (also called dexpramipexole, SND919CL2x, or RPPX) have dopaminergic activity and offer neuroprotection through accumulating in the brain, cells, and mitochondria, where they detoxify ROS. As a novel synthetic aminobenzothiazole, KNS-760704 and PPX share the benzothiazole core also present in riluzole [71]. In an SOD1G93A mouse model, treatment with KNS-760704 (100 mg/kg) increased survival by 5.6 %, though PPX (3 mg/kg) produced no significant increase. Muscle activity was measured as the time point of half-maximal motor performance: at day 103 (102–104) for the vehicle-treated group and 109 (108–110) and 106 (105–106) for KNS-760704- or PPX-treated groups, respectively. Thus, unlike PPX, KNS-760704 might permit a sufficiently high dose for reducing ROS without a dopaminergic stimulus [72]. Preclinical studies have shown that KNS-760704 is neuroprotective in vitro and in vivo in SOD1G93A mice [71, 73]. KNS-760704 has been undergoing phase III human trials but has failed such trials conducted by Biogen Idec.

Olesoxime targets the outer mitochondrial membrane proteins [74, 75] and affects microtubule dynamics [76]. Subcutaneous injections of olesoxime to G93A mice delay loss of body weight 15 days and decline in grid performance by 11 days [74]. However, the failure of European phase III clinical trials for treating symptomatic ALS patients highlights the difficulty in predicting human trial success from experimental animal models.

Creatine has neuroprotective effects that can be useful in the treatment of ALS. Creatine supplementation (2 % was the most effective) in a G93A mouse model improved body weight retention and survival [77–79]. Presymptomatic treatment delayed onset either 12 [80] or 15 days [77]. Starting at onset, administration of 2 % or 1 % creatine in diet improved motor performance and extended survival by 26 days and 14 days, respectively [79]. In a similar study, administration of 2 % creatine in the diet of G93A mice starting at 4 weeks of age delayed onset by 15 days and pro-

gression by 5 days and increased survival by 14.6% or 20 days [77]. Clinical studies have shown statistically insignificant results [81].

KNS-760704, nortriptyline, and cyclosporine all produced changes in onset that were comparable in length with the change in survival, further indicating that early administration of these agents predominately relieves symptoms and delays onset (Fig. 51.1). Once disease progression begins, they are not as effective at further attenuating symptoms. In contrast, creatine produced a very small change in onset whether given as an early or late treatment (30 days or 70 days of age) but still produced comparable increases in survival [77, 79], indicating the necessity of administering creatine beginning at onset and throughout progression. We can further assess creatine's effect in late pathogenesis when used in combination with other agents such as minocycline [82] and manganese porphyrin [62]. When given with minocycline, there was an additive effect, since minocycline alone predominately extends onset. Thus when used together, creatine and minocycline extended survival, since two different mechanisms as well as time points of effectiveness were targeted. In contrast, when given at onset with manganese porphyrin and rofecoxib, survival was decreased relative to manganese porphyrin alone [62]. This could be due to either a negative effect of rofecoxib or that creatine treatment was started late in the pathology, and the time between onset and mortality was too short for the drug to produce the effect seen in other studies, further supporting the late-treatment efficacy of manganese porphyrin.

51.3.3 Anti-apoptotic Agents

Apoptosis is a feature of chronic neurodegenerative diseases, particularly ALS [83, 84]. Understanding the pathways affected by anti-apoptotic agents is an active area for future ALS therapeutic studies. The best studied of the anti-apoptosis agents is Bcl-2, the most important protein in regulating programmed cell death [84]. Overexpression of Bcl-2 delays onset, attenuates the degeneration of spinal cord motor neurons, and prolongs survival in ALS mice [85]. Minocycline, an FDA-approved drug, is a second-generation tetracycline, a safe broad-spectrum antibiotic that crosses the blood-brain barrier. In an SOD1G93A mouse model, minocycline offers neuroprotection by delaying disease onset and prolonging survival [86, 87]. Similarly, in an SOD1G37R mouse model, minocycline improved muscle strength, increased longevity, and delayed the onset of motor neuron degeneration [82]. Furthermore, combinational therapy with minocycline and creatine offered additive neuroprotection in delaying disease onset (29.5%) and extending survival (24.4%) in SOD1G93A mice [88]. Minocycline alone delayed the disease progression as well as increased survival by 8.9%, from 125 to 136 days [86]. Minocycline affects the inflammatory pathways and inhibits caspase-independent and caspase-dependent mitochondrial cell death pathways [86, 88]. However, a large phase III clinical trial among ALS patients failed [89].

Clozapine, a third-generation antipsychotic with anti-dopaminergic and anti-serotonergic activities, also has some novel neuroprotective effects. Even though treatment of NSC-34 cells with clozapine did not reduce SOD1 protein expression, it did reduce the expression of full-length $p75^{NTR}$ in a dose-dependent manner. The neurotrophin receptor p75 ($p75^{NTR}$) has been studied for its death-signaling activity [90], which has been implicated in motor neuron degeneration in human and ALS transgenic mice [91]. Treatment with low-dose clozapine delayed impairment in locomotor performance and improved survival in ALS, but these changes were not statistically significant. High-dose clozapine induced some postural changes and extrapyramidal symptoms and accelerated progression of paralysis [91]. Clozapine seems to have no effect in preventing the loss of body weight. These results suggest it may not be a particularly promising drug for ALS.

Rasagiline, a propargylamine, exerts a neuroprotective effect in NSC-34 cells [92]. Rasagiline and other propargyl-containing molecules, such as ladostigil, activate the canonical survival pathways, MEK and PKC, associated with elevation of BDNF. In a study using SOD1G93A mice, treatment with rasagiline alone and in combina-

tion with riluzole was started at 6 weeks of age. Rasagiline administered via drinking water at either a low or high level (0.5 mg/kg or 2.0 mg/kg) elicited improvements in both motor movement and survival. The high-dose group demonstrated a marked difference on motor activity up to 29–36 weeks of age compared to controls [93]. Combining rasagiline with riluzole had decreased its effect on motor activity but increased its effect on survival.

zVAD-fmk is a broad-spectrum caspase inhibitor that extends survival in an SOD1 model by preventing motor neuron loss. Delivery was achieved through implanted osmotic pumps into the cerebral ventricle starting at 60 days of age, continuing for 56 days. Treatment with zVAD-fmk (100 ug or 300 μg/20 g body weight for 28 days) increased survival (11% and 22%, respectively). zVAD-fmk delayed onset from 103.5 ± 2.8 to 123.5 ± 6.4 days. Duration of disease progression was 23 days in the vehicle-treated group and was delayed in the treated group by 7 days, a 30% increase. Moreover, zVAD-fmk inhibits caspase-1 activity as well as caspase-1 and caspase-3 mRNA upregulation. Protection of motor neurons was predominantly more significant in the cervical than the lumbar spinal cord section in the treated group [94].

Motor neuron degeneration is also observed in the pmn mouse, an animal model for ALS. TCH346, an anti-apoptotic molecule (also known CGP 3466B), was administered orally three times a week at 10 and 100 nmol/kg and prolonged survival by 53.7 and 55.6 days, respectively. At both doses, TCH346 significantly maintained body weight and slowed disease progression by 30% compared to untreated or vehicle-treated mice. A higher dose of 1 μmol/kg did not produce any positive effect. To test more frequent administration, mice were treated with 100 nmol/kg 5 days a week, and indeed it did produce about a 10-day increase in survival compared to the former treatment [95]. Treatment with TCH346 was also studied in SOD1G93A high-copy-number mice starting at 50 days of age, via subcutaneous injection of one of four doses: 0.39, 3.9, 39, and 390 μg/kg. However, none had any effect on onset, duration, or survival [96]. The failure of preclinical studies in various animal models to find any efficacy prefigures the failure of TCH346 in clinical studies in treating PD or ALS [97].

The survival in all of the vehicle-treated groups was consistent (mortality at around 126 days of age), meaning the drug's efficacy can be more equally compared between studies. Because all the anti-apoptotic agents were given before symptom onset and elicited a delay in onset, indicating effectiveness in this earlier time frame, thus, studies of after-onset administration need to be considered. In the studies, a decrease in duration between symptom onset and survival [82, 90] was observed because the progress of the disease was actually accelerated by minocycline and clozapine. Therefore these anti-apoptotic drugs might best be used during the early stages of disease rather than throughout the course of disease. Other agents such as zVAD-fmk and rasagiline both delayed onset and slightly delayed progression compared to vehicle control, indicated by an improvement in motor performance. Minocycline with creatine had an additive effect on delaying onset but less effect in slowing the progression [88].

51.3.4 Anti-excitotoxic Agents

Another assertion for the mechanism underlying the neurodegeneration observed in ALS is glutamate-mediated toxicity [98]. G85R, A4V, and I113T SOD1-mutant mouse lines show a marked loss or inactivation of glutamate transporters [99, 100]. A disturbance in astrocytic glutamate transport was shown to elevate levels of glutamate in the cerebrospinal fluid of ALS patients, in whom glutamate-mediated toxicity has also been noted [101].

Riluzole, the only ALS treatment approved by FDA, prolongs the patient's life by only a few months [102], with the proposed mechanism being anti-excitotoxicity. Riluzole delays onset of muscle weakness and extends life span by 4–6 weeks in animal models of ALS [50, 93, 103]. Memantine is FDA approved, as this anti-glutamate and energy-buffering drug delays

disease onset and prolongs life span in mSOD1G93A mice [104, 105], but has no obvious therapeutic effects on body weight or behavioral performance.

Histone deacetylase (HDAC) inhibitors arrest growth and induce differentiation and sometimes apoptosis. Various HDAC inhibitors have emerged as potential therapeutic drugs for a broad spectrum of neurological diseases [105, 106]. Valproic acid (VPA), a HDAC inhibitor, is an antiepileptic and an antimanic drug used as a neuroprotective agent due for its additional effects on histone acetylation and cellular survival mechanisms [107, 108]. VPA protects spinal motor neurons against death from glutamate toxicity in ex vivo organotypic slice culture [108]. VPA administered via drinking water at 0.26 % (70.6 mg/kg/day/mouse) to G93A low-copy transgenic mice, beginning either pre-onset (after 45 days of age) or post-onset (the day of onset), delayed disease progression and extended survival by an average of 36 or 9 days, respectively [108]. Using a controlled delivery method in G93A high-copy-number mice, 300 mg/kg of VPA was administered twice a week starting at 30 days of age. Treatment delayed onset by 8 %, delayed progression from 19 to 23 days (23 %), and extended survival by 10.3 % [109].

Sodium phenyl butyrate, a HDAC inhibitor, significantly improved survival in G93A transgenic ALS mice. An optimal dose of 400 mg/kg/day was administered, since higher doses of 600 and 800 mg/kg hastened morbidity and mortality compared with non-treatment. When 300 mg/kg of NaPB was combined with 16 mg/kg of riluzole, survival was increased compared with either treatment alone. NaPB increased survival by 12.8 and riluzole 7.5 %; the combination extended survival 21.5 %. All groups improved in body weight and grip strength, with the combined group showing the greatest increase [110].

Trichostatin A (TSA), another HDAC inhibitor, has shown protective effects in multiple cell types implicated in ALS. Treatment in ALS animal models has received mixed reviews in its ability to extend life span. However, it does have neuroprotective ability, slowing down motor neuron death and restoring levels of acetylated histone-H3, cyclic AMP response element binding protein, and cholinergic neuron marker in the spinal cord [111, 112]. In a G93A mouse model, TSA given at 90 days of age (onset) reduced gliosis and upregulated the glutamate transporter, delaying disease progression by 18 % and prolonging life span by 7 % [113].

Topiramate is an anticonvulsant with anti-excitotoxic activity that inhibits kainite-induced seizures. It reduces AMPA-/kainite-induced excitotoxicity. Organotypic rat spinal cord cultures were treated with threo-hydroxyaspartate to selectively inhibit glutamate transport to mimic motor neuron degeneration. The motor neurons were protected when the cultures were also given topiramate. However, when topiramate was administered to SOD1 transgenic mice, no improvement was seen in life span [114].

Talampanel, an AMPA glutamate receptor antagonist, is thought to prevent glutamate from initiating motor neuron death. In a transgenic SOD1G93A mouse model, 5 mg/kg of talampanel via oral gavage once a day was administrated beginning at symptom onset. However, the study concluded that talampanel reduced motor neuron calcium levels only when administered presymptomatically [115]. Therefore, biomarkers that allow for earlier disease detection will facilitate treatment with a drug like talampanel. Glutamate transporters are important in preventing glutamate neurotoxicity and extend survival in the G93A mouse model. Ceftriaxone is a β-lactam and potent antibiotic as well as a stimulator of GLT1 expression. G93A mice were IP injected with ceftriaxone 200 mg/kg/day starting at 12 weeks of age, which extended survival by 8 %. Treated mice had a significantly delayed loss of muscle strength and body weight (4–5 weeks). Ceftriaxone treatment beginning at 6 weeks was not significantly better at extending survival compared with beginning at 12 weeks, indicating ceftriaxone's mechanism of action, since the loss of GLT1 does not begin until 90 days of age. Therapy reduced motor neuron loss and hypercellular gliosis compared to saline control [116, 117]. However, a phase III trial of ceftriaxone in ALS patients failed.

Ceftriaxone is most beneficial when given after GLT1 transporters are lost, at 90 days of age. Talampanel was only effective when given

presymptomatically. VPA and memantine treatments successfully delayed onset and progression and thus are effective treatments until the end stages of ALS.

51.3.5 Anti-aggregation Agents

Misfolded proteins and abnormal aggregation of proteins are pathologically characteristic of neurodegenerative diseases including ALS. These misfolded proteins are often thermodynamically stable and form aggresome-like structures; ALS progression may correlate with a propensity toward protein aggregation. The heat shock response (HSR) is a highly conserved cytoprotective mechanism; enhanced heat shock protein expression not only directly affects protein aggregation but also leads to more effective clearance of protein aggregates via the unfolded protein. Investigators develop drugs to modulate the HSR and parallel stress response pathways for therapeutic use [118–122].

The HSR is modulated by stress-inducible heat shock transcription factor 1. Arimoclomol, a coinducer of heat shock proteins (HSP), significantly delays disease progression in SOD1G93A mice. Mice were treated IP with 10 mg/kg of arimoclomol daily [123] at 35 days (before onset) or 70 days of age (onset) and showed extension in survival of 22 % or 18 %, respectively. Progression was measured by electrophysiological assessment of hind limb muscle function. At 120 days of age, the untreated mice showed 8.3 motor units compared to the wild-type mice with 28 units, the treatment group showed significant greater motor unit survival with 14.3 units [123], and treated animals showed 74 % greater motor neuron cell counts in the sciatic motor pool of lumbar spinal cord sections. Neuroprotection was likely associated with the prolonged activation of heat shock transcription factor-1 (Hsf-1); in spinal cord sections, HSP-70 and HSP-90 levels were increased.

Edaravone is known to have multiple effects on cellular pathology, including inhibition of the JNK-C pathway [124], and Bcl-2/Bax [125]; augmentation of nitric oxide release after acute endothelial injury [126]; suppression of ER dysfunction in cerebral ischemia [127]; and as a radical scavenger [128]; and inhibition of the production of tumor necrosis factor alpha [129]. Edaravone treatment in the SOD1G93A mouse model slowed symptom progression and motor neuron degeneration, with decreased SOD1 deposition and the amount of 3-nitrotyrosine/tyrosine in lumbar spinal cord. Female mice were treated with 15 mg/kg of edaravone after onset. Although survival was not significantly extended, symptom progression (assessed via motor tasks) did show significant delay, up to 21 days after onset [130].

Lithium prevents excitotoxic cell death of motor neurons in organotypic spinal cord cultures and in cerebellar granule cells [131, 132]. It has been shown in many mouse studies that lithium improves muscle strength and grip strength and improves reflexes greatly, although no evidence supports an effect on body weight. When lithium was combined with VPA in a G93A mouse model, synergistic effects were exhibited, significantly delaying onset and extending survival [109]. The G93A mice received IP injections with LiCl (60 mg/kg), VPA (300 mg/kg), or lithium plus VPA twice daily starting at 30 days of age. The onset of neurological symptoms was determined by the following behavioral tests: rotarod performance, postural reflex, balance beam performance, screen grasping, or tail suspension behavior. Lithium improved motor function, delayed onset by 9.2 %, and increased mortality by 8.4 %; VPA improved onset by 8 % and mortality by 10.3 %. In combination they delayed onset by 14.2 % and mortality by 14.8 %. The delay in disease progression is affected mostly by VPA, since combination therapy produced a similar increase to VPA treatment alone (18 %).

51.3.6 Anti-inflammatory

Reactive oxygen and reactive nitrogen species are not only damaging to cellular compartments but also trigger a massive inflammatory response. Although motor neuron death is the major hall-

mark of ALS, recent studies provide evidence that non-neuronal cells also contribute to pathogenesis [133–136]. The loss of motor neurons in transgenic mouse models and in human postmortem tissue is accompanied by a robust glial reaction and microglial activation [137, 138] as well as glial proliferation [139].

Neuroinflammation has emerged as a significant contributor to motor neuron damage in ALS [140]. The neuroinflammatory response associated with astrocytes and microgliosis leads to the release of pro-inflammatory cytokines [137] such as interleukin-6 [141], while ALS-linked mSOD1 activates caspase-1 and IL-1β in microglia, and microglial IL-1β has been reported as a causative event of neuroinflammation [142]. In addition, there are significant increases of TNF-α and Fas ligand immunoreactivity in ALS mice and human patients [143]. Since inflammation is a fundamental mechanism exacerbating the pathogenesis of ALS, anti-inflammatory agents may eventually play an important role in treatment.

Celastrol, derived from an herb used in traditional Chinese medicine, is a triterpene originally used as an anti-inflammatory but also has strong antioxidative effects and has been shown to increase expression of HSP-70 [144]. Celastrol was administered orally to G93A mice starting at 30 days of age. Administration of 2 and 8 mg/kg/day increased survival by 9.4 % and 13 %, respectively. The treatment significantly reduced weight loss, improved motor function, and delayed disease onset [145]. In treated animals, neuronal cell count was increased by 30 % in the lumbar spinal cord, and TNF-α, iNOS, CD40, and GFAP immunoreactivity was reduced.

Cannabinoid receptor 2 (CB2) is upregulated in microglia in response to inflammatory stimuli [146], and CB2 agonists suppress microglial activation in vitro [147]. Cannabinoids produce anti-inflammatory effects through CB2. The CB2 cannabinoid agonist AM-1241 ((R,S)-(+)-(2-Iodo-5-nitrobenzoyl)-[1-(1-methyl-piperidin-2-ylmethyl)-1H-indole-3-yl] methanone) (3 and 0.3 mg/kg) delivered at symptom onset in G93A mice prolongs survival 1.11- or 1.07-fold [148] and delays the disease progression 1.56- or 1.41-fold, respectively [149]. AM-1241 improves the motor performance of G93A mice (especially in males) but has no protective effect against body weight loss in ALS mice of either gender [149]. In addition, another CB2 agonist, WIN-55 212-2 (5 mg/kg), prolongs mortality of G93A mice 1.04-fold and delays progression 1.37-fold [148]. Cannabinol delays symptom onset in G93A mice but does not affect survival [150, 151]. WIN-55 212-2 treatment helps retain the force of the tibialis anterior (TA) muscle and the extensor digitorum longus (ETL) muscle) in the hind limb of G93A mice; it also ameliorates the normal fatigue characteristics of EDL muscles by decreasing the oxidative capacity of succinate dehydrogenase in the muscle fiber. WIN-55 212-2 also prevents the loss of motor neurons [150]. Treatment starting at 90 days' onset significantly slows disease progression as assessed at 120 days by motor neuron survival but produces no significant increase in mortality [150].

TNF-α and Fas ligand immunoreactivity is greatly elevated in motor neurons and glial cells of the lumbar spinal cord in both transgenic ALS mice and ALS patients [143]. Thalidomide works as an anti-inflammatory by destabilizing the mRNA of TNF-α and other cytokines. Thalidomide was administered orally (50 or 100 mg/kg/day) to a G93A high-copy transgenic mouse at 30 days of age. Treatment delayed onset, significantly improved in motor performance from 98 to 155 days of age [143], and attenuated weight loss from the age of 70 days. Survival was prolonged in a dose-dependent manner from 130 to 145 days (12 %, 50 mg/kg) and 151 days (16 %, 100 mg/kg), associated with its ability to reduce TNF-α and Fas ligand immunoreactivity in the spinal cords of G93A mice. Similarly, lenalidomide has been used to inhibit inflammatory signals caused by ALS pathology; it is less potent at reducing TNF-α but more potent at reducing other pro-inflammatory signals [143]. Two studies used lenalidomide treatment, one starting presymptoms [143] and the other at onset [152]. Presymptomatic oral treatment (100 mg/kg/day, started at 30 days of age) significantly increased survival from 130 to 154 days (18.5 %). In the subse-

quent study, the same dosage of lenalidomide started at symptom onset increased survival by only 11.7 % [152]. In both studies, treatment improved rotarod performance, attenuated weight loss, and reduced neuronal cell death in the lumbar spinal cord [143, 152].

Nordihydroguaiaretic acid (NDGA), an arachidonic acid 5-lipoxygenase (5LOX) inhibitor, was administered orally in SOD1G93A mice. Increased levels of 5LOX mRNA and protein were evident at 120 days of age. Oral treatment with 2,500 ppm of NDGA starting at 90 days of age significantly increased body weight, delayed disease onset, and extended survival. Lumbar spinal cord sections from treated animals revealed reduced levels of astrogliosis and cleaved microtubule-associated-tau protein. The median duration of disease was 10 days in non-treated mice and 14 days in the NDGA-treated group. NDGA extended median survival by 14 days or 9.8 %.

Pioglitazone, an agonist of the peroxisome proliferator-activated receptor, exerts anti-inflammatory effects, resulting in neuroprotection that can delay the disease process in SOD1 G93A mice [153]. Administered in food presymptomatically at 30 days of age, pioglitazone significantly decreased mortality by 13 %, delayed symptom onset by 38 % compared to control, but did not affect progression once symptoms appeared. In a similar mouse model, treatment starting at 57 days of age with an average 40 mg/kg a day was also effective. Disease onset was delayed by 10 days, and survival was extended by 10 days. Pioglitazone treatment delayed weight loss and decreased the rapid decline in muscle function observed in SOD1G93A control mice [94, 154]. Compared to untreated controls, levels of CD40, GFAP, iNOS, NFkappa-β, and 3-nitrotyrosine were all lower in the spinal cords of treated animals.

Among anti-inflammatory agents, those given after onset did produce a modest increase in survival. Greater delays in progression are observed with earlier administration; for example, pioglitazone is most effective at an early time point by reducing inflammatory response, but when administered 27 days later, the progression of the disease is more aggressive.

51.3.7 Multiple Functions of Neuroprotective Agents in Treatment of Mental Disorders

Most of neuroprotective agents above discussed have multiple functional effects in treatment of mental disorders (Tables 51.1 and 51.2). Vitamin E is used as a supplement to other treatments for Alzheimer's disease (AD) [155]. In addition to its antioxidative effects, DP109 is used in treatment of AD and has been shown to improve amyloid-β aggregates [155]. M30 has potential in treating both AD and Parkinson's disease (PD) [156, 157]. In patients with either bipolar disorder or unipolar depression, augmentation with NAC reduced depressive symptoms [158]. In schizophrenic patients, NAC treatment was associated with improvement in akathisia but no significant change in positive and negative syndrome scale [159]. In patients with trichotillomania, NAC treatment had reduced hair pulling after 9 weeks [160].

PPX is an effective treatment in improving depressive states and motor function in PD when given at 1.5 mg/day for duration of 3 months [161–163]. Olesoxime did not improve motor or metabolic function but did improve cognitive and mitochondrial pathology in a Huntington's rat model, BACHD [164]. Although creatine is known for preventing muscle fatigue, it has also been shown to reduce mental fatigue [165]. Due to its low toxicity, high dosages of creatine have been added to treatments for neuropsychiatric disorders [165, 166]. It is possible that through this mechanism, creatine supplementation improves unipolar depression but in bipolar patients might be associated with induced manic episodes [166]. However, 1 year of creatine supplementation in Huntington's disease produced no clinically significant improvement [167].

Findings support the contention that augmentation of minocycline might increase the efficacy of other antipsychotics, as seen by improved symptoms in schizophrenic patients [168–170]. Clozapine treatment for childhood-onset schizophrenia is promising, as shown by improvement

Table 51.1 Summary of agents for ALS with individual neuroprotective mechanisms

A Antioxidant agents							
	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
Melatonin	G93A/B6SJL	0.5 mg/mL in drink water	Hind limb tremor	85.4 (Veh) 89.7 5 %	131 (Veh) 136.9 4.5 %	[45]	Sleep disorders (+) [201, 202]
		30 mg/kg daily IP	No effect	110.3 ± 3.1(Veh) 121.4 ± 2.8 10.1 %	136.7 ± 2.5 (Veh) 146.8 ± 4.1 7.4 %	[43]	
Vitamin E	G93A/B6SJL 1Gur. N29/+ mice	Diet AIN-93G diet (75 and 200 IU)	Normalized daily wheel acting (+)	95 ± 13 (Veh) 108 ± 10 13.7 %	No significant statistical differences	[50]	AD (+) [155]
SS-31 peptides	G93A/B6SJL	IP 5 mg/kg/day	Motor performance (++)	88 ± 7 (Veh) 95 ± 6 7.9 %	130 ± 12 (Veh) 142 ± 12 9.2 %	[52]	N/A
FeTCPP	G93A/B6SJL G93A/C57B6	IP 1 mg/kg/day	Motor performance (++)	101.2 ± 1.4 (Veh) 104.4 ± 2.9	128 ± 1.7 (Veh) 135 ± 2.4	[53]	N/A
DP109	G93A/B6SJL	5 mg/kg	Motor performance (+)	93 ± 7 (Veh) 100 ± 6 7.5 %	133.5 ± 6 (Veh) 147 ± 10 10 %	[54]	AD (+) [155]
DP460	G93A/B6SJL	10 mg/kg	Motor performance (++)	93 ± 7 (Veh) 102 ± 9 9.6 %	133.5 ± 6 (Veh) 145 ± 10 8.6 %	[54]	N/A
M30	G93A/B6SJL	Oral gavage 1 mg/kg, 4 times/week	General muscle strength (+)	107 ± 3 (Veh) 112 ± 4	124 ± 6 (Veh) 134 ± 12	[56]	AD and PD (+) [156, 157]
NAC	G93A/B6SJL	2 mg/kg 1 % in drink water	Rotarod performance (++)	Delays 1 week 77 ± 1.33 days	127.9 ± 2.2 (Veh) 136.5 ± 2.4	[59, 60]	Depressive symptoms (+) [158] Schizophrenia (+) [159] Trichotillomania (+) [160]
EUK-8	C57BL/6 J-TgN	IP 33 mg/kg, 3 times/week	N/A	170 ± 6 (Veh) 176 ± 7 3.5 %	221 ± 5 (Veh) 238 ± 7 7.6 %	[61]	N/A

EUK-134	C57BL/6 J-TgN	IP 33 mg/kg, 3 times/ Week	N/A	170 ± 6 (Veh) 164 ± 8 –8.2 %	221 ± 5 (Veh) 244 ± 7 10.4 %	[61]	N/A
Manganese porphyrin	G93A/B6SJL	2.5 mg/kg/day IP, IP + CR SC	Slow decline in motor function	89.0 ± 0.7 (Veh) 89.0 ± 0.7 89.3 ± 0.7 90.6 ± 0.7	102.5 1.0 (Veh) 129.0 ± 3.1 (1.26-fold) 127.1 ± 3.5 (1.24-fold) 131.1 ± 1.3 (1.22-fold)	[62]	N/A

B Mitochondrial protective agents

	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
Nortriptyline	G93A/B6SJL	IP 10, 20, 30 mg/kg/ day	Motor strength and coordination (+)	108.3 ± 1.8 (Veh) 10 mg 115.9 ± 2.0 7 % 20 mg 121.8 ± 1.3 12.5 % 30 mg 112.2 ± 1.7 3.6 %	130.9 ± 1.9 (Veh) 10 mg 140 ± 1.9 6.9 % 20 mg 138.6 ± 1.7 5.8 % 30 mg 124.9 ± 2.0 4.5 %	[68]	Anti-depressant (+) [203] Sleep disorder (+) [204]
Cyclosporin A	G93A/B6SJL	ICV 20 µg/week	Delays hind limb weakness (+) Improves physical performance (+), hind limb strength and agility (+)	122.5 ± 3 (Veh) 135 ± 3.8 10.2 %	130 ± 3.4 (Veh) 146 ± 4.5 12.3 %	[69] [70]	N/A
KNS-760704	G93A/B6SJL	Diet 100 mg/kg/day	Motor performance (+)	103 (Veh) 109	125 ± 2 (Veh) 132 ± 2	[71]	PD (+), FDA approved [205]

(continued)

Table 51.1 (continued)

B Mitochondrial protective agents							
	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
PPX	G93A/B6SJL	Drink water 3 mg/kg/day	Motor performance (+)	103 (Veh) 106	125 ± 2 (Veh) 122 ± 2	[72]	Depression, anhedonia and apathy in PD (+) [161–163]
Olesoxime	G93A/B6SJL	SC 3, 30 mg/kg/day	Grip strength (+)	Body weight loss delays 15 days and decline of grid performance 11 day	138 ± 4 days 135 ± 3 days 125 ± 3 days 10 %	[74]	Cognitive, psychiatric in BACHD (+) [164]
Creatine	G93A/B6SJL	Diet 2 %	Motor performance, attenuates weight loss	110 (Veh) 114 12.7 %	133.6 ± 2.5 (Veh) 153.2 ± 2.8 14.6 %	[77]	Unipolar depression and mental fatigue (+) [165, 166] cognitive status in HD (−) [167]

C Anti-apoptotic agents							
	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
Minocycline	G93A/B6SJL	IP 10 mg/kg daily	Muscle strength (+)	90.3 ± 2.2 (Veh) 10 mg 109 ± 1.5 20.7 % 47.2 ± 1.3 (Veh) 48.45 ± 1.8	125.6 ± 3.4 (Veh) 10 mg 136.8 ± 1.2 8.9 % 103.3 ± 1.7 150.9 ± 3.7 increased the average life span of ALS mice by 3 weeks	[64, 82, 86, 88, 206] [87]	Schizophrenia, negative and cognitive symptoms in early-phase schizophrenia. (+) [168–170]

C Anti-apoptotic agents							
	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
Minocycline + creatine	G93A/B6SJL	IP 22 mg/kg minocycline and 2 % creatine daily	Muscle strength (+)	94.2 ± 6.8 (Veh) 22 mg minocycline 113.1 ± 8.8 20 % 2 % creatine 111 ± 4.7 17.8 % Minocycline + creatine 122 ± 8.9 29.5 %	126.3 ± 4.2 (Veh) 22 mg minocycline 142.2 ± 4.9 12.5 % 2 % creatine 141.9 ± 4.3 12.3 % Minocycline + creatine 157.2 ± 4.1 24.4 %	[88, 206] [64]	N/A
Clozapine	G93A/B6SJL	2.5, or 7.5 mg/kg orally by gavage thrice weekly in 0.2-ml volumes	Locomotor in low-dose treatment (+)	108.1 ± 3.4 (Veh) 2.5 mg/kg 118.0 ± 3.2 7.5 mg/kg 102.0 ± 5.1	133.5 ± 2.4 (Veh) 2.5 mg/kg 138.1 ± 2.4 7.5 mg/kg 130.6 ± 1.4	[91]	Refractory schizophrenia (++) [171, 172]
Rasagiline	G93A/B6SJL	0.5 or 2.0 mg/kg by water intake	Running wheel activity (+)	N/A	210.9 ± 7.48 (Veh) 0.5 mg/kg 223.9 ± 8.50 2.0 mg/kg 239.9 ± 4.4	[103]	Attention and executive functions in PD (+) [173] Early stage in PD (+) [174]
TCH346 (CGP 3466B)	G93A/B6SJL (pmn)	Oral 3 times/week 100 nmol 5 times/week 10 nmol 100 nmol	N/A	N/A	42.3 ± 0.8 (Veh) 3 times/week 100 nmol 45.4 ± 2.7 5 times/week 10 nmol 53.3 5 times/week 100 nmol 55.6	[95]	PD (–) [97]
z-VAD-fmk	G93A/B6SJL	ICV 300 µg/20 g body weight/28 days	Motor strength and coordination (++)	103.5 ± 2.8 (Veh) 123.7 ± 6.8 19.5 %	126.1 ± 3 (Veh) 153.3 ± 8.8 21.5 %	[94]	N/A

(continued)

Table 51.1 (continued)

D Anti-excitotoxic agents							
	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
Memantine	G93A/SOD1	30 or 90 mg/kg/day	In high-dose, rotarod and hind limb extension reflex (+)	99.2±6.3 (Veh) 103.7±6.8	134 (Veh) 141	[104, 105]	Cognition in AD, depressive disorder comorbid with alcohol dependence, OCD, vascular dementia, DLB, PDD (+) [175–179]
Valproic acid	G93A/B6SJL	IP 300 mg/kg twice l	Rotarod, postural reflex, balance beam performance, screen grasping, and tail suspension behavior (+)	106±3.0 (Veh) 114.5±4.7 8%	124.8±3.2 (Veh) 137.7±4.2 10.3%	[108, 109]	Mental retardation, concurrent affective disorders, and social anxiety disorder (+) [180, 181]
Trichostatin A (TSA)	Male G93A/C57BI6/SJL	IP 30 µl 5 day/week	Enhances motor functions as assessed by rotarod and grip strength and decreases muscle atrophy and degeneration of NMJ	Treatment at onset (90 day)	148.8±2.6 159.1±2.9 7%	[113]	Anxiety disorders Recognition memory, spatial memory, and fear memory (+) [182, 183]
Topiramate	G93A SOD1	Oral gavage (50 mg/kg)	N/A	No effect	No effect	[114]	Symptom severity in acute bipolar depression, binge-eating disorder (+) [184, 185]

D Anti-excitotoxic agents							
	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
Talampanel	G93A/B6SJL female	5 mg/kg	N/A	N/A	Prolong mouse median survival	[115]	Partial seizures and PD (+) [186, 187]
Riluzole + phenylbutyrate (PB)	G93A/B6SJL	IP 16 mg/kg 300 mg/kg	Forelimb grip strength, body weight,	N/A	126.1±2.7 (Veh) 153.2±9.1 21.5%	[110]	N/A
Riluzole	G93A/B6SJL	IP 16 mg/kg/day	Grip strength, body weight	N/A	126.1±2.7 135.5±4.4 7.5%	[110]	OCD, bipolar depression (+) [206–209]
Sodium phenylbutyrate (NaPB/SPB)	G93A/ B6SJL	IP 300 mg/kg/day 400 mg kg/day	Grip strength body weight, rotarod, stride length,	N/A	126.1±2.7 142.2±5.4 12.8% 125.7±3.0 (Veh) 153.2±6.4	[110] [210]	Psychiatric problems in IEM/ UCD (+) [211, 212]
Ceftriaxone	G93A/B6SJL	IP 200 mg/kg/day	Significantly delay loss of lumbar spinal motor neurons, muscle strength, and body weight	Delay onset by 14 days	122±2 (Veh) 132±2	[116, 117]	Cognitive function in post Lyme disease(−) [213] Depression, psychotic symptoms in neurosyphilis (+) [214, 215]

(continued)

Table 51.1 (continued)

E SOD1 aggregation clearing agents							
	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
Arimoclomol	G93A SOD1	IP 10 mg/kg	Fatigue index of EDL(+) Contractile characteristics of EDL (+) maximum force of TA and EDL (+)	70 (Veh)	125 ± 1.8 (Veh) 153 ± 2.6 22 %	[123] [216]	N/A
Edaravone	G93A SOD1	IP 15 mg/kg 5 mg/kg	Slows hind limb weakness, decreases grip strength and motor performance scores in the higher dose	100.3 ± 6.8 (Veh) 98.4 ± 4.3 1.8 %	133 (Veh) 135.8 2 %	[130]	MID and psychosis (+) [188]
Lithium	G93A/B6SJL	60 mg/kg twice daily IP	Rotarod, grip strength, and stride length (++) motor strength, coordination, and extension reflex (++)	106 ± 3.0 (Veh) 115.8 ± 1.5 9.2 %	124.8 ± 3.2 (Veh) 135.3 ± 2.1 8.4 %	[113, 114] [109, 217] [118]	Bipolar disorder, mania, euphoria/elevated mood in ASD (+) [207, 219–221]
Lithium+ valproic acid	G93A/B6SJL	Twice daily IP	Rotarod, postural reflex, balance beam performance, screen grasping, and tail suspension behavior (++)	106 ± 3.0 (Veh) 121.0 ± 2.7 14.2 %	124.8 ± 3.2 (Veh) 143.3 ± 4.1 14.8 %	[109]	Relapse prevention in bipolar I disorder (+) [222]

F Anti-inflammatory agents							
	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
Celastrol	G93A SOD1	Oral 2, 8 mg/kg 8 mg = 13 %	Motor performance (+)	N/A	2 mg = 9.4 %	[145]	Cognitive functions in AD (+) [223]

F Anti-inflammatory agents							
	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
AM1241	G93A/B6SJL	IP at onset 0.3, 3 mg/kg	Motor performance and score (+)	IP at onset 90 days IP at onset 75 days	113.7 ± 1.7 0.3 mg 123.4 ± 2.2 3.0 mg 126.9 ± 2.8 123.6 ± 2.3 (Veh, male) 126.7 ± 1.2 (male) 134.4 ± 3.4 (Veh, female) 130.9 ± 3.0 (female)	[149, 151] [149]	Nicotine seeking and taking behavior (−) [224]
WIN 55, 212-2	G93A/C57BL	IP at onset 5 mg/kg	Maximal titanic force (TA and EDL) (+), succinate dehydrogenase and the contractile characteristics (EDL) (+)	IP at onset 90 days	131 ± 1.4 (Veh) 134 ± 2.3	[150]	Fear memory and spatial memory (+) [225] decrease anxiety-like behavior (+) [226]
Thalidomide	G93A/B6SJL	Food 50, 100 mg/kg/day	Motor performance (+)	90 (Veh) Disease onset was delayed	Veh 130 ± 8.5 50 mg 145 ± 19 12 % 100 mg 151 ± 19 16 %	[143]	Increase the risk of ASD and mental retardation [227, 228]
Lenalidomide	G93A/B6SJL	Food 100 mg/kg/day	Motor performance (+)	40.8 ± 6.7 (Veh) 59.1 ± 5.8 45 %	127.7 ± 5.1 (Veh) 142.7 ± 5.4 12 %	[143, 152]	Improve language and autistic behaviors in children (+) [229]

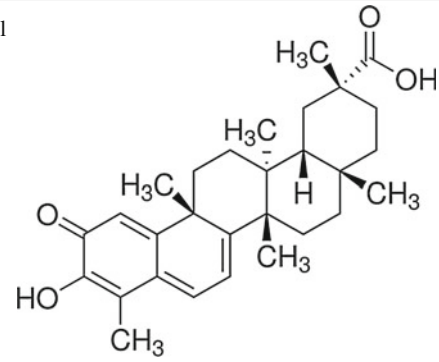
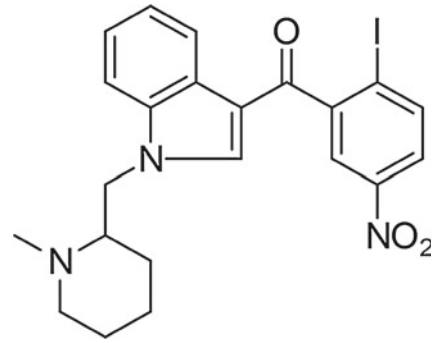
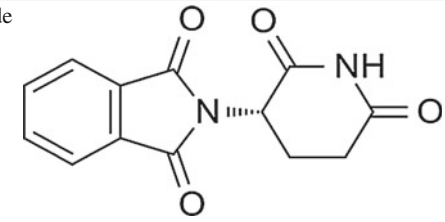
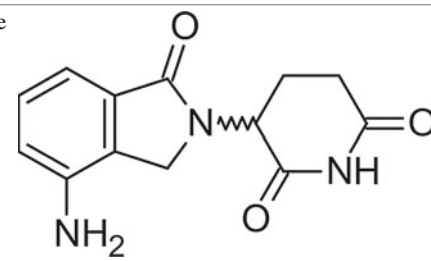
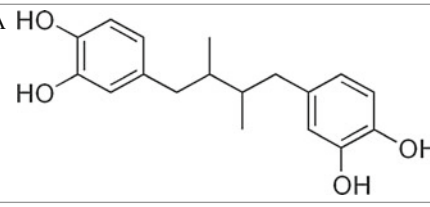
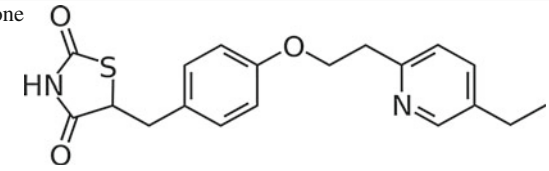
(continued)

Table 51.1 (continued)

F Anti-inflammatory agents							
	Effects on ALS						
Agent	Model	Agent Administration	Muscle function	Onset	Mortality	Ref	Effects on MD
NDGA	G93A/B6SJL	Diet 2,500 ppm	Motor performance and paralysis (+)	112 (Veh) 120 7 %	122 (Veh) 134 9.8 %	[230]	N/A
Pioglitazone	G93A/B6SJL	40 mg/kg/day by food Diet 1,200 ppm	Motor performance (+) Muscle fiber diameter in quadriceps muscle (+)	100 ± 8 110 ± 11 90 (Veh) 124 38 %	123 ± 7 133 ± 6 123.8 ± 6.8 (Veh) 139.9 ± 8.1 13 %	[154, 231]	Reduce depression severity (+) [232] reduce the risk of schizophrenia in patients with diabetes (+) [233]

The data in tables are collected, analyzed, and calculated based on original published literatures.
N/A no available data
+, improvement of behavior or muscle performance of ALS mouse after treatment of drug compared with control mouse
++, obvious protective effect of drug on behavior or muscle performance of ALS mouse

Table 51.2 Neuroprotective agents delay body weight loss in animal models of ALS

Mechanism	Bodyweight	Chemical structure of agent	Ref
Anti-inflammatory agents	(+)	Celastrol 	[145]
	(+)	AM-1241 	
	(+)	Thalidomide 	[143]
	(+)	Lenalidomide 	[143, 152]
	(+)	NDGA 	
	(++)	Pioglitazone 	[154, 231]

(continued)

Table 51.2 (continued)

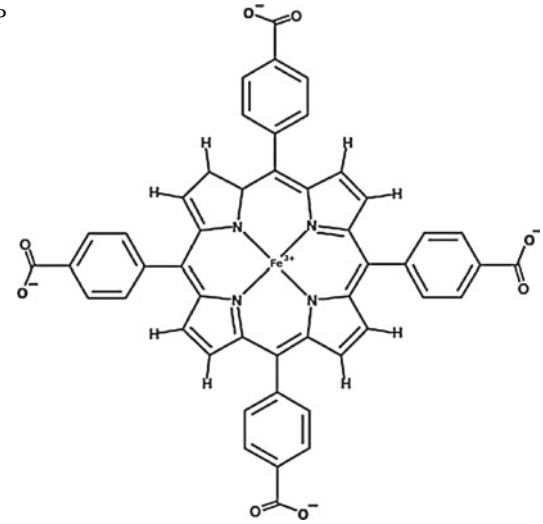
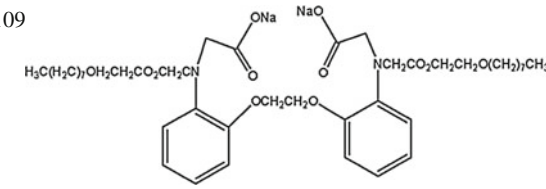
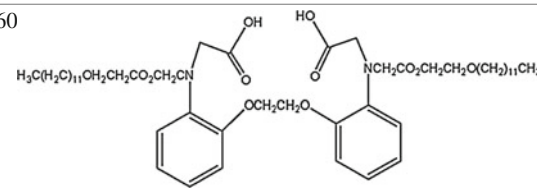
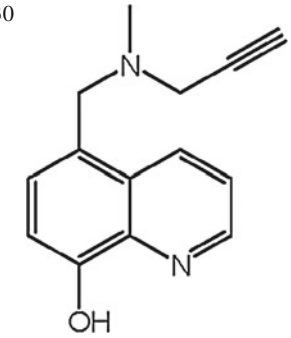
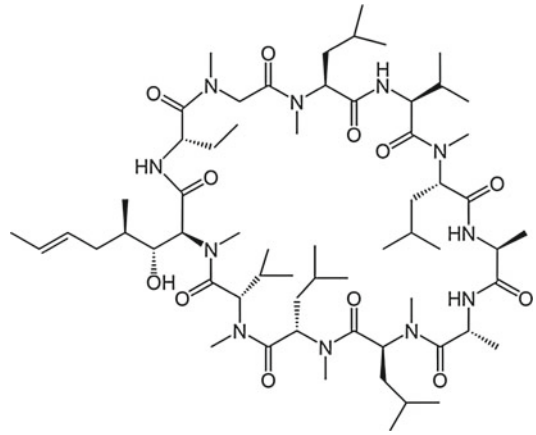
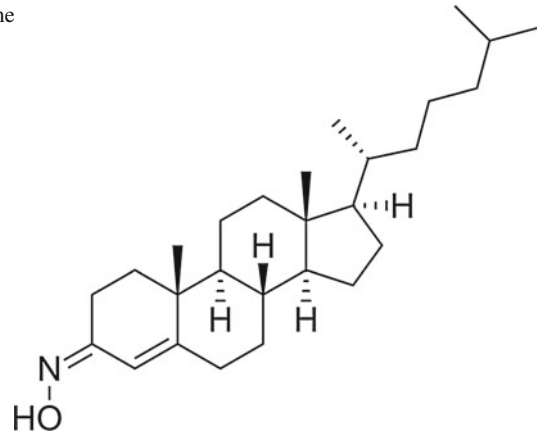
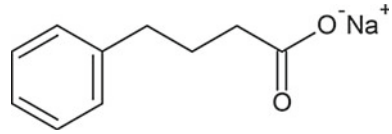
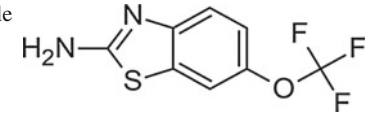
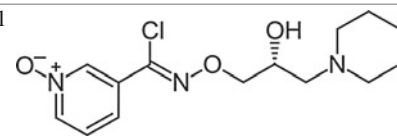
Mechanism	Bodyweight	Chemical structure of agent	Ref
Antioxidant agents	(++)	FeTCPP 	[53]
	(+)	DP109 	[54]
	(+)	DP460 	[54]
	(+)	M30 	[56]

Table 51.2 (continued)

Mechanism	Bodyweight	Chemical structure of agent	Ref
Mitochondrial protective agents	(+)	Cyclosporin A 	[69]
	(+)	Olesoxime 	[74]
Anti-apoptotic agents	(+)	NaPB 	[110, 210]
Anti-excitotoxic agents	(++)	Riluzole 	[50, 93, 110]
SOD1 aggregation clearing agents	(+)	Arimoclomol 	[123]

Neuroprotective agents inhibiting body weight loss are tabulated. Their major action mechanisms are indicated
+, drug with modest increase of body weight of ALS mouse
++, robust effect of drug on body weight gain

on positive and negative syndrome scale [171, 172]. However, due to toxicity, close monitoring is necessary. In addition to its neuroprotective effects, rasagiline has been shown to inhibit MAO-B, which enhances central dopaminergic transmission. Treatment with 1 mg/day rasagiline for 3 months ameliorated cognitive impairment in non-demented patients with PD [173] and was similarly effective for patients with early PD [174].

In Alzheimer's patients receiving stable doses of donepezil, memantine improved cognition and daily activities compared with placebo [175]. Memantine may effectively treat severe depressive disorders in patients with comorbid alcohol dependence [176]. In patients with mild to moderate vascular dementia, memantine augmentation improved obsessive-compulsive disorder behaviors [177] and memantine improved cognition [178], while in patients with PD dementia, memantine treatment showed improvement as well [179]. VPA is tolerable in long-term treatment for mental retardation, thereby reducing affective symptoms [180], and is effective for treating social anxiety disorder [181]. TSA and other HDAC inhibitors have efficacy for a broad spectrum of neurodegenerative and mental disorders [182, 183]. Preliminary data suggest that add-on treatment of topiramate reduced depressive symptoms in acute depressive bipolar patients [184]. Topiramate improved the efficacy of cognitive behavioral therapy when used to treat mental disorders such as binge eating [185]. AMPAR antagonist reduces the effects of seizures and is under investigation as an antiepileptic [186, 187]. Due to its multiple functions, edaravone holds promise in the treatment of neurological diseases like AD and mitochondrial disorders [188].

51.4 Structure-Guided Drug Screening

Loss of body weight has been suggested to predict poor prognosis in ALS patients [189], as such loss in the early stages of ALS is significantly correlated with more rapid progression of symptoms and shorter survival. We summarized neuroprotective agents that prevent body weight loss in animal models of ALS (Table 51.2). Among the seven reviewed anti-inflammatory agents, Win 55,212 had a slight protective effect on motor performance and survival in ALS mice [150]. Even though there are no reports on its effect on body weight loss in ALS animals, Win 55,212 indeed prevents the reduction in weight loss induced by chronic cisplatin in the rat [190] and increases (+50 %) glucose uptake in human fat cells [191]. Very interestingly, the other six anti-inflammatory agents (NDGA, AM-1241, pioglitazone, thalidomide, lenalidomide, and celastrol) were all found to successfully reduce body weight loss and improve motor performance and survival in ALS mice.

We further analyzed the structure-activity relationship of the small molecules in Table 51.2. Although there are differences in their action mechanism and original design, their chemical structures do share common characteristics to a certain extent.

Among the above six anti-inflammatory agents increasing body weight, the chemical structures, from core skeletons to substitution functionalities, of NDGA, AM-1241, pioglitazone, thalidomide, and lenalidomide look varied. Obviously five of the anti-inflammatory agents share some common features (Fig. 51.2): (1) they all have the potential chelating properties with cations to form 5- to 6-member rings that are thermodynamically stable; (2) they all carry antioxidation moiety, phenol, or different amines; and (3) they all possess strongly negative atoms as hydrogen-bonding acceptors.

These common characteristics are found not only in anti-inflammatory agents but also in other small molecules in Table 51.2. For instance, the chemical structures of antioxidant agents and anti-inflammatory agents share some common features. DP109, DP460, and M30 were classified as antioxidants; DP109 and DP460 were analogues, with the same core skeleton and different-length side chains; both exhibited structural features distinct from M30, whereas all three possess (1) chelating properties with cations to form 5- to 6-member rings that are ther-

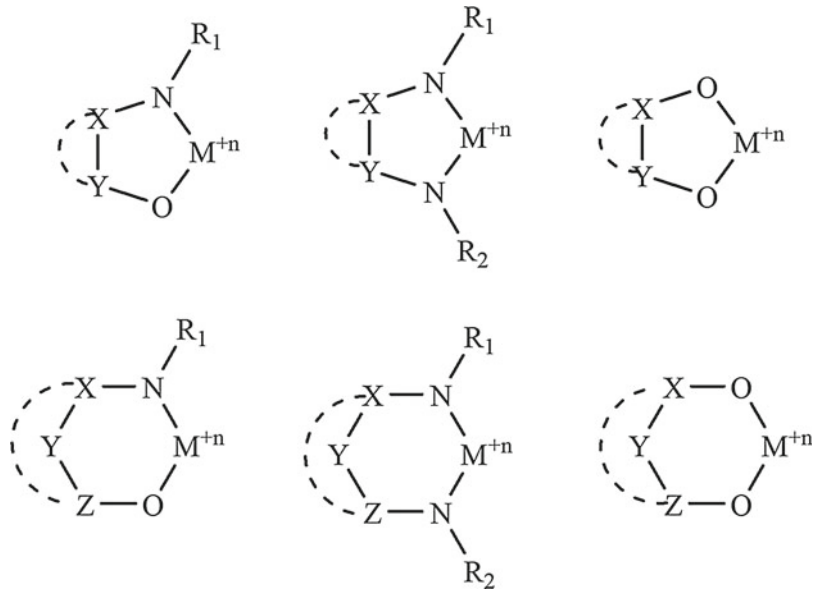


Fig. 51.2 Thermodynamic stable chelating complexes formation of positive cationic with ALS potential heteroatom therapeutics. M^{+n}: Cu^{+2}, Fe^{+2}, Cu^{+1}, and excess redox-active transition metals; X/Y/Z: aliphatic C, aromatic C, or heteroatom; R1/R2: H, aliphatic carbon side chain and aromatic carbon; X/Y/Z: aliphatic carbon, aromatic carbon and heteroatom; *Broken line*: aliphatic side chain, aliphatic cyclic ring, and aromatic fused ring

modynamically stable (Fig. 51.2), (2) antioxidation properties at phenol and aniline moieties, and (3) hydrogen-binding properties to protein or enzyme receptors.

Thus the identification of small molecules that can increase body weight and improve both motor performance and survival, accompanied by continued chemical structural analysis, may be a practical and feasible strategy to search drug candidates for ALS based on decreasing body weight loss.

51.5 Conclusions and Perspective

Riluzole has been demonstrated to have a beneficial effect in people with ALS as well as in SOD1G93A mice. At the same time, its effects are modest, and other scientifically promising therapeutic candidates exist. Unfortunately, many previously promising candidate therapeutics have shown beneficial effects in the SOD1G93A mouse model of ALS, only to meet with a lack of benefit when tested in people with ALS. Examples include thalidomide [192] and olesoxime (which was well tolerated but failed to show efficacy in a large phase II–III clinical trial [193]) and pioglitazone, which did not show any survival benefit in a phase II trial [194]. These examples highlight the poor track record of translation from survival studies in the SOD1G93A mouse model into humans. However, even as evidence mounts that survival studies in the mouse model may have poor positive predictive value, in vivo leads have also fallen short for predicting success in human trials, and the SOD1G93A mouse model has become even more entrenched as a preclinical screening tool, guiding the choice of promising therapeutic agents to carry forward to human trials.

Our review summarizes changes in body weight and muscle function, demonstrates that these outcome measures in mouse studies have been underutilized, and suggests that body weight and muscle function should be relied upon more heavily in future studies in the ALS mouse model. Rather than relying heavily on survival, more comprehensive criteria for the selection of

promising ALS therapy candidates might include (1) significant prolongation of survival and delay in onset of weakness; (2) survival prolongation after disease onset (since most people with ALS are diagnosed after onset), when the window of prevention has closed and the goal of therapy must be to slow disease progression; (3) delay in body weight loss; and (4) improvement of muscle function.

Because ALS is a multifaceted pathological disease, various pleiotropic agents including melatonin and exciting new therapies targeting one or more molecular pathogenic mechanisms are currently being studied [93, 195]. Novel therapeutic agents may even have differential effects at different points in the progression of disease. Furthermore, there may be numerous pathologically and/or genetically distinct forms of the disease, which might be best targeted with different therapies. In addition, some agents shown to improve muscle function failed to extend survival in ALS animals [196–199]. As a result, a broad range of selective therapeutics might each have a minimal effect on the whole human population with ALS or a substantial effect in one patient subgroup and virtually no effect in another subgroup. This suggests that with appropriate animal models, treatment with combinations of therapies could be better explored. Motor neuron-targeted agents might be combined with other classes of drugs, for example, skeletal muscle-targeted treatments, to increase effectiveness. Given the complexity of ALS, the likelihood of a complex solution emerging is high. Solving such a complicated problem will require collaboration among academia and the biotech and pharmaceutical industries. Furthermore, biomarker discovery may help us to recognize the earliest symptoms of ALS, ultimately improving prognosis and contributing to clinical trials with ALS patients.

Ongoing therapeutic trials in animal models of ALS will provide insight into promising therapies to decrease body weight loss, improve motor performance, extend survival, and delay disease progression [200]. Our review may imply a novel, practical, and feasible strategy for drug discovery applicable to ALS.

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